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Elimination of Micropollutants from Sewage Sludge Using Vermicomposting

(Eliminace mikropolutantů z čistírenských kalů pomocí vermikompostování)

A Doctoral dissertation work

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Declaration

I declare that this diploma thesis work **Elimination of Micropollutants from Sewage Sludge Using Vermicomposting** is my own work and all the sources cited here are listed in the References.

Prague, 2023

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Bayu Dume Gari

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1. Introduction

Sludge management remains a significant challenge in wastewater treatment today, often surpassing liquid treatment costs (Hammer & Palmowski 2021). After treatment, inorganic pollutants like heavy metals and organic micropollutants (OMPs), including pharmaceuticals and personal care products (PPCPs), endocrine disrupting chemicals (EDCs) and pollutants like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), can be discharged into the environment from wastewater treatment plants or removed through processes like sludge adsorption and microbial degradation (Hammer & Palmowski 2021). Semblante et al. (2015) reveal a staggering annual production of 45 million tons of sewage sludge dry matter from municipal wastewater treatment plants (MWWTPs) worldwide while noting a variable range of 18 to 33 million tons in dry weight for the average annual production in the EU, USA, and China. The global concern over OMPs stems from their potential as environmental contaminants (Ben et al. 2018), with various OMPs found in environmental matrices like surface water, sewage sludge, and soils (Bradley et al. 2017). PPCPs and EDCs are prevalent in the aquatic environment due to extensive research, primarily sourced from MWWTPs (Hammer & Palmowski 2021). The presence of PPCPs and EDCs in WWTPs has been extensively studied, providing valuable information about their environmental loads (Mazzeo et al. 2023). Of particular concern are PPCPs, which have become a major issue in the last decade due to potential threats to aquatic ecosystems and human health, encompassing antibiotics, hormones, and disinfectants, among others (Jiang et al. 2023). EDCs, a wide range of natural and xenobiotic compounds, are also problematic, and sewage sludge is a significant source of these substances as they are not fully removed during wastewater treatment (Nunes et al. 2021). Hu et al. (2021) further highlight the harmful biological activity of EDCs in OMP substances, even at very low concentrations.

The substantial quantity of sewage sludge generated by MWWPTs poses environmental concerns due to its hazardous content, including organic compounds (PPCPs, EDCs), heavy metals (Cd, Cr, Pb, Co, Zn, Fe, Mn), and human pathogens (bacteria, fungi) (Buta et al. 2021). Although historically disposed of through incineration, landfilling, or ocean disposal; however, now a day agricultural applications of sewage sludge have become the most common method due to its economic advantages over other options (incineration, landfilling) (Meng et al. 2021). Currently, the main methods for sewage sludge disposal in the European Union are incineration (25%), reuse in agriculture where permitted by law (27%), landfilling (9%), composting (21%), and other methods (18%) (Ferrentino et al. 2023). Despite the potential for sewage sludge to replace fertilizer and enhance crop yield, its use is limited by the presence of toxic pollutants like PPCPs, EDCs, PCBs, and PAHs (Tasselli & Guzzella 2020). To address this, aerobic transformation processes such as composting and vermicomposting have been explored to stabilize contaminated sewage sludge (Rini et al. 2020; Lv et al. 2018).

In the pursuit of sustainable waste management practices, researchers are conducting controlled experiments and data analyses to understand how different influence the emission of CO_2 and CH_4 during composting and vermicomposting (Meng et al. 2018). These greenhouse gases play crucial roles in the Earth's atmospheric balance, impacting climate change (Awasthi et al. 2018; Nigussie et al. 2016). By examining emissions under various scenarios, it is possible to provide valuable insights into the environmental implications of composting and vermicomposting sewage sludge with bulking agent (straw pellets) (Lv et al. 2018). The goal is to optimize waste management strategies, minimizing greenhouse gas emissions while effectively utilizing sewage sludge for agricultural purposes, contributing to overall sustainability and climate change mitigation (Lv et al. 2018).

Moreover, enzymes play a vital role in breaking down complex organic compounds during composting and vermicomposting, promoting nutrient cycling and humus formation (Wang et al. 2021). Researchers are investigating how the conditions applied affects enzymatic activity, seeking to enhance waste degradation and nutrient cycling, and promoting environmentally friendly agricultural practices (Błońska et al. 2017; Uzarowicz et al. 2020).

Additionally, the rising accumulation of potentially toxic elements (PTEs) in sewage sludge raises concerns for environmental and human health (Lv et al. 2016). Vermicomposting, involving earthworms and microorganisms under aerobic conditions, emerges as a promising solution to address PTEs. In this context, vermicomposting technology emerges as a promising and ecologically sound approach to tackle this issue. By employing earthworms as bio-degraders, vermicomposting holds the potential to reduce the content of PTEs in sewage sludge; thereby mitigating their adverse impacts (Singh et al. 2020).

Lastly, the carbon-to-nitrogen (C/N) ratio is a crucial factor in waste management, significantly influencing final product compost/vermicompost maturity and organic matter (Lv et al. 2018). By this reason, understanding and controlling the C/N ratio is vital for optimizing composting processes and enhancing its effectiveness (Akratos et al. 2017). Researchers are exploring the best material combinations and co-substrate additions (straw pellet) to achieve desired C/N ratios, thereby improving waste transformation processes and nutrient availability for plants (Zhang et al. 2016; Akratos et al. 2017).

This thesis primarily aims to assess organic micropollutant (OMPs) contents (including PPCPs and EDCs) during vermicomposting of sewage sludge mixed with varying proportions of straw pellets. Additionally, the study examines parameters such as carbon dioxide (CO_2) and methane (CH_4) emissions, changes in enzymatic activity, concentrations of potentially toxic elements (PTEs) like As, Cd, Cr, Cu, Pb, and Zn, as well as PTE content in earthworm tissues. Moreover, the research seeks to evaluate the feasibility and quality of end-products (compost/vermicompost) derived from sewage sludge under diverse C/N ratios.

2. Literature review

2.1. Organic pollutants and micro-pollutants in sewage sludge, soil and plants

Global anthropogenic chemical production has increased from 1 to 400 million tonnes since World War II (Naidu et al. 2021). Several of these chemicals, particularly persistent organic pollutants (POPs), have harmful effects. Currently, increasing attention is being paid toward understanding the fates and effects of OMPs in the ecosystem (Gavrilescu et al. 2015). OMPs is an operational definition for a group of compounds, which are not covered by existing water quality regulations due to their lower concentrations (i.e., ng/L to µg/L) but are thought to be potential threats to environmental ecosystems (Farré et al. 2008). The group includes more than 20 classes, which are found in the European aquatic (Geissen et al. 2015). The prominent classes are pharmaceutically active compounds (PACs), personal care products (PCPs), endocrine-disrupting chemicals (EDCs), pesticides, industrial chemicals, disinfection by products (DBPs), perfluorinated compounds (PFCs), additives, preservatives, detergents, surfactants, flame retardants, plasticizers, and their transformation products. These OMPs are commonly removed via biodegradation and sorption on sewage sludge in a biological wastewater treatment system (Menon et al. 2020). These OMPs, which are characterized by strong sorption properties, tend to accumulate on soil particles, e.g., trimethoprim, indomethacin, propranolol, metoprolol, and carbamazepine (Barron et al. 2009). Moreover, the translocation of OMPs depends on several factors, such as physicochemical properties of

OMPs (acid dissociation constant (pKa), octanol-water partition coefficient (K_{ow}), molecular size, etc.), properties of soil (soil pH, temperature, organic carbon content, etc.), and environmental conditions (temperature, relative humidity, photo-period, etc.) (Madikizela et al. 2018; Martín et al. 2012).

pKa is a measure of the strength of an acid in a solution. It represents the negative logarithm (base 10) of the acid dissociation constant (Ka) of a compound. The Ka is a measure of the extent to which an acid dissociates into its conjugate base and a hydrogen ion (H^+). Mathematically, the relationship between pKa and Ka is as follows (Martín et al. 2012):

$$pKa = -\log_{10}(Ka) \tag{1}$$

In simpler terms, the pKa value quantifies how readily an acid donates a proton (H^+) in a solution. Lower pKa values indicate a stronger acid, meaning it dissociates more readily and releases more H^+ ions. Conversely, higher pKa values indicate weaker acids, which do not dissociate as readily in solution (Jensen et al. 2017). K_{ow} is a measure of the relative solubility of a chemical compound in octanol (a non-polar organic solvent) and water (a polar solvent). It quantifies the tendency of a compound to preferentially partition into either of these phases. The K_{ow} value is defined as the concentration of a chemical in octanol divided by its concentration in water at equilibrium. Mathematically, the K_{ow} value is expressed as (Golovko et al. 2020):

$$K_{ow} = \text{[Compound] in Octanol} / \text{[Compound] in Water} \tag{2}$$

The K_{ow} value provides essential information about a chemical's hydrophobicity or lipophilicity. A higher K_{ow} value indicates that the compound is more soluble in octanol, which means it is more hydrophobic and tends to accumulate in fatty tissues or non-polar environments. On the other hand, lower K_{ow} values suggest that the compound is more soluble in water and is less likely to accumulate in fatty tissues. Both pKa and K_{ow} are crucial in various fields, including medicinal chemistry, environmental science, and drug development. They help scientists understand how chemicals interact with biological systems, their distribution in different body compartments, and their potential environmental fate (Jensen et al. 2017; Golovko et al. 2016).

Sorption of OMPs to the soil, apart from the soil pH and organic carbon content, also depends on the mineral parts of the soil (Thiele-Bruhn 2003). Mechanisms that may affect the adsorption of micropollutants are ion exchange, hydrogen bonds, or the formation of complexes with Ca^{2+}, Mg^{2+}, Fe^{3+}, or Al^{3+} ions (Díaz-Cruz et al. 2009; Xia et al. 2015). Some commonly detected organic compounds in sewage sludge are shown in Table 1. The translocation of OMPs can be referred to as the passage of OMPs from the roots to the areal parts of the plants, such as leaves, stems, fruits and rhizomes. Figure 1 shows plant uptake and translocation of PPCPs and EDCs from the environment to different aerial parts.

Table 1. Commonly detected organic compounds in sewage sludge

Name of Group	Concentration (no. of compounds, no. of samples)	Reference
Polycyclic aromatic hydrocarbons (PAH)	67-370 mg/kg dw (24 compounds, n =14); 19-219 µg/L (13 compounds, n=5); 5.2-11.57 mg/kg dw (13 compounds, n=5)	Barret et al. 2010
Organophosphorus compounds(OPs) Phthalates	0.620-6.90 mg/kg (12 compounds, n-17) DEHP-130 mg/kg dw, DBP-1094 mg/kg dw; 23.9-506.3 mg/kg dw (5 compounds, n=3)	Salaudeen et al. 2018
Polychlorinated biphenyls (PCBs)	0-5100 mg/L (35 compounds, n=9); 0.11-0.44 mg/kg dw (37 compounds, n=14)	Stevens et al. 2003
Polychloinated dibenzo-p-dioxins and-furans(PCDD/Fs)	TEQ 0.73 and 7348.40 pg/g dw (compounds, n=14), 104.0-1661 pg/g dw (17 compounds, n=24)	Balasubramani & Rifai 2015
Polybrominated diphenyl ethers (PBDEs)	0.197-1.185 mg/kg (11 compounds, n=5), 0.071-1.02 mg/kg (dw)	Xia et al. 2015
Polychlorinated alkanes	7-200 mg/kg (short chained) and 30-9700 mg/kg (medium chained) (4 compounds, n=14); 0.065-0.157 mg/kg dw (29 compounds, n=8)	Gomez-Canela et al. 2012

Source: Dubey et al. 2021. TEQ- Toxic equivalency PCDD/F, dw= dry weight

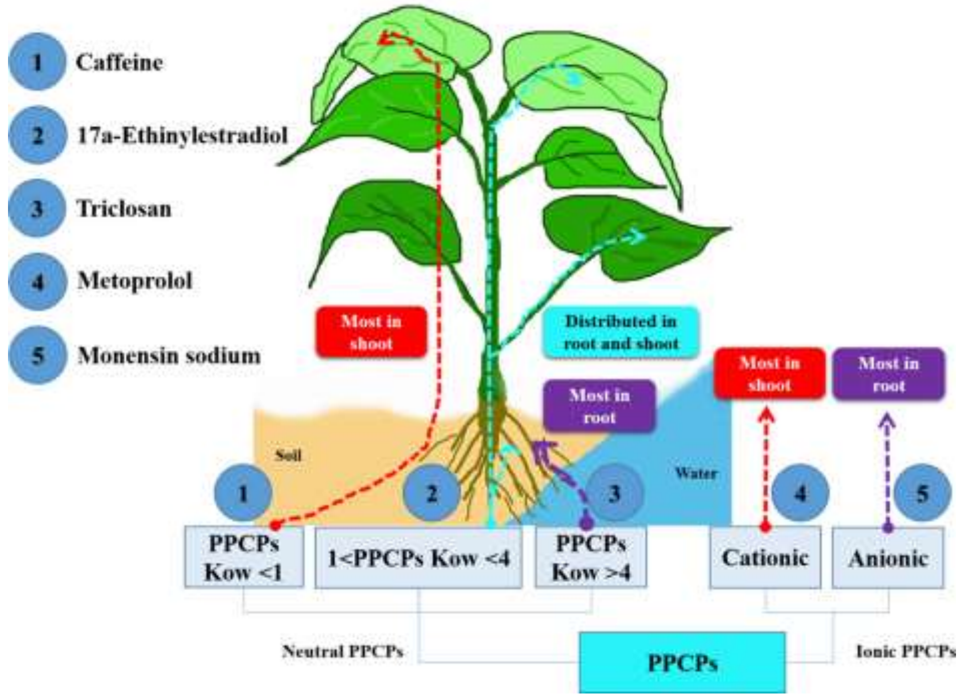

Figure 1. Plant uptake and translocation of PPCPs from the environment to different aerial parts (Chuang et al. 2019)

2.1.1. Endocrine disruptors

Various chemicals are known to act as endocrine disrupting compounds, i.e., exogenous chemicals which disrupt normal endocrine activity (Tabb & Blumberg 2006) in wildlife (Vos et al. 2000) and potentially in humans. This phenomenon has attracted attention from both the public and scientific communities (Vidaeff & Sever 2005). Endocrine disrupting chemicals (EDCs) are either naturally produced, such as 17b-estradiol (E2) and estrone (E1) which enter the waste water treatment system from the excreta of livestock and humans, or are synthetic, such as 17a-ethynylestradiol (EE2), which is an ingredient of the human contraceptive pill. Some synthetic EDCs, e.g. bisphenol-A, which is widely used for the production of epoxy resins and polycarbonate plastics (Fuerhacker 2003), may be consumed by humans through leaching from polycarbonate plastic products used in the packaging of food and beverages (Timms et al. 2005). Equally, phthalates which serve as plasticizers are found in many commercial products (McDowell & Metcalfe 2001). These ubiquitous chemicals are known to be taken up by both animals and humans; for example, phthalates have been found in human breast milk and urine (Calafat et al. 2005). These EDCs may therefore enter the wastewater treatment plant, following the discharge of urine and faeces into waste water. Concentrations of selected endocrine disruptors in sewage sludge are shown in Table 2. If they are not removed by the wastewater treatment process, their subsequent entry into freshwater systems may modify the normal endocrine activity of wildlife resulting in, for example, the feminisation effects reported in fish and demasculinization.

Table 2: Selected endocrine disruptors in sewage sludge compounds in sewage sludge, China

Sludge sample	BPA (μg/g)	4-n-NP (μg/g)	E2 (μg/g)	EE2(μg/g)
Anaerobic tank	0.65	0.51	0.16	2.12
Anoxic tank	1.62	0.53	0.39	3.23
Aerobic tank	0.30	0.05	0.02	0.51
Returned activated sludge	0.72	0.45	0.05	0.80

Source: Zhou et al. (2012), BPA= bisphenol A, 4-n-NP =4-n-nonylphenol, E2 =17b-estradiol, EE2 = 17aethinylestradiol

2.1.2. Pharmaceutical and personal care products (PPCPs)

Active pharmaceutical ingredients (APIs) may be released into the soil environment when contaminated sewage sludge, sewage effluent, or animal manure is applied to land (Monteiro & Boxall 2010). Veterinary pharmaceuticals may also be excreted directly to soils by pasture animals. Consequently, a range of APIs has been detected in agricultural soils, with reported concentrations ranging from 0.02 to 15 μg/kg dry soil (Vazquez-Roig et al. 2010). A number of studies have explored the uptake of APIs into aquatic invertebrates and fish (Meredith-Williams et al. 2012). However, much less work has been done to assess the uptake of APIs into terrestrial organisms. The work that has been done has focused on the uptake of human and veterinary APIs into plants (Carter et al. 2014) with only a few studies looking at uptake into terrestrial invertebrates such as earthworms (Kinney et al. 2012). The limited data on uptake of APIs into earthworms originates from studies of the bioaccumulation of anthropogenic waste indicators (including the APIs trimethoprim, caffeine, carbamazepine, thiabendazole, and diphenhydramine) from agricultural soil amended with biosolids or swine manure (Kinney et al. 2008). Trimethoprim was the only API detected in the earthworms, at concentrations of 127 μg/kg dry weight in earthworms from a biosolid-amended field and 61μg/kg dry weight in earthworms from the manure amended field (Kinney et al. 2008). Given the paucity of the data and the potential for pharmaceuticals to end up in the soil, further research is therefore required to fully characterize the potential for pharmaceutical uptake into terrestrial invertebrates.

Pharmaceuticals and Personal Care Products (PPCPs) are listed below included: Veterinary and human antibiotics (Trimethoprim, erythromycine, lincomycin, sulfamethaxole, amoxycillin, chloramphenicol), analgesics and anti-inflammatory drugs (Ibuprofen, diclofenac, fenoprofen, acetaminophen, naproxen, paracetamol, acetylsalicylic acid, fluoxetine, ketoprofen, indometacine), antidepressants (Diazepam, Carbamazepin, Primidon, Salbutamol), fat regulators (Clofibric acid, bezafibrate, fenofibric acid, etofibrate, gemfibrozil), beta-blockers (Metoprolol, propranolol, timolol, sotalol, atenolol), X-rays contrast (Iopromid, iopamidol, diatrizoate), Steroids and hormones (Estradiol, estrone, estriol, diethylstilbestrol). Personal care Products: Sun-screen agents (Benzophenone, methylbenzylidene camphor) Scents, UV filters, Insect repellents (N, N-diethyltoluamide), surfactatnts (surface-active substances), antiseptics (Triclosan, chlorophene). Table 3 shows concentration of selected PPCPs in sewage sludge.

Table 3: Concentration of selected PPCPs in sewage sludge

Organic material	Triclosan	Caffeine	Citalopram	Diclofenac	Telmisartan
Sewage sludge (ng/g)	543.2±62.07	141.8±21.6	440.28±4.9	284.2±16.97	10,161.6±391.7

Source: Dume et al. 2023

2.1.3. Polycyclic aromatic hydrocarbons (PAH)

Organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), have been identified as a significant group of contaminants found in sewage and subsequently in sludge. These pollutants find their way into wastewater from multiple sources and become absorbed by solid particles. Consequently, during processes like biological treatment, they tend to accumulate in the sludge due to their parasitic nature (Xie et al. 2012). PAHs possess relatively high water solubility and exhibit strong lipophilic characteristics. These compounds predominantly find their way into the environment through the release of waste from industrial facilities like refineries, wastewater treatment plants, as well as from natural sources. Additionally, household and municipal usage of certain products containing PAHs contributes to their presence in the environment. Subsequently, these PAH compounds can be discharged into sewage through the mentioned sources and ultimately undergo concentration and accumulation in sewage sludge (Mokhtari et al. 2017). Figure 2 shows some example of polycyclic aromatic hydrocarbon (PAH) in sewage sludge.

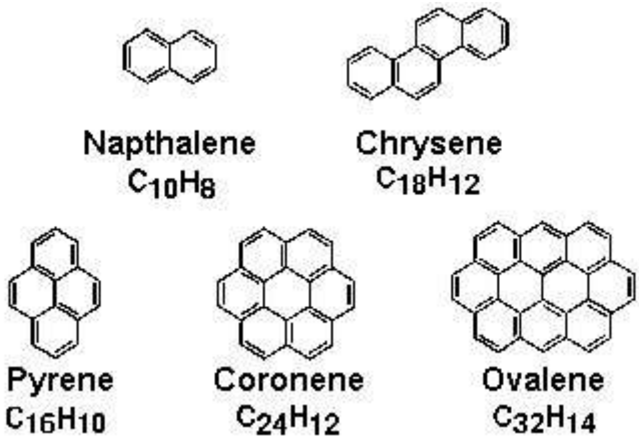

Figure 2. Some example of polycyclic aromatic hydrocarbon (PAH)

Most PAHs are absorbed to particles at low vapour pressure in the air. In the case of dispersing in water or being absorbed on a particulate material, they may be decomposed when exposed to ultraviolet light of sunshine. In the atmosphere, PAHs can produce nitro, di-nitro-PAHs, and sulfonic acids with pollutants including ozone, nitrogen, and sulphur oxides, respectively. Moreover, PAHs may be decomposed by some microorganisms in the soil (Zhang et al. 2020). Polycyclic aromatic hydrocarbons are common pollutants in soils and typically tend to be persistent in soils because of their relatively low mobility and high resistance to degradation. As a result, prior to the ultimate treatment of sludge, it becomes crucial to determine the concentration levels and risk assessment of pollutants, including PAHs. This information plays a significant role in assessing the potential fate and environmental impact of these specific compounds. Table 4 shows comparison of PAH concentrations in sewage sludge from wastewater treatment plants across the world.

Table 4. Comparison of PAH concentrations in sewage sludge from wastewater treatment plants across the world

Location	Simple size (No. of WWTPs)	Sludge type	∑PAHs (ng/g)	References
China(Honk Kong)	n = 2	Municipal	796 – 839	Man et al. 2016
China(Zhejiang)	n = 3	Municipal	16,090 – 20,900	Hu et al. 2014
China(Shaanxi)	n = 18	Various	778 –3,265	Wu et al. 2019
Korea(Ulsan)	n = 1	Industrial	2,100	Oh et al. 2016
Japan(Hiroshima)	n = 1	Municipal	69	Osaki et al. 2015
Poland(Opole)	n = 2	Municipal	10,060 –30,540	Poluszyńska et al. 2017
Italy(Lombardy)	n= 1	Municipal	2,325 –2,890	Torretta & Katsoyiannis 2013

Source: Wu et al. 2019

2.1.4. Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are a family of non-polar, aromatic chlorinated hydrocarbons that were produced commercially by direct chlorination of biphenyl. There are 209 possible different PCB compounds referred to as congeners, depending on the biphenyl rings capacity of 1-10 possible chlorine sites. Health concerns arose from PCBs suspected toxic and carcinogenic properties, as well as its endocrine disruptive effects, including effects on the immune system, reproductive system, nervous system, and endocrine system. PCBs also bio-accumulates in the food chain, with concentrations increasing by several orders of magnitude at succeeding trophic levels. The transport of PCBs between agricultural soils and earthworms has also been studied, with the primary objective of using the earthworms as monitors and indicators of soil contamination. Concentration of selected PCBs in sewage sludge is shown in Table 5. Research has also focused on investigation of the changes in metabolic activity of earthworms as a consequence of their exposure to PCBs.

Table 5. Concentration of selected PCBs in sewage sludge

Organic material	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
Sludge(ng/g,)	1,317.1	26.4	3.101	2.763	3.002	2.893	0.2893

Source: Yao et al. 2014

The risk posed by these organic contaminants to soil animals and plants, as well as to human health, is concerning. The pollutants in soil may undergo mineralization, degradation, sorption, bound-residue formation, and transformation to various metabolites. These processes can accelerate the dissipation of pollutions in soils, but the metabolites may be more toxic to soil organism. Soil organisms and plants can accumulate organic pollutants from soils and affect the environmental fate of the pollutions. They increase sorption of pollutants to soil (especially on fresh cast) and degradation of various organic pollutants in soil but decrease mineralization of the pollutants and produce more metabolites. Both laboratory and field studies showed that degradation by plants and their associated microbes are efficient to remove variety organic pollutants including PCB from soil. Wetland plants can stimulate metabolism and dissipation of TBBPA in submerged soil, and willow plants increase the removal of PPCP from soil (Zhang et al. 2020).

2.2. Vermicomposting

Vermicomposting is gaining popularity due to its versatility (Soobhany 2019). Recent research has also shown that a controlled vermicomposting process reduces greenhouse gas (GHG) emissions (Lv et al. 2018). As a result, vermicomposting can use to recover nutrients from organic solid wastes such as sewage sludge in a more secure and environmentally sound manner. Vermicomposting, in which earthworms and the bacterial community in their alimentary canal promote material transformation, may be used as an alternative or supplement to composting (Hait & Tare 2012). Vermicomposting is also a bio-oxidative process that engages earthworms and microorganisms. Microorganisms, both in the guts of the earthworms and in the feedstock, are responsible for biochemical decomposition of organic matter; in contrast, the earthworms fracture the substrate, increasing the surface area exposed to the microorganisms. As a result, earthworms directly alter the material’s physical qualities while indirectly altering its chemical properties. Vermicomposting is a mesophilic biological process in which earthworms and microorganisms interact to treat organic waste. Earthworms act as mechanical blenders in this technique, accompanied by microorganisms that act as biochemical degraders of organic waste.

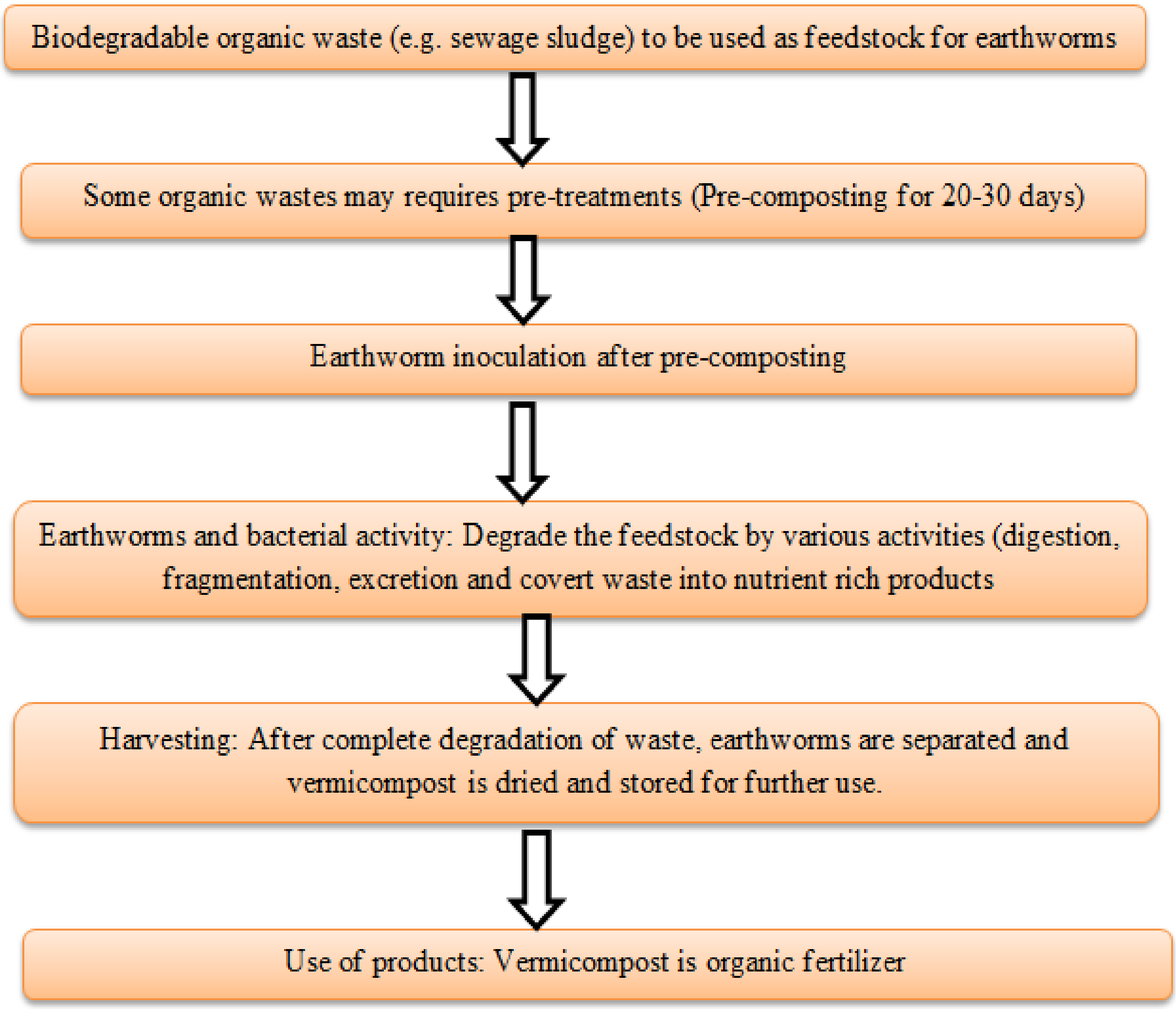

Figure 3. Step-wise procedure for vermicomposting (Sharma & Garg 2023)

Earthworms and microbes work together to degrade waste and recycle it into vermicompost, a humus-rich product. Vermicompost has a high porosity and surface area, as well as a good nitrogen (N), phosphorus (P), potassium (K) and C/N ratio. It also has excellent antibacterial properties. Earthworms and microorganisms work differently during vermicomposting, and earthworms are the process's dynamic operators, acting as waste degraders and fragments. This action increases the surface area of the waste and exposes it to microorganisms, making it more suitable for microbial activity. The earthworm's intestine contains microbial flora that aids in the biochemical degradation of waste (Dominguez & Edwards 2011). Vermicomposting has two stages: active and maturation. Earthworms change the physical state and microbial composition of organic waste during the first phase (Lores at al. 2006). During the maturation phase, earthworms migrate to new layers of waste, and microbes continue to degrade

(Dominguez & Edwards 2011). The step-by-step procedure for vermicomposting is shown in Figure 3. To achieve optimal conditions for earthworms, a variety of waste residues such as animal excreta, sugarcane trash, sewage sludge, paper waste, crop straw, and so on are used as bedding materials. Vermicomposting bedding should have a high C/N ratio, good bulking potential and high absorbency (Garg et al. 2006).

2.3. Factors affecting vermicomposting

The essential factors on which process of vermicomposting depends are abiotic and biotic. The most important abiotic factors which affect vermicomposting process include temperature, moisture content, C/N ratio, pH value, and oxygen availability; feed quality, light, ammonia and salt content. The biotic factors are earthworm stocking density, micro-organism, and enzymes.

2.3.1. Temperature

Earthworms have fairly complex responses to changes in temperature, the optimum temperature range may be 25-37 °C, which favours the activity, growth, metabolism, respiration, reproduction, and cocoon production for earthworms and also favours the microorganisms associated with earthworms. Worms tolerate at a temperature range of 5-29 °C (Sinha et al. 2002). Different tolerant temperature ranges are documented by several researchers from 0 to 40 °C (Nagavallemma et al. 2004). At higher temperatures (above 30 °C), the chemical and microbial activity enhances in the substrate, which leads to the reduction of oxygen level and thus had negative effects on earthworms (Sinha et al. 2002). Different earthworm species showed different responses against temperature. For example, *Eisenia fetida* grows optimally at 25 °C with 0-35 °C temperature tolerance, whereas *Dendrobaena veneta* showed optimum growth at lower temperature and found less tolerance of extreme temperatures. *Eudrilus eugeniae* and *Perionyx excavatus* also showed optimum growth at about 25 °C; however, their tolerance temperature range was generally found between 9 and 35 °C. In another study, *Dendrobaena veneta* showed poor reduction with decreasing temperature. A study conducted by Frederickson & Howell 2007 to assess the earthworm population in unheated (at ambient temperature of 6.3 °C ±2.3 °C) and heated beds (at 13.7 °C±0.8 °C) showed greater earthworm biomass and higher numbers of hatchlings and cocoons in heated beds when compared with unheated beds. However, another study found that *Eudrilus eugeniae* and *Perionyx excavates* showed higher hatching percentage at lower temperatures (20-24 °C) when compared with high temperatures (27-30 $^{\circ}$C) (Giraddi et al. 2008). This discussion may lead to the conclusion that different earthworm species responded differently to different temperature ranges. Vermicomposting systems, when compared with composting process, are greatly affected by extreme temperature conditions, that is, low or high temperature. For example, higher temperatures in vermicomposting systems are responsible for the loss of nitrogen as the consequence of NH_3 volatilization. On the other hand, lower temperatures in vermicomposting process fail to destroy pathogenic organisms (Ndegwa & Thompson 2001).

2.3.2. Moisture content

A strong relationship exists between the moisture content of organic wastes and the growth rate of earthworms. The earthworms need to be in a moist ambient because they need to keep their skin wet for respiration through it. It's known that, there is a relationship between moisture content and temperature in effecting on vermicompost process. Adequate moisture content is one of the most important factors necessary for the working of earthworms and microorganisms in vermicomposting system. Earthworms breathe through their skin; therefore the system must have adequate moisture content. In a comparative study on vermicomposting process and earthworm's growth at different temperature and moisture ranges showed that 65–75% is most suitable range of moisture at all ranges of vermicomposting temperature (Amaravathi & Reddy 2014). The bedding used for vermicomposting must be able to hold sufficient moisture as earthworms respire through their skins and moisture content in the bedding of less than of 45% can be fatal to the worms. Although *epigenic species, E. fetida and E. andrei* can survive moisture ranges between 50% and 90%, but they grow more rapidly between 80% and 90% (Dominguez & Edwards 1997). The bacteria also plays vital role in vermicomposting. Its activity decreases in moisture content lower than 40% and it almost stops in lower than 10% (Tchobanoglous et al. 1993).

2.3.3. C/N ratio

C/N ratio of feed material affects the earthworms' growth and reproduction. Higher C/N ratio in the feed material accelerates the growth and reproduction of worms. If C/N ratio is too high or too low, waste degradation is slowed. Major nutrients requirements for growth sustenance of composting microorganisms are carbon (C), nitrogen (N), phosphorus (P) and potassium (K) and are acquired during breakdown of organic compounds in order to obtain the needed energy for their metabolism (Chen et al. 2011). Carbon (energy source) and nitrogen (build cell structure) are two most important nutrients, and their ratio indicates the rate of organic matter breakdown in the composting stock (Chen et al. 2011). According to Igoni et al. (2008), limited amount of N reduces microbial growth resulting in slower rate of carbon decomposition while presence of excess N than required by available microbial population. When there is a decrease in C to N ratio during composting, the frequency of organic N mineralization is lower compared to rate of organic carbon mineralization hence the need to adjust the initial raw materials to give the optimum C/N ratio definitive of sustainable composting. The C/N ratios usually indicate that available C is used up by microorganisms 30 to 35 times quicker than the N conversion rate (Igoni et al. 2008). Different C/N are documented in literature which includes C/N: 25-30:1 (Huang et al. 2008); C/N ratio: 20-40:1; C/N ratio 25-50:1 (Petric et al. 2015). Low C/N ratio releases huge sum of soluble salt thereby rendering it non-favourable for growing plant and also makes the compost stock to generate a characteristic foul-smell (Mohee et al. 2015). Meanwhile high proportion of C/N indicates insufficient N for optimum growth of microbial population leading to slow rate of decomposition (Chen et al. 2011). The C/N ratio could be adjusted with the use of different types of bulking agents which are mixed with organic material before composting in order to improve the porosity as well as C/N ratio of the initial feedstock (Zhang et al. 2016). Examples of notable bulking agents includes, rice husk, wood chip, sawdust, and peanut shells, and urea and they are known to absorb moisture thereby controlling odour during co-composting as itself becomes degraded along the process.

2.3.4. pH

Neutral pH is suitable for the proper working of earthworms, but the acceptable range reported is 4.5-9.0. It mostly depends on earthworms sensitivity and physicochemical characteristics of the waste. The difference in physicochemical characteristics of waste mainly alters the pH of vermicomposting process. The microbial activity changes physicochemical characteristics of waste during decomposition process along with mineralization of nitrogen and phosphorus into nitrites/nitrates and orthophosphates (Suthar 2009). Because of the decomposition of organics, some intermediates are produced; such as ammonium and humic acids, during the decomposition process that alter the change of pH based on the fact that negatively and positively charged groups led to either neutral or acidic pH (Pramanik et al. 2007). Different types of substrates also affect the pH of the vermicomposting system because of different intermediate species production, and hence, different types of waste show different behaviors in pH shift, and the overall pH in vermicomposting process drops from alkaline to acidic nature. Many studies (Yadav & Garg 2011) reported acidic pH during vermicomposting process; however, at initial stages, the pH value is in alkaline range (8.3-7.2), which slightly shifts to acidic or neutral range (6.3-7.1) at the end of vermicomposting process (Garg et al. 2006) owing to the intermediate products produced during the process. Yadav & Garg (2011) also reported decreased pH from alkaline (7.2-8.1) to slightly acidic (6.4-6.8), which was supported by the results of Khwairakpam & Bhargava (2009). Another experiment conducted by Yadav & Garg (2009) also showed decreasing trend in vermicomposting process with initially alkaline (6.5-7.9) to finally acidic (5.8-6.7). The results of Suthar (2009) showed 3.5-9.5) overall decrease in pH value during vermicomposting. The final lower pH of waste when compared with the initial characteristics was largely due to the evolution of CO_2 and the accumulation of organic acids (Usman et al. 2015).

2.3.5. Oxygen content

As the earthworms are aerobic organisms, oxygen is essential for vermicomposting. Oxygen consumption is a function of microbial and earthworm activity, oxygen levels are also related to substrate temperatures. In a vermicomposting system excessive moisture can cause poor availability of oxygen and may affect the oxygen supply to the worms. Earthworms are oxygen breathers and cannot survive in anaerobic conditions. They operate best when compost material is porous and well aerated. Earthworms also help themselves by aerating their bedding by their movement through it. *E. fetida* have been reported to migrate in high numbers from oxygen depleted water saturated substrate, or in which carbon dioxide or hydrogen sulfide has accumulated (Meng et al. 2018).

2.3.6. Feed quality

Suitable feed material for earthworms is a primary need in the vermicomposting process. The amount of food that can be consumed daily by earthworm varies with a number of factors such as particle size of food, state of decomposition of the food, C/N ratio of food, salt content in food etc. Small particle size of feed waste will ensure the earthworms to speed up the vermicomposting process. This small particle size allows the proper aeration through the pile of waste material and available to worms. The quantity of food taken by a earthworms varies from 100 to 300 mg/g body weight/day. Earthworms derive their nutrition from organic materials, living microorganisms and by decomposing macro-fauna.

2.4. Properties of vermicompost and its uses

Various organic wastes (animal dung, crop residues, sewage sludge, waste paper, rot food etc.) are used to produce vermicompost. Vermicompost application as organic fertilizer improves plant growth, enhanced microbial population in soil and suppresses plant diseases.

Table 6. Physicochemical properties of vermicompost prepared from different organic waste

Organic wastes	pH	EC(dS/m)	TOC (g/kg)	TN (g/kg)	C/N ratio	Reference
Paper mill 1^0 waste	8.01–8.10	1.75–1.89	281–295	15.26–22.17	12.71–19.67	Ganguly & Chakraborty 2018
Paper mill 2^0 waste	8.31–8.39	1.78–191	152–165	16.37–23.15	6.61–10.07	
Municipal solid waste	7.25	2.79	364.8	16.84	21.66	Srivastava et al.2020
Sea weeds, cow dung	7.14–7.38	1.97–2.11	21.93–22.02	1.01–1.39	16–21.70	Ananthavalli et al. 2019
Fruit & vegetable waste	6.55–7.29	3.38–3.94	235.4–340.3	15.46–20.43	11.35–22.02	Sharma & Garg 2017
Cow manure, mushroom residue	7.57	2.98	257.6	23.03	11.32	Song et al. 2014
Pig manure, mushroom residue	7.35	3.26	273.6	26.21	10.43	

Source: Sharma & Garg 2023.TOC= Total organic carbon, TN = Total nitrogen, EC = Electrical conductivity

Soil quality is also improved by vermicompost as it can enhance soil porosity, water retention and oxygen content. If vermicompost is a component of integrated nutrient management then dependency on chemical fertilizers can be reduced significantly. Table 6 summarizes various physico-chemical properties of vermicompost prepared from different wastes and uses of vermicompost are given in Figure 4. Vermicompost is a finely divided peat-like material with excellent structure, porosity, oxygen, drainage, and moisture-holding capacity (Edwards et al. 2011). Vermicompost, an organic fertilizer rich in N, P, K, micronutrients, and beneficial soil microbes (nitrogen-fixing and phosphate solubilizing bacteria and actinomycetes), is a sustainable alternative to chemical fertilizers, which is an excellent growth promoter and protector for crop plants (Chauhan & Singh 2015). Today vermicompost is an important component of organic farming systems, because it is easy to prepare, has excellent properties and is harmless to plants. Vermicompost improves the physical, chemical and biological properties of the soil as well contribute to organic enrichment (Chauhan & Singh 2013).

Figure 4. Benefits of vermicompost as organic fertilizer

3. Hypotheses and objectives of the work

3.1. Hypotheses

- The addition of different proportions of bulking agent (straw pellets) during sewage sludge composting and vermicomposting will significantly affect carbon dioxide (CO_2) and methane (CH_4) emissions.
- The enzymatic activity during composting and vermicomposting of sewage sludge mixed with bulking agent (straw pellets) will be significantly different, with vermicomposting showing higher enzymatic activity compared to composting.
- Different mixing mass ratios of bulking agent (straw pellets) reduced the potentially toxic element (PTEs) among variants, and earthworms (*Eisenia andrei*) reduced the PTEs among variants during vermicomposting.
- The content of organic micropollutants (OMPs) (PPCPs and EDCs) is significantly reduced during vermicomposting.
- The feasibility and end-product quality of compost/vermicompost produced from sewage sludge will vary under different carbon-to-nitrogen (C/N) ratios.

3.2. Objectives

- To assess the carbon dioxide (CO_2) and methane (CH_4) emissions from sewage sludge composting and vermicomposting under the influence of different proportions of bulking agent (straw pellets).
- To compare and evaluate the differences in enzymatic activity during composting and vermicomposting of sewage sludge mixed with bulking agent (straw pellets).
- To evaluate the content of PTEs (As, Cd, Cr, Cu, Pb and Zn) during the vermicomposting of sewage sludge in varying proportions with the bulking agent and the content of the PTEs in earthworm tissues with the aim of evaluating the ability of earthworms to remove monitored elements from sewage sludge during vermicomposting.
- To evaluate the contents of organic micropollutants (OMPs) (PPCPs and EDCs) during the vermicomposting of sewage sludge in varying proportions with the bulking agent (straw pellets).
- To evaluate the feasibility and end-product quality of compost/vermicompost produced from sewage sludge under different C/N ratios.

4. Materials and Methods

4.1. Initial raw materials and earthworms

The experiments used freshly deposited sewage sludge collected from a wastewater treatment plant (WWTPs) in a small town in the Czech Republic (3,500 population equivalents). The WWTP was operated on the mechanical-biological principle with an activation process applied for biological (secondary) treatment. The sludge was digested under aerobic conditions. Before being used in the experiment, it was kept at 4 $^{\circ}$C for one week. Dried straw pellets were provided by Granofyt Ltd. Company (Chrášťany, Czechia) with a diameter of 10 mm. Earthworms were collected from a private vermiculture stock in the Czech Republic with grape marc substrate as survival media. Of the determined 32 organic micropollutants, only 6.05 ng g^{-1} of caffeine (CAF) and 2.24 ng g^{-1} of telmisartan (TE) were detected in earthworm tissues. Due to its high tolerance for environmental variables such as pH, moisture, and temperature, as well as accepting a wide variety of feeds, a high growth rate, and the capability of converting semi-composted biomass into stable products, the *epigeic* earthworm species *Eisenia andrei* was used in the experiments (Yadav & Garg 2016). The selected physicochemical properties of the initial materials, organic micropollutants and microorganisms before vermicomposting are presented in Table 7.

Table 7. Physicochemical properties of the initial materials, organic micropollutants and microorganisms before vermicomposting

Parameters	Sewage sludge	Straw pellet	Earthworms
Dry matter (%)	13.3±0.19	21.2±0.56	
pH-H_2O	6.9±0.03	8.3±0.52	
Electrical conductivity (mS/cm)	0.617±0.11	0.68±0.07	
Total carbon (%)	32.9±0.26	42.59±0.36	
Total nitrogen (%)	5.36±0.03	0.80±0.12	
C/N ratio	6.1±0.04	53.67±7.60	
Micropollutants (ng g^{-1})			
Bisphenol A	88.78±18	n.d	n.d
Caffeine	141.81±12.5	10.23±2.56	6.05±0.69
Carbamazepine	38.51±0.73	n.d	n.d
Cetirizine	78.95±0.69	n.d	n.d
Citalopram	440±2.84	n.d	n.d
Diclofenac	284.22±9.8	n.d	n.d
Ibuprofen	87.33±6.68	n.d	n.d
Mirtazapine	63.26±2.83	1.75±0.07	n.d
Sulfapyridine	15.22±1.03	1.69±0.04	n.d
Telmisartan	10,161.60±226	3.74±0.20	2.24±0.11
Triclosan	543.24±36	n.d	n.d
Venlafaxine	33.97±3.74	1.67±0.03	n.d
Acetaminophen	85.72±10.65	n.d.	n.d.
Amitriptyline	5.19±0.22	n.d.	n.d.

Atenolol	5.11±0.11	n.d.	n.d.
Atorvastatin	12.37±0.67	n.d.	n.d.
Carbamazepine 10,11-epoxide	3.72±0.11	n.d.	n.d.
Clarithromycin	89.02±1.01	n.d.	n.d.
Daidzein	14.73±1.08	n.d.	n.d.
Equol	8.85±1.03	n.d.	n.d.
Estrone	15.64±0.98	n.d.	n.d.
Gabapentin	15.45±0.93	n.d.	n.d.
Genistein	14.81±0.25	n.d.	n.d.
Hydrochlorothiazide	103.93±24.65	n.d.	n.d.
Lamotrigine	104.84±30.89	n.d.	n.d.
Metoprolol	82.56±23.88	n.d.	n.d.
Omeprazole	1.27±0.12	n.d.	n.d.
Paraxanthine	28.60±4.41	n.d.	n.d.
Sulfamethoxazole	21.42±0.66	n.d.	n.d.
Sulfanilamide	15.82±0.98	n.d.	n.d.
Tramadol	36.55±1.89	n.d.	n.d.
Trimethoprim	45.06±5.19	n.d.	n.d.
Microorganisms (μg PLFA/g dw)			
Fungi	146±2.0	24±3.0	n.a.
Bacteria	3,150±83.0	53±6.0	n.a.
Actinobacteria	34±0.0	1±0.0	n.a.
G+ bacteria	1,159±34.0	20±3.0	n.a.
G- bacteria	1,766±47.0	22±4.0	n.a.
Total microbial biomass	4,145±93.0	122±9.0	n.a.

dw = dry weight, PLFA, phospholipid fatty acids, n.d. = not detected, n.a. = not analysed, values indicate mean ± standard error (n = 3), G+ = gram positive bacteria, G- = gram negative bacteria.

4.2. Experimental set-up

The experiment included eight variants with three replications at different mixing proportions of sewage sludge (SS) and straw pellets (SP) with earthworms (+EW) and without earthworms (-EW). Table 8 shows the composition of composting/vermicomposting materials in various proportions. In all variants, the additive material was homogenised and transferred to worm-bins (40 × 40 × 15cm) for 120 days of vermicomposting and also the same variants were transferred to aerobic composters (fermenter barrels) with a working volume of 70 L and a diameter of 56 cm, which were constructed with the aim to ensure optimal conditions for composting. The substrate (3 L grape marc) containing earthworms were placed into the tray from the side to avoid earthworm mortality and to allow earthworms to return to optimum condition (Hanc et al. 2022). In the substrate, the average earthworm density (*E. andrei*) was 126 pieces/L, with each piece weighing 0.2 g. The vermicomposting process was carried out at a constant temperature of 22 °C. The moisture level of the material was maintained at around 70%–80% by spraying the surface with water every 2 days during vermicomposting. The experiment was carried out at the Faculty of Agrobiology, Food, and Natural Resources experimental station in Cerveny Ujezd, Czech University of Life Sciences Prague.

Table 8: Composition of composting/vermicomposting materials in different proportions

Variants	SS (%)	SS(kg)	SP (%)	SP (kg)	Mixing ratio	Total weight Material (kg)	Earthworm substrate (L)
T1	100	9	0	0	4:0	9	3
T2	100	9	0	0	4:0	9	0
T3	75	6.75	25	2.25	3:1	9	3
T4	75	6.75	25	2.25	3:1	9	0
T5	50	4.5	50	4.5	2:2	9	3
T6	50	4.5	50	4.5	2:2	9	0
T7	25	2.25	75	6.75	1:3	9	3
T8	25	2.25	75	6.75	1:3	9	0

SS = sewage sludge, SP = straw pellets

4.3. Measurements of carbon dioxide (CO_2) and methane (CH_4) during composting and vermicomposting

During the composting and vermicomposting process, a closed chamber technique was employed to assess CO_2 and CH_4 concentrations. This method involved using a tightly sealed chamber equipped with two ports, one for sampling the headspace gas and measuring air temperature, and the other for connecting to data collection instrument. One end of a plastic tube was linked to the closed composting barrels and vermicomposting worm bin, while the other end was connected to the measuring instruments. Over a period of 60 days, gas measurements were taken twice daily, with 12-hour intervals, using the Gasko Infrared Gas Analyzer (Chan et al. 2011). To determine the cumulative CO_2 and CH_4 emissions, the daily values were summed, providing the total gas emissions over the entire duration of the experiment (Nigussie et al. 2016).

$$A_{t(ab)} = \frac{(t_b - t_a).(F_{ta} + F_{tb})}{2} \qquad (3)$$

Where $A_{t(ab)}$ is the cumulative emission (g kg^{-1} dry matter) between the measurement days (between ta and t_b), ta and t_b are the measurement dates, and F_{ta} and F_{tb} are the gas fluxes on the two measurement dates. Therefore, the total cumulative emissions were calculated as the sum of cumulative emissions on each day using Equation (2):

$$Total\ cumulative\ emission = \sum A_{t(ab)} \qquad (4)$$

C losses during composting and vermicomposting were calculated as:

$$C\ loss(\%) = \frac{(C_{initial} - C_{ending})}{C_{initial}} \qquad (5)$$

4.4. Enzymatic activity and phospholipid fatty acid (PLFA)

The activities of eight hydrolytic enzymes were also determined. In 96-well microplates, the activities of eight hydrolytic enzymes were measured: β-D-glucosidase, acid phosphatase, arylsulphatase, lipase, chitinase, cellobiohydrolase, alanine aminopeptidase, and leucine aminopeptidase. The enzymes were fed with the following substrates: 4-methylumbellyferyl-β-D-glucopyranoside (MUFG, 2.75 mmol L^{-1}) for β-D-glucosidase; 4-methylumbellyferyl-phosphate (MUFP, 2.75 mmol L^{-1}) for acid phosphatase; 4-methylumbellyferyl sulphate potassium salt (MUFS, 2.50 mmol L^{-1}) for arylsulphatase; 4-methylumbellyferyl-caprylate (MUFY, 2.50 mmol L^{-1}) for lipase; 4-methylumbellyferyl *N*-acetylglucosaminide (MUFN, 1.00 mmol L^{-1}) for chitinase; 4-methylumbellyferyl-*N*-cellobiopyranoside (MUFC, 2.50 mmol L^{-1}) for cellobiohydrolase; L-alanine-7-amido-4-methylcoumarin (AMCA, 2.50 mmol L^{-1}) for alanine aminopeptidase; and L-leucine-7-amido-4-methylcoumarin (AMCL, 2.50 mmol L^{-1}) for leucine aminopeptidase. Hydrolytic enzyme activities were measured by a change in fluorescence 5 and 125 minutes after incubation in microplates at 40 °C, with an excitation wavelength of 355 nm and an emission wavelength of 460 nm, in accordance with (Hřebečková et al. 2019; Hanc et al. 2017; Košnář et al. 2019).

For phospholipid fatty acid (PLFA) determination, samples were extracted using a mixture of phosphate buffer, chloroform, and methanol (0.8:1:2; v/v/v). The extracted fatty acids were converted to methylated esters and analyzed using gas chromatography-mass spectrometry (450-GC, 240-MS Varian, Walnut Creek, CA, USA). The analysis method has been described in detail by Hanc et al. (2022).

4.5. Analysis of potentially toxic elements (PTEs)

To analyze the content of PTEs (As, Cd, Cr, Cu, Pb, and Zn) in inatioal feedstocks (SS, SP and earthworms), variants and compost/vermicompost, a decomposition technique using wet digestion (65% HNO_3 + 30% H_2O_2) was employed. Firstly, the samples were subjected to this wet digestion process to release and quantify the PTEs present. After separating the earthworms from the samples, they were manually counted. Subsequently, the earthworms underwent washing and weighing to determine their biomass. To assess the PTE content in the earthworms, the same wet digestion method (65% HNO_3 + 30% H_2O_2) was used. The wet digestion process was carried out in a closed system with microwave heating using the Ethos 1 system from MLS GmbH, Germany. For quantifying the PTEs (As, Cd, Cr, Cu, Pb, and Zn) in both vermicompost and earthworms, inductively coupled plasma optical emission spectrometry (ICP-OES) was utilized, employing an axial plasma configuration. The ICP-OES instrument used for this purpose was the VARIAN VistaPro from Varian, Australia. In summary, the PTE content in vermicompost and earthworms was determined through wet digestion using a specific acid mixture, followed by analysis using ICP-OES in the axial plasma configuration (He et al. 2016).

4.6. Extraction and analysis of PPCPs and EDCs

The PPCPs and EDCs in the samples were analysed using LC-MS/MS after they had been homogenized. Subsequently, 1-2 g samples were moved to an extraction cell and placed in an accelerated solvent extractor (ASE, Dionex). The extraction process included preheating the methanol solvent and the cell to 80 °C and then performing three cycles with five-minute fixed intervals between each cycle. The evaporated extracts were spun in a centrifuge at 6000 g for 10 minutes, and the supernatants were collected and transferred to 2 mL vials for further analysis. The Agilent 1260 infinity liquid chromatography system and Agilent 6470 LC/TQ triple quadrupole mass detector were used to examine the samples. Separation was carried out using a Poroshell 120 EC-C18 column (2.7 m, 3 mm x 100 mm, Agilent) and a Poroshell 120 EC-C18 pre-column (2.7 m, 3 mm x 5 mm, Agilent), both of which were heated to 40°C. The mobile phase was made up of phase A (0.5 mM ammonium fluoride in MQ water plus 0.01% formic acid, LC-MS grade) and phase B (100% methanol, LC-MS grade). The elution schedule of the gradient was such that the % phase B was as follows (time [min]): 0, 5; 4, 50; 6, 50; 18, 100; 21, 100; 22, 5, and 23, 5. The mobile phase had a flow rate of 0.4 mL/min, the duration of the run was 23.50 minutes, and the amount injected was 2 L. The matrix effect was diminished by the use of automatic standard additions of 1, 5, and 25 ng/mL to measure the samples. Innemanová et al. (2022) utilized MassHunter Source Optimizer and Workstation Optimizer (Versions 10.0, SR1, Agilent) to optimize the mass spectrometric parameters. After the experiment the analyses were carried out at the Institute of Microbiology of the Czech Academy of Sciences. The analysis was done as part of a planned procedure called "scheduled analysis". The target of this study consisted of 32 OMPs, 28 of which were PPCPs and four were EDCs (Bisphenol A, Estrone, Daidzein and Genistein). Based on the available data analysed, the following 32 OMPs were included (Table 7).

4.7. The calculation for a percentage reduction of OMPs and PTEs

The reduction percentage R (%) of each variant was calculated for the content of all OMPs and PTEs using the following equation (Ashfaq et al. 2017).

$$\mathrm{R}(\%) = \frac{\mathrm{Ci} - \mathrm{Cf}}{\mathrm{Ci}} \quad x\,100 \qquad (6)$$

Where Ci is the concentration of OMPs/PTEs in mg kg^{-1} on the initial (day 0) variants) and Cf denotes the same for the final concentration of OMPs/PTEs after 120 days of vermicomposting.

4.8. Analysis of agrochemical properties

Various agrochemical parameters were analyzed. These parameters included pH, EC, total and available macronutrients (K, Mg, and P), mineral nitrogen forms ($N\text{-}NO_3^-$, $N\text{-}NH_4^+$), micronutrients (B, Cu, Fe, Mn, and Zn), total nitrogen (TN), and total carbon (TC). To determine the pH and EC values, a WTW pH 340i and WTW Cond 730 instrument were used, respectively, following the BSI EN 15933 (2012). For the analysis of total macronutrients (K, Mg, and P), a closed system with microwave heating using an Ethos1 system (MLS GmbH, Germany) was employed. The contents of $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, readily available macronutrients (K, Mg, and P), and available micronutrients (B, Cu, Fe, Mn, and Zn) were determined using the CAT solution (0.01 mol.l^{-1} $CaCl_2$ and 0.002 mol.l^{-1} diethylene triamine pentaacetic acid (DTPA) at a ratio of 1:10 (w/v), following the BSI EN 13651 (2001) standard. To assess the total and available element contents, optical emission spectrometry using inductively coupled plasma (ICP-OES) with axial plasma configuration was conducted, using the VARIAN VistaPro instrument from Varian, Australia. To determine the C/N ratio, a CHNS Vario MACRO cube analyser (Elementar Analysensysteme GmbH, Germany) was utilized, following the methodology described by Hanc et al. (2017). The CHNS Vario MACRO cube analyser is known for its high accuracy and reliability in determining total carbon and total nitrogen content, which enables the calculation of the C/N ratio. Regarding the earthworms, they were manually sorted and counted after being separated from the samples. The earthworms were further washed with water and weighed to determine their weight for subsequent analysis.

4.9. Statistical analyses

Normality analyses were performed (Kolmogor-Smirnov, Lilliefors, Shapiro- Wilk's W test) and homogeneity (Cochran's, Hartley's, Bartlett's test). Based on the results for normality and homogeneity, analysis of variance followed by one-factor ANOVA was chosen Tukey's HSD test ($p < 0.05$) using Statistica 12 software (StatSoft, Tulsa, USA). Multiple linear regression and correlation analyses Pearson's correlation coefficient ($p < 0.05$) and principal component analysis (PCA) were performed using R version 4.0.2.

5. Published papers

5.1. **Dume et al. (2021).** Carbon Dioxide and Methane Emissions during the Composting and Vermicomposting of Sewage Sludge under the Effect of Different Proportions of Straw Pellets.

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Carbon Dioxide and Methane Emissions during the Composting and Vermicomposting of Sewage Sludge under the Effect of Different Proportions of Straw Pellets

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Abstract: Owing to rapid population growth, sewage sludge poses a serious environmental threat across the world. Composting and vermicomposting are biological technologies commonly used to stabilize sewage sludge. The objective of this study was to assess the carbon dioxide (CO_2) and methane (CH_4) emissions from sewage sludge composting and vermicomposting under the influence of different proportions of straw pellets. Four treatments were designed, by mixing the initial sewage sludge with varying ratio of pelletized wheat straw (0, 25%, 50%, and 75% (w/w)). The experiment was conducted for 60 days, and *Eisenia andrei* was used for vermicomposting. The results revealed that the mixing ratio influenced CO_2 (F = 36.1, p = 0.000) and CH_4 (F= 73.9, p = 0.000) emissions during composting and CO_2 (F= 13.8, p = 0.000) and CH_4 (F= 4.5, p= 0.004) vermicomposting. Vermicomposting significantly reduced CH_4 emissions by 18–38%, while increasing CO_2 emissions by 64–89%. The mixing agent (pelletized wheat straw) decreased CO_2 emission by 60–70% and CH_4 emission by 30–80% compared to control (0%). The mass balance indicated that 5.5–10.4% of carbon was loss during composting, while methane release accounted for 0.34–1.69%, and CO_2 release accounted for 2.3–8.65%. However, vermicomposting lost 8.98–13.7% of its carbon, with a methane release of 0.1–0.6% and CO_2 release of 5.0–11.6% of carbon. The carbon loss was 3.3–3.5% more under vermicomposting than composting. This study demonstrated that depending on the target gas to be reduced, composting and vermicomposting, as well as a mixing agent (pelletized wheat straw), could be an option for reducing greenhouse gas emissions (i.e. CH_4, CO_2).

Keywords: thermophilic; earthworms; biosolids; sewage sludge; composting

1. Introduction

The world generates approximately 1.3 billion metric tons of solid waste, which is nearly double the amount generated a decade ago [1]. Solid waste generation is expected to be more than double by 2025 [2]. The annual increase in solid waste generation is inextricably linked to the global population's rapid growth and urbanization rate. Municipal solid waste (MSW) has primarily been disposed of in urban areas around the world through landfilling, incineration, and centralized composting and anaerobic digestion facilities. These processes result in direct and indirect emissions of greenhouse gases (GHGs) such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and non-methane hydrocarbons (NMHCs), accounting for approximately 3–4% of anthropogenic GHG emissions in terms of the CO_2-equivalent (CO_2-e) [3]. The anaerobic decomposition of these wastes in landfills produces CH_4 emissions, which contribute significantly to the global greenhouse budget [4].

Sewage sludge is a residual, semi-solid material produced as a by-product of biological wastewater treatment or municipal wastewater treatment [5,6]. Due to the putrescible characteristics of sewage sludge, a large amount produced in recent decades represents a rising trend, and improper disposal or management has resulted in serious environmental pollution, posing a waste management challenge [5,7].

Inadequate sewage sludge management causes secondary pollution such as pathogenic microbes, organic micropollutants, and toxic heavy metals; thus, sustainable and eco-friendly sewage sludge management is urgently needed [8].

According to He et al. [9], the European Union currently produces more than 10.96 million tons of sewage sludge per year and China produces 40 million tons of sewage sludge with an 80% moisture content [10], both of which are increasing due to both accelerated urbanization and the increased capacity of municipal wastewater treatment facilities [11,12]. In the past, sewage sludge was disposed of through incineration, landfilling, or ocean disposal [13].

Composting and vermicomposting are effective and low-cost methods for managing and reusing sewage sludge because the products are safe and stable, and can be used as organic fertilizer or soil conditioner for farming [14–16]. However, harmful gases such as ammonia (NH_3), nitrous oxide (N_2O), and methane (CH_4) emitted because of poor composting process management reduce not only the agronomic value of compost as a soil fertilizer or amendment but also the environmental benefits of composting [17,18]. Researchers have become interested in N_2O and CH_4 emissions during the composting process as global warming worsens and the greenhouse effect intensifies [19,20].

Two of the most significant greenhouse gases in the atmosphere are methane (CH_4) and carbon dioxide (CO_2). On a mass basis, methane is more radiatively powerful than CO_2 and the current global warming potential of CH_4 is 34 times greater than that of CO_2 over a 100-year period [21].

Concerning the aforementioned issues, substantial research on sewage sludge composting has been conducted in recent decades, with a particular emphasis on the use of various additives to reduce greenhouse gas emissions [22,23]. Although earthworms do not produce these gases, they can have a significant impact on the physicochemical properties of the feeding substrate, thereby indirectly affecting gas-producing processes and thus CO_2 and CH_4 emissions.

The effects of earthworms on greenhouse gas emissions are complicated and no agreement has been reached. Earthworms, for example, increased N_2O and CO_2 emissions from soils by 42% and 33%, respectively [24]. Others found that earthworms increased CO_2 emissions but had no effect on N_2O fluxes from soils [25,26]. Similarly, the study in [27] demonstrated that vermicomposting of household waste produced more CO_2 and CH_4, but produced less N_2O than traditional composting.

The majority of previous composting and vermicomposting studies focused on the feasibility of different organic wastes, the factors influencing earthworm growth and reproduction rates, and the quality of composts and vermicompost [28,29]. Furthermore, several recent studies [24,30] have focused on the effects of earthworms on GHG emissions from soils. However, there are limited studies on carbon dioxide and methane emissions during composting and vermicomposting of organic wastes, specifically sewage sludge, with varying ratio of additive materials. As a result, the goal of this study was to assess the carbon dioxide (CO_2) and methane (CH_4) emissions from sewage sludge composting and vermicomposting under the influence of different proportions of straw pellets.

2. Materials and Methods

2.1. Raw Materials

The study made use of unstabilized sewage sludge and straw pellets mixed with water. The freshly deposited sewage sludge (SS) used in the experiments originated from a wastewater treatment plant in the Czech Republic, where thousands of people live, and had a dry matter content of 13.3%. A dried pelletized wheat straw (PWS) with a diameter of 10 mm was provided by the Granofyt Ltd. Company (Chrášťany, Czechia). Dry straw pellets were mixed with hot water at a rate of 4 L per 1 kg of straw pellets. After mixing, the wet pellets were added to the sludge. The resulting material was put into aerobic fermenters for composting and the same mixing materials (treatments) were also transferred to worm bins for vermicomposting. The experiment was carried out at the Research Station of the Czech University of Agriculture in Červený Újezd, with samples subsequently analyzed at the Life Science laboratories of the Czech University in Prague. The selected chemical properties of the sewage sludge and pelletized wheat straw are listed in Table 1, while the treatments on the initial day (day 0) are listed in Table 2.

Table 1. Selected chemical properties of the sewage sludge and pelletized wheat straw.

Parameters	Sewage Sludge (SS)	Pelletized Wheat Straw (PWS)
pH	6.99 ± 0.017	8.30 ± 0.300
EC (mS/cm)	0.617 ± 0.064	0.680 ± 0.040
TC (%)	32.95 ± 0.150	42.6 ± 0.207
TN (%)	5.36 ± 0.017	0.8 ± 0.069
C:N	6.15 ± 0.011	53.2 ± 4.388

Values indicate mean ± standard error (n = 3).

Table 2. Selected chemical properties of the treatments on the initial day (day 0).

Treatments	pH	EC(mS/cm)	TC (%)	TN (%)	C:N
T1	6.99 ± 0.017	0.617 ± 0.064	32.9 ± 0.150	5.36 ± 0.017	6.15 ± 0.023
T2	7.32 ± 0.064	0.633 ± 0.046	35.36 ± 0.133	1.98 ± 0.121	18.03 ± 1.11
T3	7.64 ± 0.144	0.649 ± 0.035	37.77 ± 0.139	1.34 ± 0.040	28.17 ± 0.826
T4	7.97 ± 0.219	0.664 ± 0.029	40.18 ± 0.167	1.05 ± 0.029	38.36 ± 1.172

T1= 100% SS; T2= 75% SS + 25 % PWS; T3= 50 % SS + 50 % PWS; and T4 =25 % SS + 75 % PWS (w/w). Values indicate mean ± standard error (n = 3).

2.2. Experimental Setup

Composting and Vermicomposting

The experiment consisted of four treatments obtained by mixing the sewage sludge (SS) with pelletized wheat straw (PWS) at different mixing ratio including, T1 (100% SS (control)), T2 (75% SS + 25% PWS), T3 (50% SS + 50% PWS), and T4 (25% SS + 75% PWS; w/w). To avoid earthworm mortality and to allow earthworms to return to suitable conditions, the substrate (3 L of apple pomace) containing earthworms was placed into the tray from the side. After mixing the materials (SS and PWS) at different percentage proportions, the treatments were transferred to worm-bins for vermicomposting in a specially adopted laboratory with controlled conditions (temperature 22 °C, relative humidity 80%) for 60 days. Each worm-bin received 377 (57.4 g) pieces of adult *Eisenia andrei* earthworms per treatment, with the initial average weight and number of earthworms at 19.13 g/kg and 126 pieces/kg, respectively, of the substrate. The moisture level of the material was maintained at about 70–80% of the wet mass throughout the vermicomposting stage by spraying the surface with water at two-day intervals and the same treatments used for vermicomposting were also transferred to the fermenter barrels for 60 days of composting. Three replications were conducted for all the treatments.

2.3. Carbon Dioxide (CO_2) and Methane (CH_4) Measurements during Composting and Vermicomposting

The CO_2 and CH_4 concentrations were measured using a closed chamber technique during composting and vermicomposting. A tight-fitting lid with two ports for headspace gas-sampling and air temperature measurement was used to connect one side of a plastic tube to closed barrels for composting and to a worm bin for vermicomposting, while the other side of the plastic tube was connected to instruments during the data collection. For 60 days, measurements were taken twice per day at 12 h intervals using the Gasko Infrared

Gas Analyzer [31]. To calculate the cumulative CO_2 and CH_4 emissions, we added daily values to obtain the total cumulative gas emissions over the course of the experiment [31].

$$A_{t(ab)}=\frac{(t_b-t_a)\cdot(F_{ta}+F_{tb})}{2} \quad (1)$$

where $A_{t(ab)}$ is the cumulative emission between the measurement days (between ta and t_b), ta and t_b are the measurement dates, and F_{ta} and F_{tb} are the gas fluxes on the two measurement dates. Therefore, the total cumulative emissions were calculated as the sum of cumulative emissions on each day using Equation (2):

$$Total\ cumulative\ emission=\sum A_{t(ab)} \quad (2)$$

C losses during composting and vermicomposting were calculated as:

$$C\ loss(\%)=\frac{(C_{initial}-C_{ending})}{C_{initial}} \quad (3)$$

2.4. Analysis of Total Carbon (TC), Total Nitrogen (TN), pH, and EC

The representative composite samples (about 150 g of wet basis per treatment) were taken, freeze-dried (−25 °C), lyophilized, and ground for the total carbon (TC) and total nitrogen (TN) analysis, whereas a 30 g sample was frozen at 4 °C for the pH and EC determination. Standard methods were used to determine TC, TN, pH, and EC from the samples. The pH and electrical conductivity (EC) were measured in distilled water at a 1:5 (w/v) ratio. The pH-H_2O and the electrical conductivity (EC) were tested using a WTW pH 340i and WTW cond 730 (1:5 w/v dry basis) according to [32]. Inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia) with axial plasma configuration was used to determine TC and TN in accordance with [33].

2.5. Statistical Analyses

The statistical analyses were carried out using the R version 4.0.2 statistical package. ANOVA was used to test whether there was a significant difference between the composting method and mixing ratio in GHGs (i.e. CO_2 and CH_4) emissions and properties of final product. Tukey HSD test was used to compare the treatment means if the effect of the factors was significant at $p < 0.05$.

3. Results and Discussions

3.1. Temperature during Composting and Vermicomposting

During the composting process, the temperature in each treatment reached its maximum, with significant ($F = 18.6$, $p = 0.000$) differences among the treatments (Figure 1a). On days 3 and 2, the temperatures of two treatments (T3 and T4) rapidly reached the thermophilic stage (>50 °C). T4 reached a maximum thermophilic phase of 65.5 °C in four days, while T3 reached 57.4 °C in four days. The thermophilic phase of T4 lasted 14 days, while that of T3 lasted 10 days. The maximum temperature for the remaining treatments was 37.6 °C for T2 and 29.55 °C for T1, with temperatures gradually decreasing until the experiment ended. Thus, the addition of pelletized wheat straw resulted in a more intensive decomposition in the thermophilic phase, but the degradation process resulted in less heat in these mixtures during the cooling phase due to the depletion of easily degradable organic compounds [34]. T1 (control) and T2 (25% PWS) delayed reaching the thermophilic stage and had no thermophilic phase at all; the maximum temperature for T2 was 37.6 °C and 29.55 °C for the control, and they matured within the mesophilic temperatures. This may have been because of the high moisture content of these treatments.

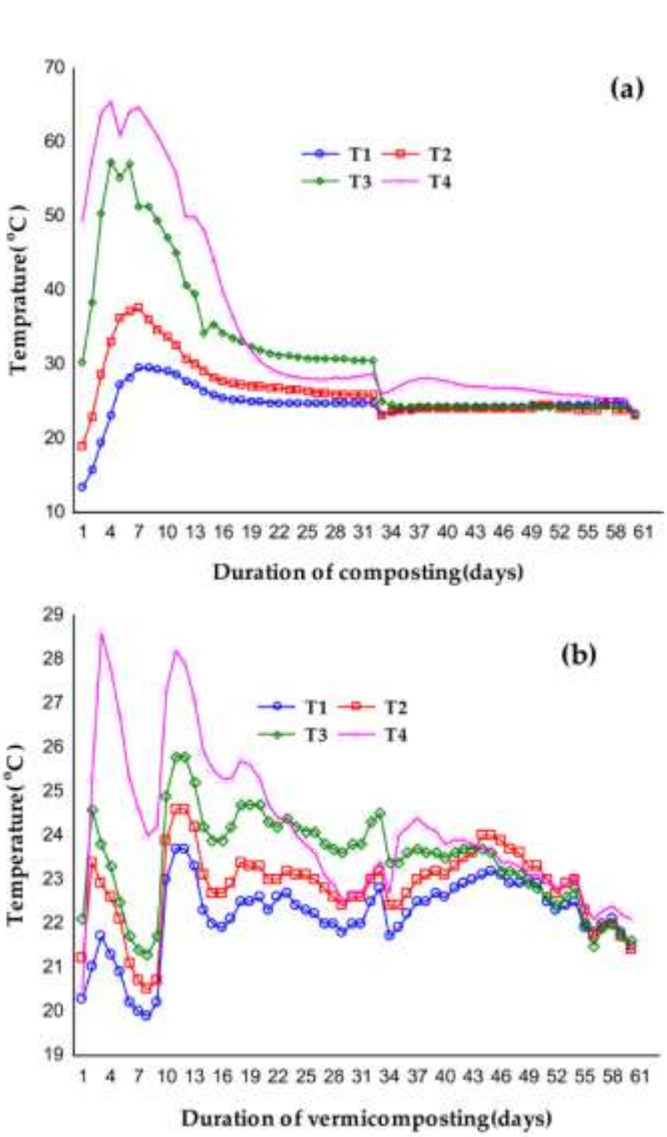

Figure 1. Evolution of temperatures during the composting (a) and vermicomposting (b) processes.

In the laboratory, the vermicomposters were kept at 22 °C. The temperatures recorded during the vermicomposting are shown in Figure 1b. The temperature of the vermicomposting was in the 19 °C to 28 °C range, which was obviously less than the thermophilic compost range and was favorable for earthworms [35]. Statistical analysis revealed that there were significant temperature differences among the treatments during the vermicomposting period ($F = 31$, $p = 0.000$). At the start of the process, the temperature of the vermicomposting material rose to 28.6 °C only for T4.

3.2. pH and EC

The pH of the final compost and vermicompost for each treatment is shown in Table 3. The proportions of pelletized wheat straw in the mixtures resulted in lesser pH values during vermicomposting [36]. This was probably due to the high content of organic acids (e.g., succinic and maleic acid) and was directly proportional to the amount of straw in the treatments [36]. Other researchers [36–39] reported similar pH behaviors during the vermicomposting of sewage sludge, crop straw, municipal solid waste, and livestock manure. Gigliotti et al. [40] reported that the mineralization of organic matter generally leads to the release of ammonium and volatile ammonia, which increases pH levels. The release of low-molecular weight organic acids from organic decomposition, as well as the increase in nitrification may reduce the pH during vermicomposting [37]. The pH of the vermicompost might indicate that a more intense decomposition reaction occurs during vermicomposting than composting.

Table 3. Selected chemical properties of the end-product compost and vermicompost.

Composting Method	Treatments	pH	EC (mS/cm)	TC (%)	TN (%)	C:N
Composting	T1	8.4 ± 0.069	1.90 ± 0.098	29.52 ± 0.421	4.55 ± 0.081	6.50 ± 0.012
	T2	8.3 ± 0.052	1.43 ± 0.052	32.43 ± 0.456	3.69 ± 0.017	8.84 ± 0.185
	T3	8.4 ± 0.046	1.94 ± 0.081	34.45 ± 0.883	3.27 ± 0.029	10.57 ± 0.375
	T4	8.0 ± 0.035	0.80 ± 0.035	37.95 ± 0.012	2.76 ± 0.087	13.88 ± 0.462
Vermicomposting	T1	6.7 ± 0.670	0.644 ± 0.023	28.43 ± 0.185	4.22 ± 0.127	6.77 ± 0.150
	T2	6.5 ± 0.866	1.186 ± 0.127	31.96 ± 0.514	3.58 ± 0.023	8.94 ± 0.202
	T3	6.5 ± 0.081	0.802 ± 0.225	34.38 ± 0.652	2.95 ± 0.087	11.72 ± 0.537
	T4	6.6 ± 0.179	1.21 ± 0.069	35.32 ± 0.214	3.08 ± 0.035	12.15 ± 0.185

T1= 100% SS; T2= 75% SS + 25 % PWS; T3= 50 % SS + 50% PWS; and T4 =25 % SS + 75 % PWS (*w*/*w*). The values indicate mean ± standard error (n = 3).

The EC value of compost was greater than that of the vermicompost made from the same raw materials and treatments (Table 3). The EC increased in all treatments, which could be explained by the release of bonded elements during earthworm digestion [41,42], as well as by the mineral release during organic matter decomposition in the form of cations in the vermicompost [43]. The final EC for all treatments was less than 2 dS/m [44], indicating that the vermicompost/compost was suitable for plant application. The increased EC during the vermicomposting processes is consistent with that of previous researchers [45,46] and is most likely due to organic matter degradation, which releases minerals such as exchangeable Ca, Mg, K, and P in the available forms, that is, in the form of cations in the vermicompost and compost [43].

3.3. Carbon Dioxide(CO_2) and Methane(CH_4) Emissions during Composting and Vermicomposting

3.3.1. Carbon Dioxide (CO_2)

The CO_2 emissions increased at the start of the composting (Figure 2a) and vermicomposting (Figure 2c) due to the rapid decomposition of easily degradable organic matter, and then gradually decreased until the end of the composting/vermicomposting. This finding confirms those reported by Awasthi et al. [47] and Meng et al. [15] during sewage sludge composting. During the first 13 days of composting, CO_2 emissions in the control (T1) were greater than in the other treatments (T2, T3, and T4). However, CO_2 emissions were less in the T1 (control) during vermicomposting. As the earthworms inhibited microbial activity and reduced the readily available OM, this result was possible [48]. There were significant differences in CO_2 ($F = 36.1$, $p = 0.000$) emissions among the treatments during the composting and vermicomposting CO_2 ($F = 13.8$, $p = 0.000$). These findings imply that pelletized wheat straw may be lost in the inhibition after the thermophilic stage, most likely as a result of high-temperature self-degradation [49]. This conclusion is supported by the temperature and pH of T1, T2, T3, and T4. In all treatments, there was a significant decrease in CO_2 emissions on day 14 and a minor peak on day 20 (Figure 2a). This finding could be attributed to the anaerobic environment created by the rapid decomposition of OM during the first 14 days. The anaerobic conditions were destroyed by the subsequent turn on day 10. Previous studies [47] on sewage sludge composting reported similar results, in which CO_2 emissions were higher at the start of the composting period, with the highest levels observed on day 2, and then gradually decreased until the end of the thermophilic phase.

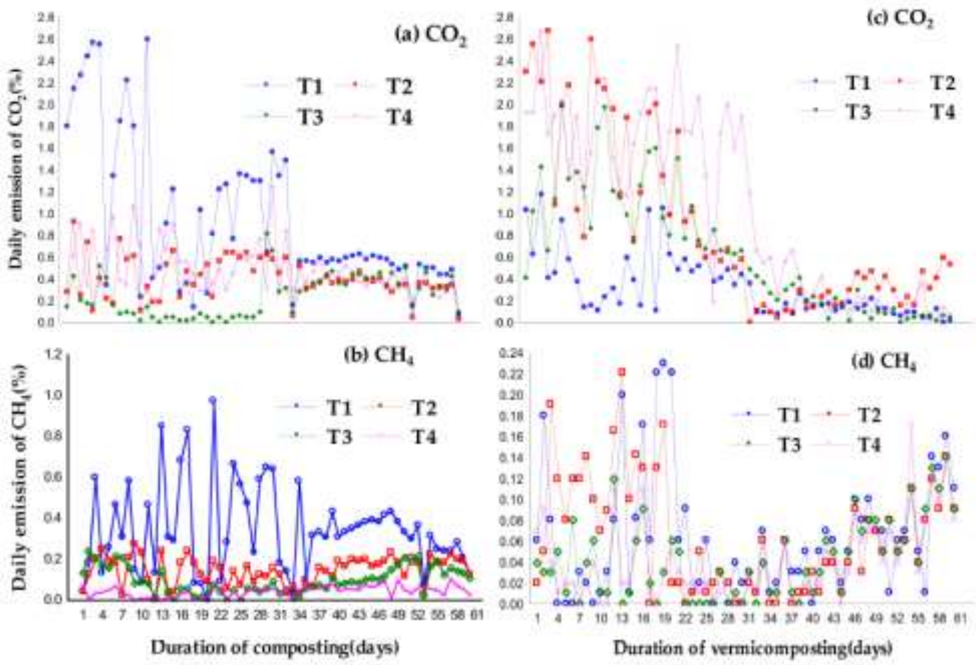

Figure 2. Daily emissions of CO_2 and CH_4 during composting (a,b) and vermicomposting (c,d).

3.3.2. Methane (CH_4)

The amount of CH_4 produced by all the treatments used during the composting (Figure 2b) and vermicomposting (Figure 2d) processes are shown in Figure 2. There were significant differences in the CH_4 ($F = 73.9$, $p = 0.000$) emitted during composting and the CH_4 ($F = 4.5$, $p = 0.004$) emitted from all the treatments during vermicomposting. The CH_4 concentrations in all the treatments peaked relatively early (within 1–3 weeks) in both the composting and vermicomposting processes, and then gradually declined until the experiment ended. As a result, it is reasonable to assume that the CH_4 emissions occur at the beginning of the process. Several studies have discovered that the greatest levels of CH_4 emissions occur at the beginning of the composting and vermicomposting processes [50]. CH_4, a major GHG produced during composting and vermicomposting, significantly contributes to global warming. CH_4 production is attributed to the methanogen deoxidization of CO_2/H_2 and acetic acid in the presence of low oxygen [51]. Following that, as the organic matter (OM) decomposed and oxygen was replenished through turning, the CH_4 emissions of all the treatments fell sharply and remained lowered throughout the composting and vermicomposting maturation phases.

The observed pattern of CH_4 emissions in this study is similar to the patterns reported by Ma et al. [52] and Wang et al. [53]. As microorganisms can rapidly degrade organics in the thermophilic phase, there is a dramatic reduction in O_2 levels in the compost [54]. Composting emitted more CH_4 than vermicomposting in all the treatments and the greater results were measured in the control area.

Total cumulative CO_2 levels differed significantly ($p < 0.001$) by the composting method (Figure 3). Vermicomposting increased total cumulative CO_2 emissions in comparison to thermophilic composting. Composting reduced total cumulative CH_4 emissions ($p < 0.001$). When compared to thermophilic composting, vermicomposting reduced CH_4 emissions by 74.5% from a high proportion of pelletized wheat straw T4 treatments.

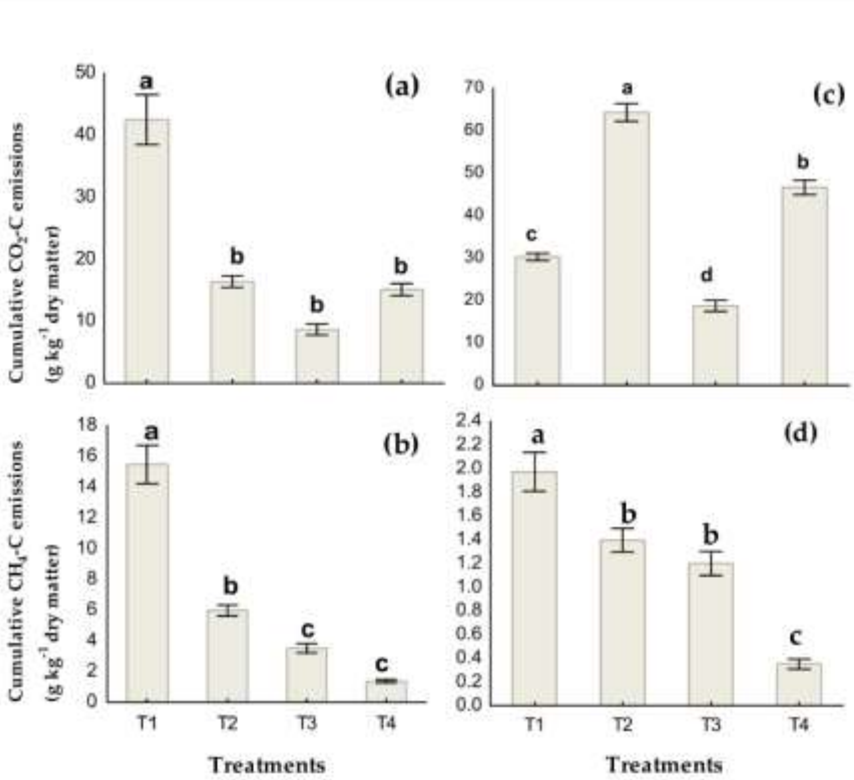

Figure 3. Total cumulative emissions of CO_2-C (a) and CH_4-C (b) after 60 days of composting, and CO_2-C (c) and CH_4-C (d) during vermicomposting. The bars indicate the standard error of the mean (n = 3). Different letters indicate significant differences among the treatments ($p < 0.05$).

3.4. Total Carbon(TC), Total Nitrogen(TN), and the C:N Ratio

The content of TC, TN, and C:N ratio for all the treatments is shown in Table 3. When compared to the initial treatments, the TC and C:N contents of both compost and vermicompost decreased. However, the TN content of both compost and vermicompost increased. The loss of ammonia volatilization at relatively high temperatures, combined with a pH unsuitable for nitrification and denitrification, resulted in an increase in TN content [55]. According to Zhang et al. [56], the increase in TN during sludge vermicomposting was due to worm activity. Composting and vermicomposting both reduced the C:N ratio for all the treatments. Considering that it reflects stabilization and mineralization rates during vermicomposting, the C:N ratio indicates the maturity of compost/vermicompost [57]. The C:N ratio is an important metric for determining whether the compost/vermicompost product has been thoroughly stabilized. Microorganisms decompose biodegradable components and convert them to CO_2, H_2O, and to other small molecules during the composting/vermicomposting process. However, the rate of loss for organic N is less than that for organic C, resulting in a decrease in the C:N ratio during the composting/vermicomposting process. In general, the C:N ratio of fully decomposed compost/vermicompost should be between 15 and 20 [58]. The C:N ratio of all mixtures in this study followed the same trend, with statistically significant differences between the two composting processes (Table 3). Previous research [59] found that vermicomposting cow dung with vegetable waste reduced the C:N ratio by up to 50.86% and 48.88%. The final C:N ratio recorded for all the treatments was less than 20, which is within the recommended value for soil applications [60].

3.5. Carbon Balances

The mass balance analysis revealed that composting lost 5.54–10.42% of the total carbon across all treatments; total methane release accounted for 0.34–1.69%; and CO_2 release accounted for 2.3–8.65%. However, vermicomposting lost 8.98–13.73% of the total carbon, with a total methane release of 0.1–0.6% and CO_2 release of 5.03–11.61% of the initial total carbon (Table 4). These findings agree with those of Nigussie et al. [61] who demonstrated that organic carbon is lost during composting/vermicomposting. Thus, when compared to thermophilic composting, vermicomposting increased the total C loss by 3.3–3.5% (Table 4).

Table 4. Carbon loss (CH_4-C and CO_2-C) during composting and vermicomposting.

Total C Emission during Composting								
Trts	Initial C ($g\ kg^{-1}$)	Ending C ($g\ kg^{-1}$)	CH_4-C ($g\ kg^{-1}$)	CO_2-C ($g\ kg^{-1}$)	C Loss (%)	CH_4-C Loss (%)	CO_2-C Loss (%)	Unaccounted C (%)
T1	329.53	295.2	5.48	28.51	10.42	1.66	8.65	0.11
T2	353.62	324.38	5.97	16.42	8.29	1.69	4.64	1.96
T3	377.70	344	3.51	8.68	8.92	0.93	2.30	5.69
T4	401.78	379.5	1.37	15.11	5.54	0.34	3.76	1.44
Total C Emission during Vermicomposting								
Trts	Initial C ($g\ kg^{-1}$)	Ending C ($g\ kg^{-1}$)	CH_4-C ($g\ kg^{-1}$)	CO_2-C ($g\ kg^{-1}$)	C Loss (%)	CH_4 -C Loss (%)	CO_2-C Loss (%)	Unaccounted C (%)
T1	329.53	284.3	1.97	30.28	13.73	0.60	9.19	3.94
T2	353.62	305.6	0.35	44.31	13.58	0.1	12.53	0.95
T3	377.70	343.8	1.20	18.73	8.98	0.32	5.03	3.63
T4	401.78	353.2	1.40	46.64	12.09	0.35	11.61	0.13

T1= 100% SS; T2= 75% SS + 25 % PWS); T3= 50% SS + 50 % PWS; and T4 =25 % SS + 75 % PWS (*w*/*w*).

Earthworms decomposing organic matter [24]; earthworms mixing the substrate and increasing the accessibility of the materials for decomposers (e.g. Fungi, bacteria); and earthworm casts increasing the decomposition [62] all contributed to greater C loss after vermicomposting. Unaccounted C ranged from 0.11 to 5.69% during composting and 0.13 to 3.94% during vermicomposting, which is consistent with previous research [63,64]. Unaccounted C indicates that C was not measured between sampling dates [63] and C losses due to volatile compounds [64].

3.6. Population and Biomass of Earthworms

The population (number) and biomass of earthworms (g) in all the treatments are shown in Figure 4.

The substrate ratio (pelletized wheat straw) had no effect on the relative change of the earthworm biomass ($p = 0.49$) and population ($p = 0.36$). The earthworm biomass increased in mixtures containing a high percentage of pelletized wheat straw (T4). Increased earthworm abundance reduced CH_4 emissions and accelerated the decomposition process. Vermicomposting increased CO_2 emissions, implying that vermicompost is further along in its decomposition process than thermophilic compost. These findings are consistent with those of Nigussie et al. [61] who found that vermicomposting reduced CH_4 while increasing CO_2 emissions.

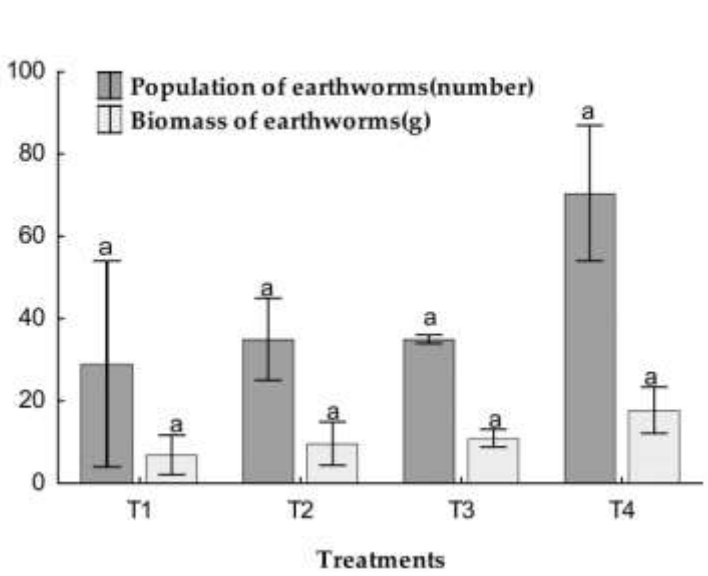

Figure 4. Population (number) and biomass of earthworms (g) after 60 days of vermicomposting. Bars indicate the standard error of the mean (n = 3).

4. Conclusions

The composting and vermicomposting of sewage sludge produced significant amounts of CO_2 ($F = 36.1$, $p = 0.000$) and CH_4 ($F = 73.9$, $p = 0.000$), which were emitted during composting, and CO_2 ($F = 13.8$, $p = 0.000$) and CH_4 ($F = 4.5$, $p = 0.004$), which were emitted from all the treatments during vermicomposting. The greatest values were obtained at the start of the experiment and gradually decreased. The fate of C in the waste substrate is linked to the emission of CH_4 and CO_2 during composting and vermicomposting. Vermicomposting reduced CH_4 emissions while also accelerating the decomposition process. CO_2 and CH_4 emissions were increased during composting at various proportions of added pelletized wheat straw. Vermicomposting increased CO_2 emissions, implying that vermicompost is further along in its decomposition process. Vermicomposting significantly reduced CH_4 emissions by 18–38%, while increasing CO_2 emissions by 64–89%. The mixing agent (pelletized wheat) decreased CO_2 emission by 60–70% and CH_4 emission by 30–80% compared to control (0%). Increased earthworm abundance reduced CH_4 emissions and increased CO_2 emissions. The mass balance analysis indicated that 5.5–10.4% of carbon was lost by composting, methane release accounted for 0.34–1.69%, and CO_2 release accounted for 2.3–8.65%. However, 8.98–13.7% of carbon was lost by vermicomposting with a methane release of 0.1–0.6% and CO_2 release of 5.0–11.6% of C. Thus, when compared to thermophilic composting, vermicomposting increased the total C loss by 3.3–3.5%. This study demonstrated that depending on the target gas to be reduced, composting and vermicomposting, as well as a mixing agent (pelletized wheat straw), could be an option for reducing greenhouse gas emissions (i.e. CH_4, CO_2).

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5.2. Hanc, A., **Dume, B., et al. (2022).** Differences of enzymatic activity during composting and vermicomposting of sewage sludge mixed with straw pellets.

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Differences of Enzymatic Activity During Composting and Vermicomposting of Sewage Sludge Mixed With Straw Pellets

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The study aims were focused on profiling eight hydrolytic enzymes by fluorescence method using a multifunctional modular reader and studying the proportion of basic microorganism groups during composting and vermicomposting of sewage sludge mixed with straw pellets in several proportions (0, 25, 50, 75, and 100%). The greatest decrease in enzymatic activity occurred in the first half of composting and vermicomposting. After 4 months of these processes, the least enzymatic activity was observed in the sludge with 50% and also 25% straw addition, indicating that straw is an important means for the rapid production of mature compost from sewage sludge. Enzymatic activity was usually less in the presence of earthworms than in the control treatment because some processes took place in the digestive tract of the earthworm. For the same reason, we observed reduced enzyme activity during fresh feedstock vermicomposting than precomposted material. The final vermicompost from fresh feedstocks exhibited less microbial biomass, and few fungi and G− bacteria compared to precomposted feedstock. The enzymatic activity during composting and vermicomposting of sewage sludge and their mixtures stabilized at the following values: β-D-glucosidase—50 μmol MUFG/h/g dw, acid phosphatase—200 μmol MUFP/h/g dw, arylsulphatase—10 μmol MUFS/h/g dw, lipase—1,000 μmol MUFY/h/g dw, chitinase—50 μmol MUFN/h/g dw, cellobiohydrolase—20 μmol MUFC/h/g dw, alanine aminopeptidase—50 μmol AMCA/h/g dw, and leucine aminopeptidase—50 μmol AMCL/h/g dw. At these and lesser values, these final products can be considered mature and stable.

Keywords: enzymatic activity, sewage sludge, straw pellets, composting, vermicomposting, microorganisms, earthworms

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INTRODUCTION

The global production of sewage sludge is estimated at 45 million tons per year [expressed in dry matter (Zhang et al., 2017)]. The most common methods of handling dewatered treated sludge include direct application to agricultural land, landfilling, and composting. In the Czechia, sludge production was 228 thousand tons of dry matter in 2018, i.e., 21 kg per capita. Approximately 1/3 of this amount was composted (Eurostat, 2021).

The course of the composting process and the physico-chemical and biological parameters of the final compost are especially affected by the addition of various bulking agents (Wang et al., 2021). Sludge composting with many bulking agents was previously described, e.g., sawdust, coffee husks, and brewery waste (Manga et al., 2021), yard trimming wastes (Robledo-Mahón et al., 2020), and cornstalks (Yuan et al., 2016). Composting is characterized by a thermophilic phase and the need to turn over or to aerate the composted material. Conversely, vermicomposting requires that the temperature does not exceed 35°C, in which case the earthworms die. The optimal temperature is between 20 and 25°C. Aeration is provided directly by earthworms by creating passages in the material. Earthworms act as mechanical blenders, and by disintegrating the organic matter, they change its physical and chemical properties, especially by gradually reducing the C:N ratio and increasing the surface area exposed to microorganisms. Thus, earthworms make feedstocks much more favorable for microbial activity and further decomposition (Dominguez and Edwards, 2011). At first, ingestion, digestion, assimilation, and the influence of earthworm gut microorganisms are involved in organic matter decomposition, and the processes associated with casting follow (Gómez-Brandón et al., 2011). Casting processes are more closely related to aging in maturation phase when the vermicompost is expected to reach optimum levels in terms of its biological parameters, thereby promoting plant growth and suppressing plant diseases. Little is known about determining the optimum levels (Domínguez and Gómez-Brandón, 2012). Degradation or utilization of substrate by microorganisms involves significant processes like nutrient cycling and transforming organic matter.

In the process, microbial activity could be assessed effectively with the analysis of enzymatic activity and microbial respiration. Soil enzyme activities provide information on the potential of soils to perform biochemical reactions (Błońska et al., 2017; Uzarowicz et al., 2020). Enzymatic activity was also explored as a possible tool for compost characterization and determination of compost maturity (Mondini et al., 2004). The biochemical reactions are brought about by enzyme catalytic action. Enzymes are divided into seven classes based on the reaction type that they catalyze, namely:

- Oxidoreductases—acting on the CH-OH group of donors, the aldehyde or oxo group of donors, CH-CH group of donors, CH-NH_2 group of donors, CH-NH group of donors, etc.
- Transferases—transferring one-carbon groups, aldehyde, or ketonic groups, alkyl or aryl groups, other than methyl groups, nitrogenous groups, phosphorus-containing groups, etc.
- Hydrolases—acting on ester bonds, ether bonds, peptide bonds, carbon-nitrogen bonds, carbon-carbon bonds, phosphorus-nitrogen bonds, carbon-sulfur bonds, etc.
- Lyases—e.g., carbon-carbon lyases, carbon-oxygen lyases, carbon-nitrogen lyases, carbon-phosphorus lyases.
- Isomerases—e.g., racemases and epimerases, intramolecular isomerases and lyases.
- Ligases—forming carbon-oxygen bonds, carbon-sulfur bonds, carbon-nitrogen bonds, etc.
- Translocases—catalyzing the translocation of hydrons, inorganic cations, inorganic anions and their chelates, etc. (Nomenclature Committee of the International Union of Biochemistry and Molecular Biology [NC-IUBMB], 2021).

Some enzymes are important in organic material decomposition, organic matter transformation, nutrient cycling, nitrogen fixation, detoxification of hazardous substances such as xenobiotics, pesticides, pharmaceutical and personal care products, etc., and thus regulate the ecosystem (Gunjal et al., 2019). We studied eight hydrolase enzymes because they participate in metabolic processes during organic matter decomposition. These are as follows:

- β-glucosidase is widely distributed in nature and is related to the carbon cycle, acting in the cleavage of cellobiose into glucose molecules. Because of its sensitivity, this enzyme is considered as soil quality indicator and is directly related to the quantity and quality of soil organic matter. Furthermore, the addition of soil organic residues, such as biosolids, manure, urban sludge, and poultry litter, increases the activity of this enzyme in soil (Almeida et al., 2015). A long-term field experiment utilizing barley received four different treatments prior to sowing: municipal solid waste compost at either 20 t/ha (C20) or 80 t/ha (C80), cow manure at 20 t/ha (MA), and mineral fertilizer either NPK 400 kg/ha or NH_4NO_3 150 kg/ha (MIN). The enzymatic activity of β-glucosidase was higher by 38, 62, 87, and 6% for C20, C80, MA, and MIN, respectively, compared with control unfertilized variant (García-Gil et al., 2000).
- Acid phosphatase—Phosphate-solubilizing micro-organisms are crucial for the transformation of organically bounded phosphorus into bioavailable forms by excreting extracellular phosphatase in the form of acid and alkaline phosphatase (Zheng et al., 2021).
- Arylsulphatase hydrolyzes aromatic sulfate esters and releases SO_4^{2-}. It is an indicator of sulfur mineralization in soils and also is important in the cycling of this element (Uzarowicz et al., 2020).
- Lipases are ubiquitous enzymes that catalyze the breakdown of fats and oils with the subsequent release of free fatty acids, diacylglycerols, monoglycerols, and glycerol. Besides this, they are also efficient in various reactions, such as esterification, transesterification, and aminolysis, in organic solvents. Therefore, those enzymes are nowadays extensively studied for their potential industrial applications. Examples in the literature concerning their use in different fields are numerous, such as resolution of racemic mixtures, synthesis of new surfactants and pharmaceuticals, oil and fat bioconversion, and detergency applications (Villeneuve et al., 2000).
- Chitinases are able to degrade the chitin chain. Chitinases are classified as exochitinases or endochitinases. Exochitinases cleave chitin from the open ends, while

endochitinases cleave chitin at random positions. Fungal chitinases belong to the glycoside hydrolase family 18 and are divided into three phylogenetic groups—A, B, and C—and further subdivided into several subgroups. Chitinases are involved in different aspects of fungal biology, including fungal–fungal interactions, nutrient acquisition, cell wall remodeling, and autolysis (Tzelepis and Karlsson, 2021).

- Cellobiohydrolases are among the most important enzymes functioning in crystalline cellulose hydrolysis, significantly contributing to the efficient biorefining of recalcitrant lignocellulosic biomass into biofuels and bio-based products. Filamentous fungi are recognized as both well-known producers of cellulolytic enzyme commercial preparations and efficient hosts for heterologous protein secretion (Zoglowek et al., 2015).
- Aminopeptidases belong to exopeptidases, proteolytic enzymes that remove amino acids from the termini of peptides and proteins. They attack their substrates exclusively from the amino terminal end. Most remove one amino acid at a time, but a small group cleaves two or three residues. Alanine and leucine aminopeptidases release terminal nitrogen from amino acids (mainly from alanine and leucine), peptides, amides, and arylamides (Bradshaw, 2013).

Commercially derived hydrolytic enzymes are very expensive, whereas enzymes produced from mixed biosolids are very cheap and perform similarly to commercially produced enzymes (Nabarlatz et al., 2012). Enzymes secreted by microbial species or commercially produced enzymes were used in biosolid pretreatment for increasing methane production (Sethupathy et al., 2020).

In the literature, there are hydrolytic enzyme activity values, which were determined by classical colorimetric methods. We used a fluorescence method with multifunctional modular reader, which is a fast, modern, and economically advantageous method for determining the enzymatic activity in a large sample number. The enzymatic activity trends and values of eight hydrolytic enzymes identified in this study can be a guide for research and control institutes and for producers of composts and vermicomposts based on sewage sludge. The values can be used to predict compost and vermicompost maturity and to determine the time when the enzymatic activity no longer changes. The aims of the study were as follows: (i) to compare the enzymatic activity of eight hydrolytic enzymes and a proportion of microorganism groups during composting and vermicomposting of sewage sludge mixed with straw pellets, (ii) to find the impact of precomposting on enzymatic activity, microorganisms, and earthworm presence, (iii) to determine the effect of addition of straw pellets on the above-mentioned biological properties.

MATERIALS AND METHODS

Feedstocks

Sewage sludge was obtained from a sewage treatment plant located in a small town in the Czechia. The sludge did not undergo any stabilization process. The dry matter content was 13.3%, and the total content of C, N, P, K, Ca, and Mg was 32.9, 5.3, 1.6, 0.5, 1.4, and 0.5% in dry matter, respectively. The pH/H_2O (1:5, w/v) value was 7.0. The content of pollutants did not exceed the valid legislation. Pelletized straw pellets (PWS) were bought at the Granofyt Ltd., company. The pellet diameter was 10 mm. Because of very reduced pellet moisture, they were mixed with hot water (60°C) at the rate of 1:4 (w/v) before experimental use. The pellets were added to the sludge to plump the structure with enough air and an increased C:N ratio (53.2). The total content of C, N, P, K, Ca, and Mg was 42.6, 0.8, 0.1, 0.6, 0.4, and 0.1% in dry matter, respectively. The enzymatic activity and content of the main microorganism groups in feedstocks are shown in **Table 1**.

Experimental Design

Composting

For composting, five treatments were established:

(1) sludge 100%
(2) sludge 75% + PWS 25%
(3) sludge 50% + PWS 50%
(4) sludge 25% + PWS 75%
(5) PWS 100%

The feedstocks and its mixtures were thoroughly stirred and then were composted for 4 months in aerobic composters with 70-L working volume and 56-cm diameter. Aeration was provided from the bottom of the composter by a compressor. For the first 14 days (thermophilic phase), the air flow was set at 4 L/min for 5 min every half an hour and then for 3 min every

TABLE 1 | Enzymatic activity and content of microorganisms in input raw materials.

	Sewage sludge	Peletized wheat straw
β-D-glucosidase (μmol MUFG/h/g dw)	679 ± 78	22 ± 3
Acid phosphatase (μmol MUFP/h/g dw)	4,411 ± 690	80 ± 3
Arylsulphatase (μmol MUFS/h/g dw)	173 ± 32	4 ± 0
Lipase (μmol MUFY/h/g dw)	9,020 ± 131	283 ± 31
Chitinase (μmol MUFN/h/g dw)	556 ± 81	1 ± 2
Cellobiohydrolase (μmol MUFC/h/g dw)	270 ± 2	4 ± 4
Alanine aminopeptidase (μmol AMCA/h/g dw)	1,185 ± 116	0 ± 0
Leucine aminopeptidase (μmol AMCL/h/g dw)	880 ± 67	2 ± 0
Fungi (μg PLFA/g dw)	146 ± 2	24 ± 3
Bacteria (μg PLFA/g dw)	3,150 ± 83	53 ± 6
Actinobacteria (μg PLFA/g dw)	34 ± 0	1 ± 0
G+ (μg PLFA/g dw)	1,159 ± 34	20 ± 3
G− (μg PLFA/g dw)	1,766 ± 47	22 ± 4
Total microbial biomass (μg PLFA/g dw)	4,145 ± 93	122 ± 9

Values are means ± SD (n = 3).
dw, dry weight; MUFG, 4-methylumbellyferyl-β-D-glucopyranoside; MUFP, 4-methylumbellyferyl-phosphate; MUFS, 4-methylumbellyferyl sulfate potassium salt; MUFY, 4-methylumbellyferyl-caprylate; MUFN, 4-methylumbellyferyl-N-acetylglucosaminide; MUFC, 4-methylumbellyferyl-N-cellobiopyranoside; AMCA, L-alanine-7-amido-4-methylcoumarin; AMCL, L-leucine-7-amido-4-methylcoumarin; PLFA, phospholipid fatty acids.

half an hour. A temperature probe was inserted from the top of the composter to reach half the material height. The temperature was recorded every hour, and the values were collected in a data logger. Prior to sampling at the end of each month, any leachate was poured back into the composted material to achieve a closed loop of substances, and then the composted material was mixed thoroughly to achieve maximum homogeneity. Six samples ($n = 6$) of 0.5 kg each were taken.

Vermicomposting of Fresh and Precomposted Feedstock

For vermicomposting, five treatments were established as in the case of composting. Each treatment was carried out in triplicate without (as a control) and with earthworms as follows:

(1) sludge 100% without earthworms.
(2) sludge 100% with earthworms.
(3) sludge 75% + PWS 25% without earthworms.
(4) sludge 75% + PWS 25% with earthworms.
(5) sludge 50% + PWS 50% without earthworms.
(6) sludge 50% + PWS 50% with earthworms.
(7) sludge 25% + PWS 75% without earthworms.
(8) sludge 25% + PWS 75% with earthworms.
(9) PWS 100% without earthworms.
(10) PWS 100% with earthworms.

Fresh feedstocks were used in one set and precomposted feedstocks in the second set. Precomposting was conducted in composters, as described above, for 14 days. Vermicomposting trays measuring 40 × 40 × 15 cm were used for this part of the experiment. For vermicomposting with earthworms, 2/3 of the trays were filled with fresh or precomposted feedstocks (9 kg) and 1/3 with earthworm substrate based on grape marc (3 L). The bulk density of this fresh substrate was 685 g/L. The average earthworm density in the substrate was 125 pieces/L, with an average weight of 0.2 g per piece. The materials were separated by a mesh with 6-mm hole diameter. The earthworm substrate was utilized from the side to allow earthworms to move freely between materials in case of unsuitable conditions in the tested feedstocks, especially in case of ammonia formation in sewage sludge, which is toxic to earthworms. To ensure homogeneity, subsamples were taken from five sites in the tray (near the corners and in the middle). The material in the tray could not be mixed before sampling, as there would be a risk of death of the earthworms present. The total sample weight taken up at the end of each month was 0.5 kg from each of the three trays ($n = 3$).

Sample Analyses

With respect to vermicomposting samples, earthworms were taken, counted, washed, and weighed. The cocoon number was also determined. To determine the enzymatic activity and the presence of microorganism groups, 150 g of the sample was frozen at −25°C, which was subsequently lyophilized.

For hydrolytic enzyme determination, a suspension was prepared by homogenizing 0.2 ± 0.002 g of a lyophilized compost or vermicompost sample and 20 ml of acetate buffer (pH = 5) at a concentration of 50 mmol L^{-1} for approximately 30 s using an Ultra-Turrax instrument (IKA Labortechnik, Germany). Then, 200 μl of the homogenized suspension was pipetted into a microtiter plate, and then the appropriate substrate at the given concentration (c) was added depending on the enzyme [β-D-glucosidase: 4-methylumbellyferyl-β-D-glucopyranoside (MUFG) at c = 2.75 mmol/L, acid phosphatase: 4-methylumbellyferyl-phosphate (MUFP) at c = 2.75 mmol/L, arylsulphatase: 4-methylumbellyferyl sulfate potassium salt (MUFS) at c = 2.50 mmol/L, lipase: 4-methylumbellyferyl-caprylate (MUFY) at c = 2.50 mmol/L, chitinase: 4-methylumbellyferyl-N-acetylglucosaminide (MUFN) at c = 1.00 mmol/L, cellobiohydrolase: 4-methylumbellyferyl-N-cellobiopyranoside (MUFC) at c = 2.50 mmol/L, alanine aminopeptidase: L-alanine-7-amido-4-methylcoumarin (AMCA) at c = 2.50 mmol/L, leucine aminopeptidase: L-leucine-7-amido-4-methylcoumarin (AMCL) at c = 2.50 mmol/L]. The microtiter plates were then placed in an incubator heated to 40°C for 5 min. Afterward, the substrate fluorescence was measured using a Tecan Infinite® M200 (Tecan, Austria). Subsequently, the plates were again placed into an incubator for 2 h, and the fluorescence was measured again. From the difference between the initial and final value, the enzymatic activity was calculated and is usually given in micromoles of the respective substrate per hour and per gram of sample (Baldrian, 2009; Štursová and Baldrian, 2011). Fluorescence determination using a multifunctional modular reader is fast, modern, and an economically advantageous method for determining the enzymatic activity in a large sample number.

Samples for phospholipid fatty acid (PLFA) determination were extracted using phosphate buffer, chloroform, and methanol (0.8:1:2; v/v/v). Gas chromatography–mass spectrometry (450-GC, 240-MS Varian, Walnut Creek, CA, United States) was employed for determination of fatty acid methylated esters. The details of the analyses are described in Hanc et al. (2021).

A CHNS Vario MACRO cube analyzer (Elementar Analysensysteme GmbH, Germany) was used to determine total carbon and nitrogen *via* a thermal conductivity detector according to Hanc et al. (2017). The total contents of P, K, Ca, and Mg were determined by decomposition obtained by pressurized wet-ashing ($HNO_3 + H_2O_2$) of dried samples in a closed system of Ethos 1 (MLS GmbH, Germany). Afterward, the element content was determined using ICP-OES (Agilent 720, Agilent Technologies Inc., United States) according to García-Sánchez et al. (2017).

Statistical Analysis

Values are arithmetic means of three to six values (according to treatment) ± standard deviations. Based on the results of normality and homogeneity tests, the non-parametric Kruskal–Wallis test ($P \leq 0.05$) was chosen for statistical analyses.

RESULTS

Composting

After the first month of composting 100% sludge, there was a sharp 64% decrease in the enzymatic activity of the eight monitored enzymes. The smallest decrease by 16% was recorded

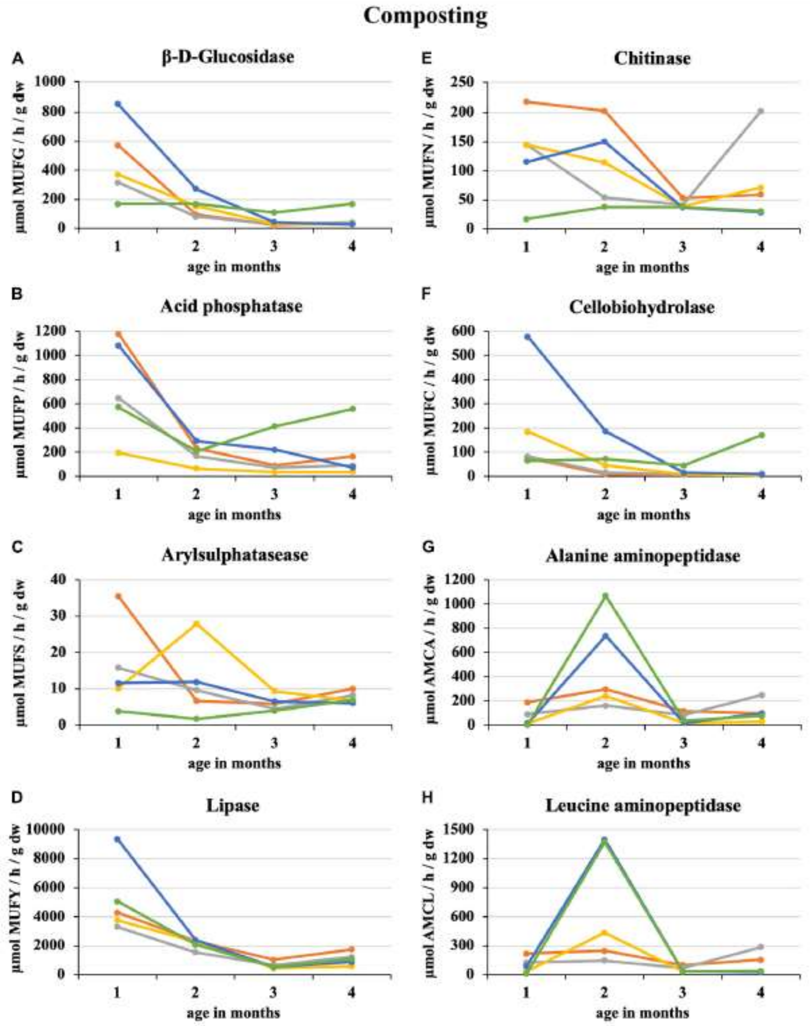

FIGURE 1 | Course of enzymatic activity during composting of fresh feedstock. sludge 100%; sludge 75% + PWS 25%; sludge 50% + PWS 50%; sludge 25% + PWS 75%, PWS 100%; PWS, pelletized wheat straw; dw, dry weight; MUFG, 4-methylumbellyferyl-β-D-glucopyranoside; MUFP, 4-methylumbellyferyl-phosphate; MUFS, 4-methylumbellyferyl sulfate potassium salt; MUFY, 4-methylumbellyferyl-caprylate; MUFN, 4-methylumbellyferyl-N-acetylglucosaminide; MUFC, 4-methylumbellyferyl-N-cellobiopyranoside; AMCA, L-alanine-7-amido-4-methylcoumarin; AMCL, L-leucine-7-amido-4-methylcoumarin.

for β-D-glucosidase, and the greatest by 84% was for alanine aminopeptidase. The least enzymatic activity at the composting end was recorded for 50% sludge (acid phosphatase, lipase, cellobiohydrolase, and alanine aminopeptidase) and 25% straw addition (β-D-glucosidase, arylsulphatase, chitinase, and leucin aminopeptidase) as illustrated in **Figure 1**. Conversely, the 100% straw was the least mature because of still ongoing decomposition characterized by a great activity of β-D-glucosidase, acid phosphatase, and cellobiohydrolase. Similarly, arylsulphatase and lipase increased in the second half of the composting period in the case of 100% sludge. The greatest enzymatic activity was recorded in the first month of composting, except for alanine and leucine

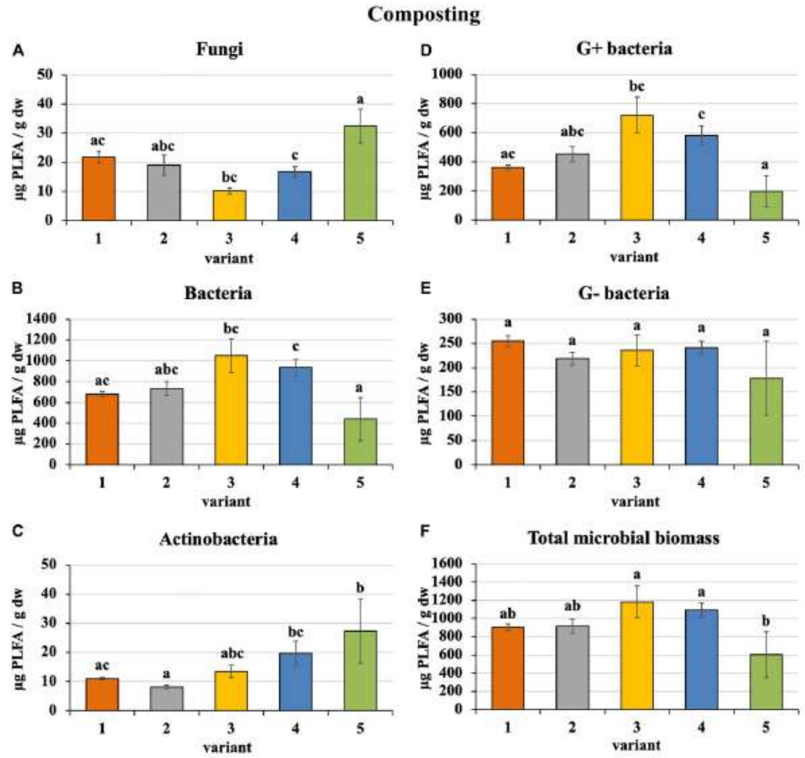

FIGURE 2 | Content of microorganisms after composting feedstock. Treatments: (1) sludge 100%, (2) sludge 75% + PWS 25%, (3) sludge 50% + PWS 50%, (4) sludge 25% + PWS 75%, and (5) PWS 100%. Values are means ± SD ($n = 3$). Different letters indicate significant differences among treatments (Kruskal–Wallis test, $P \leq 0.05$). PWS, pelletized wheat straw; PLFA, phospholipid fatty acids; dw, dry weight.

aminopeptidase, which had their distinct peaks in the second month, especially in PWS 100% and sludge 25% + PWS 75%. For most enzymes, a statistically significant difference was found between the first and second halves of the composting process. The statistical differences among the treatments varied according to the individual enzymes and on a time course basis.

Sewage sludge 100% differed from PWS 100% in the content and proportion of microorganism groups expressed by PLFA. During composting of 100% sewage sludge, total microbial biomass, fungi, bacteria, actinobacteria, and G+ and G− bacteria decreased and accounted for 22, 15, 22, 32, 31, and 14% of used feedstock, respectively. Conversely, microorganism content increased in PWS 100% at the end with respect to total microbial biomass, fungi, bacteria, actinobacteria, and G+ and G− bacteria by 4. 9-, 1. 4-, 8. 3-, 27. 3-, 9. 8-, and 9.1-fold, respectively. In spite of that, total microbial biomass, bacteria, and G+ and G− bacteria were found to a greater extent in sewage sludge 100% than in PWS 100% after 4 months of composting. Conversely, fungi and actinobacteria were greater in PWS 100% (**Figure 2**). The most bacteria and, conversely, the least fungi were found in sludge 50% + PWS 50%, followed by sludge 25% + PWS 75%.

Vermicomposting of Fresh and Precomposted Feedstock

Enzymes

During the first month of 100% fresh sewage sludge vermicomposting, there was a 65% decrease in the enzymatic activity of eight enzymes (**Tables 2, 3**). The enzymatic activity of alanine aminopeptidase decreased the most among enzymes during this period (by 87%). In contrast, acid phosphatase activity decreased by only 9%. The average activity of all monitored enzymes decreased in this variant by 95% after 4 months of vermicomposting. After 1 month of vermicomposting, no difference was found between the treatment with earthworms and without earthworms. However, after the 2nd and 3rd months, the enzymatic activity was greater in treatments with earthworms by 51 and 32%, respectively. After this time, no differences

TABLE 2 | Activities of β-D-glucosidase, acid phosphatase, arylsulphatase, and lipase during vermicomposting fresh feedstock.

	Sludge 100% without e.	Sludge 100% with e.	Sludge 75% + PWS 25% without e.	Sludge 75% + PWS 25% with e.	Sludge 50% + PWS 50% without e.	Sludge 50% + PWS 50% with e.	Sludge 25% + PWS 75% without e.	Sludge 25% + PWS 75% with e.	PWS 100% without e.	PWS 100% with e.
β-D-glucosidase (μmol MUFG/h/g dw)										
1 month	341 ± 94 aAB	248 ± 17 aAB	914 ± 196 aA	250 ± 71 aAB	139 ± 11 aAB	59 ± 28 aB	844 ± 202 aAB	779 ± 303 aA	118 ± 54 aAB	215 ± 34 aAB
2 months	26 ± 1 abA	25 ± 4 abA	50 ± 4 aAB	36 ± 13 abAB	140 ± 52 aB	44 ± 3 aAB	60 ± 18 abAB	38 ± 3 abAB	35 ± 11 bAB	57 ± 14 abAB
3 months	24 ± 8 abAB	14 ± 8 bAB	51 ± 13 aAB	31 ± 12 abAB	47 ± 16 aAB	4 ± 1 aA	39 ± 12 bAB	18 ± 8 bAB	81 ± 18 abB	27 ± 4 bAB
4 months	24 ± 5 bA	26 ± 8 abA	40 ± 24 aA	18 ± 8 bA	61 ± 18 bA	5 ± 1 aA	41 ± 14 abA	29 ± 5 abA	58 ± 1 abA	57 ± 27 abA
Acid phosphatase (μmol MUFP/h/g dw)										
1 month	1,734 ± 289 aAB	4,045 ± 1,107 aAB	3,111 ± 701 aAB	1,867 ± 626 aAB	1,413 ± 520 aAB	899 ± 208 aB	3,890 ± 1,403 aAB	7,065 ± 2,047 aA	970 ± 290 aB	1,296 ± 342 aAB
2 months	280 ± 56 bA	308 ± 140 abA	1,859 ± 86 abB	517 ± 65 abAB	1,319 ± 312 aAB	458 ± 41 abAB	749 ± 207 abAB	551 ± 83 aAB	893 ± 218 aAB	739 ± 190 abAB
3 months	385 ± 121 abAB	212 ± 41 bAB	378 ± 106 abAB	90 ± 19 bA	613 ± 152 aB	372 ± 103 abAB	421 ± 37 bAB	275 ± 36 aAB	235 ± 87 aAB	457 ± 112 abAB
4 months	423 ± 177 abA	243 ± 16 abA	331 ± 112 bA	209 ± 50 abA	572 ± 237 aA	317 ± 136 bA	516 ± 42 abA	254 ± 57 aA	280 ± 30 aA	269 ± 84 bA
Arylsulphatase (μmol MUFS/h/g dw)										
1 month	31 ± 5 aA	31 ± 3 aA	27 ± 12 aAB	19 ± 9 aAB	5 ± 2 aAB	0 ± 0 aB	13 ± 0 aAB	12 ± 6 aAB	10 ± 0 aAB	9 ± 0 aAB
2 months	3 ± 0 abAB	4 ± 0 bA	3 ± 1 aAB	3 ± 2 abAB	0 ± 0 bB	0 ± 0 aB	2 ± 1 bAB	1 ± 1 abAB	4 ± 0 bAB	3 ± 1 bAB
3 months	1 ± 0 bAB	3 ± 2 abAB	4 ± 3 aAB	4 ± 3 abAB	3 ± 1 abAB	5 ± 7 aAB	4 ± 3 abAB	0 ± 0 bA	7 ± 3 abB	4 ± 1 abAB
4 months	5 ± 3 abA	6 ± 0 abA	3 ± 2 aA	0 ± 0 bA	2 ± 1 abA	4 ± 3 aA	6 ± 3 abA	1 ± 1 abA	8 ± 2 abA	6 ± 1 abA
Lipase (μmol MUFY/h/g dw)										
1 month	3,375 ± 772 aAB	2,976 ± 353 aAB	4,153 ± 1,009 aAB	2,449 ± 780 aAB	5,372 ± 1,490 aB	3,282 ± 268 aAB	2,837 ± 624 aAB	3,116 ± 763 aAB	1,333 ± 375 aA	1,489 ± 914 aA
2 months	748 ± 113 abAB	640 ± 345 aAB	1,438 ± 275 abA	422 ± 118 aB	1,234 ± 263 abAB	830 ± 333 abAB	892 ± 148 abAB	830 ± 357 abAB	360 ± 155 aB	914 ± 197 aAB
3 months	396 ± 72 bA	477 ± 53 aA	615 ± 160 abA	582 ± 408 aA	406 ± 8 bA	527 ± 250 abA	325 ± 181 bA	213 ± 93 bA	404 ± 206 aA	440 ± 153 aA
4 months	716 ± 157 abA	515 ± 289 aA	490 ± 189 bA	382 ± 52 aA	462 ± 128 abA	371 ± 39 bA	439 ± 110 abA	386 ± 182 abA	426 ± 157 aA	445 ± 92 aA

Values are means ± SD (n = 3). Different lowercase letters in a column indicate significant differences between months, while capital letters indicate significant differences among treatments (Kruskal-Wallis test, $P \leq 0.05$).

dw, dry weight; PWS, pelletized wheat straw; e., earthworms; MUFG, 4-methylumbelliferyl β-D-glucopyranoside; MUFP, 4-methylumbelliferyl phosphate; MUFS, 4-methylumbelliferyl sulfate potassium salt; MUFY, 4-methylumbelliferyl caprylate.

TABLE 3 | Activities of chitinase, cellobiohydrolase, alanine aminopeptidase, and leucine aminopeptidase during vermicomposting fresh feedstock.

	Sludge 100% without e.	Sludge 100% with e.	Sludge 75% + PWS 25% without e.	Sludge 75% + PWS 25% with e.	Sludge 50% + PWS 50% without e.	Sludge 50% + PWS 50% with e.	Sludge 25% + PWS 75% without e.	Sludge 25% + PWS 75% with e.	PWS 100% without e.	PWS 100% with e.
Chitinase (μmol MUFN/h/g dw)										
1 month	526 ± 90 aA	329 ± 7 aAB	381 ± 131 aAB	191 ± 21 aAB	243 ± 50 aAB	380 ± 64 aAB	297 ± 121 aAB	183 ± 30 aAB	59 ± 11 aB	57 ± 30 aB
2 months	42 ± 22 abAB	30 ± 12 aAB	47 ± 8 abAB	15 ± 7 abA	125 ± 30 abB	50 ± 4 abAB	83 ± 30 abAB	43 ± 8 abAB	17 ± 4 bAB	39 ± 17 aAB
3 months	29 ± 10 bAB	26 ± 10 aAB	37 ± 7 abAB	22 ± 10 abAB	41 ± 1 abA	20 ± 6 bAB	36 ± 16 bAB	11 ± 5 abB	33 ± 15 abAB	34 ± 3 aAB
4 months	60 ± 21 abA	30 ± 6 aAB	23 ± 4 bAB	13 ± 1 bAB	33 ± 4 bAB	29 ± 9 abAB	42 ± 19 abAB	10 ± 0 bB	30 ± 15 abAB	47 ± 9 aAB
Cellobiohydrolase (μmol MUFC/h/g dw)										
1 month	57 ± 21 aA	40 ± 6 aA	311 ± 46 aA	63 ± 21 aA	111 ± 29 aA	134 ± 22 aA	300 ± 168 aA	326 ± 1 aA	38 ± 22 aA	106 ± 36 aA
2 months	9 ± 5 abA	7 ± 5 aA	13 ± 5 abA	7 ± 3 abA	18 ± 7 abA	11 ± 0 abA	23 ± 8 abA	9 ± 6 abA	16 ± 2 abA	12 ± 0 aA
3 months	4 ± 3 bA	16 ± 9 aA	7 ± 3 abA	10 ± 6 abA	12 ± 3 abA	5 ± 2 abA	9 ± 4 bA	3 ± 2 bA	12 ± 2 bA	10 ± 2 aA
4 months	8 ± 3 abAB	6 ± 2 aAB	8 ± 2 bAB	3 ± 1 bA	8 ± 1 bAB	4 ± 3 bAB	12 ± 5 abAB	6 ± 3 abAB	35 ± 3 abB	10 ± 5 aAB
Alanine aminopeptidase (μmol AMCA/h/g dw)										
1 month	98 ± 2 aAB	161 ± 43 aA	143 ± 57 aAB	88 ± 21 aAB	27 ± 7 aB	32 ± 4 aAB	81 ± 10 aAB	69 ± 37 aAB	44 ± 1 aAB	28 ± 3 aB
2 months	34 ± 2 aAB	28 ± 12 aAB	58 ± 11 abAB	33 ± 14 abAB	42 ± 9 aAB	31 ± 1 abA	78 ± 4 aAB	47 ± 3 abAB	43 ± 5 aAB	82 ± 4 bB
3 months	28 ± 16 aA	25 ± 11 aA	30 ± 19 abA	27 ± 7 abA	41 ± 10 aA	19 ± 1 abA	25 ± 12 aA	15 ± 1 abA	38 ± 16 aA	44 ± 19 abA
4 months	25 ± 4 aAB	23 ± 4 aAB	22 ± 5 bAB	12 ± 7 bAB	10 ± 7 aAB	13 ± 2 bAB	20 ± 7 aAB	3 ± 1 bA	46 ± 2 aB	45 ± 3 abB
Leucine aminopeptidase (μmol AMCL/h/g dw)										
1 month	112 ± 14 aA	197 ± 65 aAB	147 ± 45 a	90 ± 14 aA	166 ± 15 aAB	286 ± 63 aAB	292 ± 56 aAB	419 ± 97 aB	194 ± 59 aAB	258 ± 86 aAB
2 months	91 ± 37 abAB	87 ± 22 abAB	66 ± 37 abAB	26 ± 11 abA	78 ± 4 abAB	224 ± 25 abAB	210 ± 60 aAB	237 ± 19 abA	56 ± 5 abAB	216 ± 64 aAB
3 months	38 ± 2 bAB	15 ± 9 bAB	29 ± 2 abAB	20 ± 8 abAB	26 ± 8 abAB	86 ± 37 abAB	12 ± 4 aA	12 ± 1 abA	47 ± 9 abAB	129 ± 7 aB
4 months	43 ± 5 abAB	32 ± 7 abAB	22 ± 1 bAB	9 ± 2 bA	9 ± 4 bA	21 ± 9 bAB	9 ± 4 aAB	11 ± 2 bAB	44 ± 7 bAB	114 ± 36 aB

Values are means ± SD (n = 3). Different lowercase letters in a column indicate significant differences between months, while capital letters indicate significant differences among treatments (Kruskal-Wallis test, P ≤ 0.05).

dw, dry weight; PWS, palletized wheat straw; e. = earthworms; MUFN, 4-methylumbelliferyl-N-acetylglucosaminide; MUFC, 4-methylumbelliferyl-N-cellobiopyranoside; AMCA, L-alanine-7-amido-4-methylcoumarin; AMCL, L-leucine-7-amido-4-methylcoumarin.

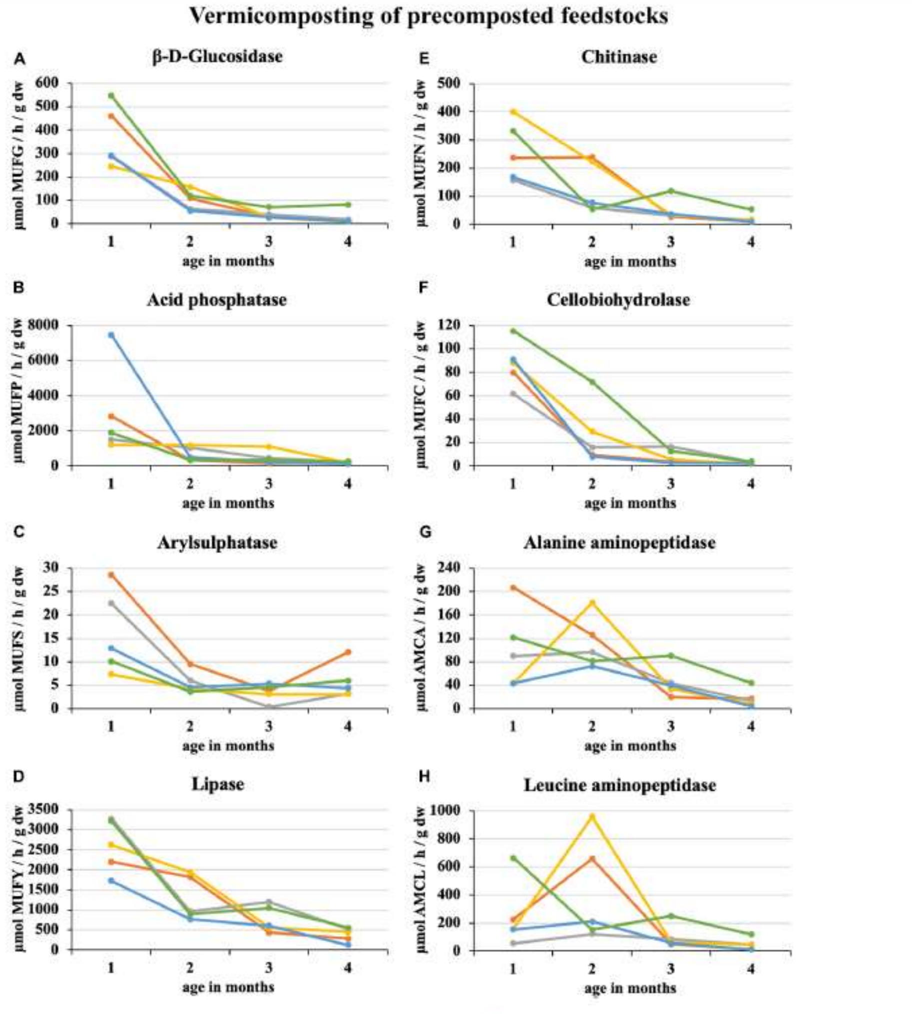

FIGURE 3 | Course of enzymatic activity during vermicomposting of precomposted feedstock. , sludge 100% with earthworms; , sludge 75% + PWS 25% with earthworms; , sludge 50% + PWS 50% with earthworms; , sludge 25% + PWS 75% with earthworms; , PWS 100% with earthworms; PWS, pelletized wheat straw; dw, dry weight; MUFG, 4-methylumbellyferyl-β-D-glucopyranoside; MUFP, 4-methylumbellyferyl-phosphate; MUFS, 4-methylumbellyferyl sulfate potassium salt; MUFY, 4-methylumbellyferyl-caprylate; MUFN, 4-methylumbellyferyl-N-acetylglucosaminide; MUFC, 4-methylumbellyferyl-N-cellobiopyranoside; AMCA, L-alanine-7-amido-4-methylcoumarin; AMCL, L-leucine-7-amido-4-methylcoumarin.

in average enzymatic activity were apparent. However, the situation was different for individual enzymes. PWS 100% was characterized by a significantly greater enzymatic activity in the earthworm treatments, especially in the case of β-D-glucosidase, acid phosphatase, lipase, chitinase, and leucin aminopeptidase. The least enzymatic activity was found at the vermicomposting end in mixtures, especially in sludge 75% + PWS 25%.

During 2 weeks of precomposting, the enzymatic activity of eight enzymes in 100% sewage sludge decreased by an average of 45%. A further decrease of 25% was recorded in the first month of subsequent vermicomposting (**Figure 3**). At the end of the 1st month of vermicomposting, some enzyme values were greater than at the beginning (i.e., immediately after precomposting). Specifically, these enzymes were PWS (all enzymes), sludge

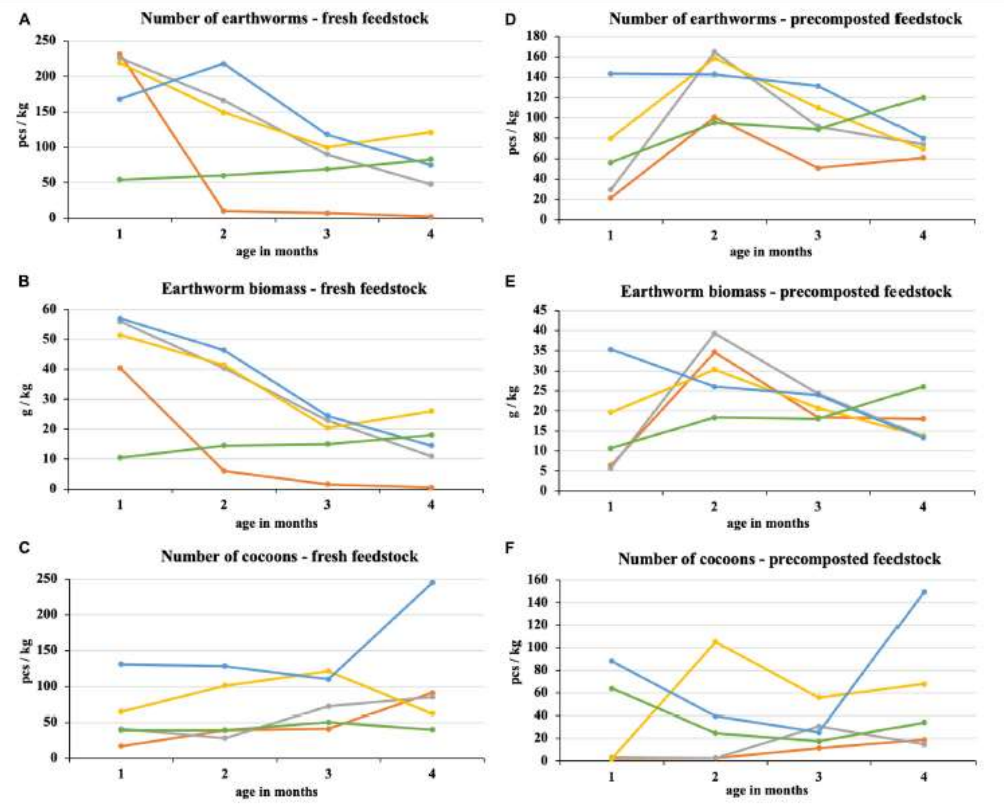

FIGURE 4 | Course of number of earthworms, earthworm biomass, and number of cocoons in treatments with vermicomposting of fresh feedstock (**A–C**) and with vermicomposting of precomposted feedstock (**D–F**). orange, sludge 100% with earthworms; grey, sludge 75% + PWS 25% with earthworms; yellow, sludge 50% + PWS 50% with earthworms; blue, sludge 25% + PWS 75% with earthworms; green, PWS 100% with earthworms; PWS, pelletized wheat straw.

25% + PWS 75% (acid phosphatase, arylsulphatase, chitinase, cellobiohydrolase, alanine aminopeptidase, and leucin aminopeptidase), sludge 50% + PWS 50% (acid phosphatase, chitinase, alanine aminopeptidase, and leucin aminopeptidase), sludge 75% + PWS 25% (acid phosphatase), and sludge 100% (acid phosphatase and cellobiohydrolase). Thus, acid phosphatase increased in all treatments. Since the end of the 1st month, enzymatic activity decreased (the same **Figure 3**). During vermicomposting of precomposted feedstocks, the enzymatic activity decreased. For most enzymes, it was significant after the 1st month. As in the case of composting, alanin and leucin aminopeptidase, in some treatments (e.g., Sludge 50% + PWS 50%), increased after 1 month and decreased later. Of the monitored treatments, the greatest decrease (91%) was in the sludge 25% + PWS 75% treatment, followed by the sludge 100% treatment (by 89%), sludge 50% + PWS 50% (by 83%), sludge 25% + PWS 75% (by 79%), and PWS 100% (by 78%).

Earthworms

After the first month of fresh feedstock vermicomposting, most earthworms were in 100% sewage sludge and mixtures with its high proportion (**Figure 4**). In these treatments, there was a subsequent decrease in earthworm number, which was directly proportional to the sewage sludge proportion. The difference between the process beginning and end was statistically significant in the sewage sludge 100% and sewage sludge 75% + PWS 25%. After the first month, the earthworm number in sludge 25% + PWS 75% increased (**Figure 4A**), but the earthworm biomass decreased (**Figure 4B**), which was due to the reduced weight of individual earthworm pieces. PWS 100% showed four times less earthworm number and five times less earthworm biomass than other treatments at the process beginning. During the following months, however, these parameters increased, and this treatment was included among the other mixed treatments. The earthworms decreased significantly in 100% sewage sludge, where only two pieces per kilogram were present at the end of vermicomposting. The cocoon number (**Figure 4C**) in the vermicomposted material increased during the process (at the beginning, 17–131 pieces/kg; in the end, 40–245 pieces/kg). Sewage sludge 25% + PWS 75% was characterized by the greatest number of loaded cocoons.

In the case of precomposted feedstock, the greatest number and earthworm biomass were found in three treatments with the greatest sludge proportion at the end of the 2nd month of vermicomposting (**Figures 4D,E**). An approximately six-fold increase was found in the 75% sludge treatment. The earthworm number in the 25% sludge treatment was the greatest of all tested treatments at the process beginning (143 pieces/kg) and differed statistically from the 100% sludge treatment. It was the most stable treatment with a slight decrease. Conversely, PWS 100% showed a continuous increase in earthworm number and biomass. The earthworm number in this treatment varied statistically at the process beginning and in the end and reached the greatest values among all treatments (120 pieces/kg). PWS addition to the sludge and PWS itself caused an increase in the cocoon number in the 1st month and at the process end (**Figure 4F**). The cocoon number in the 100% sludge and the 75% sludge treatments increased slightly in the 2nd half of vermicomposting. Nevertheless, these treatments contained fewer cocoons at the end of vermicomposting compared to treatments with a greater PWS proportion.

Microorganisms

Similarly, as in the case of composting, fresh sewage sludge 100% differed from PWS 100% in the content and proportion of microorganism groups. During vermicomposting of 100% sewage sludge, total microbial biomass, fungi, bacteria, actinobacteria, and G+ and G− bacteria decreased and accounted for 9, 4, 9, 31, 13, and 5% of used feedstock, respectively. Conversely, the microorganism content increased in PWS 100% at the end, including total microbial biomass, fungi, bacteria, actinobacteria, and G+ and G− bacteria by 7. 4-, 1. 7-, 13. 4-, 10. 5-, 17. 6-, and 15.2-fold, respectively. After 4 months of vermicomposting, the earthworm treatments contained 60% of fungi, 80% of bacteria, 112% of actinobacteria, 86% of G+ bacteria, 73% of G− bacteria, and 71% of total microbial biomass compared to treatments without earthworms (**Table 4**). However, PWS 100% contained a greater number of microorganisms, except fungi, than the control treatment. Within treatments with and without earthworms, the greatest number of fungi, bacteria, actinobacteria, G+ bacteria, G− bacteria, and total microbial biomass was found in PWS 100% without earthworms, sludge 100% without earthworms, sludge 100% with earthworms, PWS 100% with earthworms, PWS 100% with earthworms, and sludge 75% + PWS 25% without earthworms, respectively.

Microorganism content in precomposted treatments at the process end was slightly greater (bacteria—410 μg/g, actinobacteria—9 μg/g, G+ bacteria—183 μg/g, G− bacteria—187 μg/g, and total microbial biomass—508 μg/g), as shown

TABLE 4 | Content of microorganisms after 4 months of vermicomposting fresh feedstock.

	Sludge 100% without e.	Sludge 100% with e.	Sludge 75% + PWS 25% without e.	Sludge 75% + PWS 25% with e.	Sludge 50% + PWS 50% without e.	Sludge 50% + PWS 50% with e.	Sludge 25% + PWS 75% without e.	Sludge 25% + PWS 75% with e.	PWS 100% without e.	PWS 100% with e.
Fungi (μg PLFA/g dw)	12 ± 8 a	6 ± 0 ab	13 ± 5 ab	6 ± 1 ac	23 ± 5 ab	11 ± 1 ab	28 ± 13 ab	21 ± 7 ab	48 ± 16 b	41 ± 3 bc
Bacteria (μg PLFA/g dw)	514 ± 258 ab	272 ± 8 ab	430 ± 248 ab	194 ± 14 a	364 ± 128 ab	180 ± 23 a	331 ± 116 ab	252 ± 14 ab	422 ± 18 ab	741 ± 18 b
Actinobacteria (μg PLFA/g dw)	8 ± 0 ab	11 ± 1 a	8 ± 1 ab	9 ± 0 ab	7 ± 0 ab	6 ± 0 b	7 ± 1 ab	7 ± 0 ab	7 ± 2 ab	10 ± 1 a
G+ (μg PLFA/g dw)	215 ± 77 ab	150 ± 3 ab	180 ± 83 ab	97 ± 10 a	167 ± 50 ab	87 ± 23 a	160 ± 59 ab	115 ± 8 ab	193 ± 13 ab	353 ± 12 b
G− (μg PLFA/g dw)	254 ± 166 ab	85 ± 6 ab	208 ± 152 a	65 ± 1 ab	160 ± 77 ab	74 ± 6 a	135 ± 54 ab	109 ± 7 ab	193 ± 4 ab	335 ± 8 b
Total microbial biomass (μg PLFA/g dw)	671 ± 319 ab	362 ± 14 ab	1,517 ± 1,083 a	263 ± 16 ab	479 ± 147 ab	233 ± 18 b	446 ± 155 ab	339 ± 28 ab	566 ± 12 ab	898 ± 16 a

Values are means ± SD (n = 3). Different letters in a column indicate significant differences between treatments (Kruskal–Wallis test, $P \leq 0.05$).
dw, dry weight; PWS, pelletized wheat straw; e., earthworms; PLFA, phospholipid fatty acids.

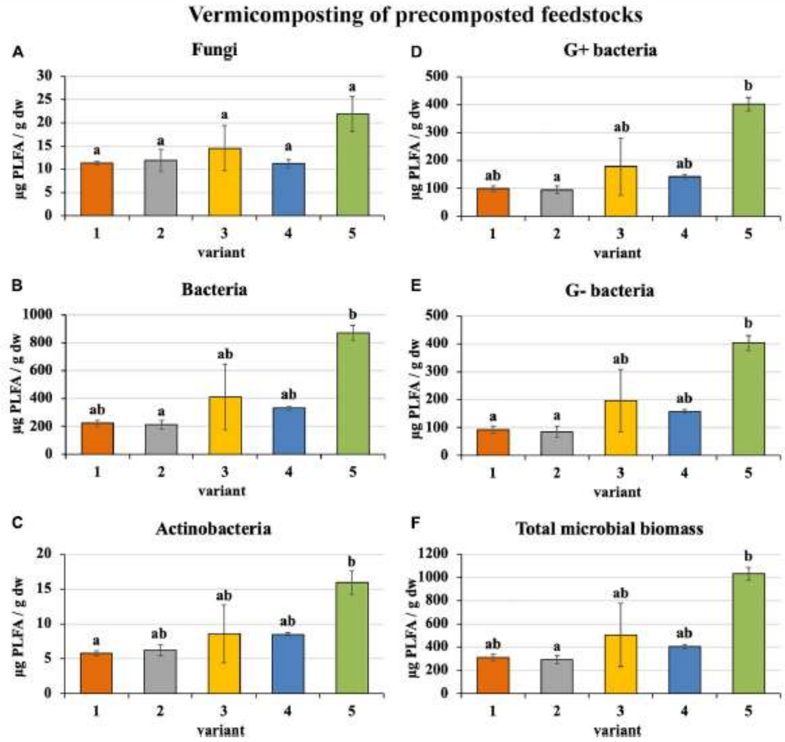

FIGURE 5 | Content of microorganisms after vermicomposting precomposted feedstock. Treatments: (1) sludge 100% with earthworms, (2) sludge 75% + PWS 25% with earthworms, (3) sludge 50% + PWS 50% with earthworms, (4) sludge 25% + PWS 75% with earthworms, and (5) PWS 100% with earthworms. Values are means ± SD ($n = 3$). Different letters indicate significant differences among treatments (Kruskal–Wallis test, $P \leq 0.05$). PWS, pelletized wheat straw; PLFA, phospholipid fatty acids; dw, dry weight.

in **Figure 5**, than in the case of freshly used feedstocks described above (bacteria—328 µg/g, actinobacteria—8 µg/g, G+ bacteria—160 µg/g, G− bacteria—134 µg/g, and total microbial biomass—419 µg/g). Fungi were exceptions (in precomposted treatments—14 µg/g and in treatments with fresh feedstocks—17 µg/g). The greatest content of individual microorganism groups were unequivocally found in PWS 100%, followed by sludge 50% + PWS 50%, sludge 25% + PWS 75%, and almost identically sludge 100% and sludge 75% + PWS 25%.

DISCUSSION

During sludge composting and treatments with its addition, there was a significant and permanent decrease in β-D-glucosidase activity. In the case of straw pellets, there was a reduced but constant enzyme activity because of slow degradation. Plant residues primarily composed of cellulose, hemicellulose, or lignin are difficult to biodegrade and increase composting time, which is associated with longer enzymatic activity (Hemati et al., 2021). Estrella-González et al. (2019) determined profiles of β-glucosidase, amylase, cellulase, and xylanase activities during large-scale composting of vegetable waste, urban solid waste, sewage sludges, agrifood waste, and olive oil mill wastewater. The results revealed very different profiles. The evolution profiles of the enzymes involved in the degradation of lignocellulosic fractions in sewage sludges coincided with greater biodegradation rates of these fractions at the process end. The β-D-glucosidase decrease in sewage sludge copied the trend we found. Unfortunately, it is not possible to compare specific values because we utilized the more effective and modern fluorescence method, and the previous study used classical colorimetric estimation of p-nitrophenol released by p-nitrophenyl-β-D-glucopiranoside hydrolysis.

The greatest acid phosphatase activity was found at composting beginning in 100% sewage sludge, which was because of the high phosphorus content in this feedstock. This statement is confirmed by Albrecht et al. (2010) who co-composted green waste and sewage sludge for 5 months. Acid phosphatase activities were high during the first month of composting and then declined. Large nutrient quantities stimulated the growth of total aerobic bacteria and subsequent phosphatase activity.

Ma et al. (2019) found that arylsulphatase content in sewage sludge (75%) + sawdust (25%) initially increased and subsequently decreased from the initial rate of 10.05 to 15.51 μmol/(h × g) at the end of composting. By contrast, the arylsulphatase content in sewage sludge (75%) + sawdust (12.5%) + matured compost (12.5%) decreased continuously. The arylsulphatase content in the matured compost treatment was significantly greater than in the treatment without matured compost at the mesophilic phase. After entering the thermophilic phase, the arylsulphatase content in sewage sludge (75%) + sawdust (12.5%) + matured compost (12.5%) was significantly greater than that without matured compost, and this difference was maintained until the composting end ($P < 0.05$). Therefore, the arylsulphatase content increased during the mesophilic phase and decreased during the cooling phase when the matured compost was added. In our experiment, the arysulphatase activity increased after the 2nd month of composting in the treatments with 50 and 75% straw pellets and in the case of the pellets themselves in the 2nd half of the process. This may be because of the gradual release of organic sulfur from straw with the degradation of cellulose and other refractory organic matter, resulting in increased arylsulphatase content (Tejada et al., 2006).

Gea et al. (2007) co-composted sewage sludge with 30 and 50% fat addition. Lipase from a thermophilic composting environment showed a high stability for mesophilic temperature values and slightly alkaline pH values. The maximum lipolytic activity was observed at the thermophilic phase, which is consistent with our findings where the greatest lipase activity was during the first month of composting and then declined. Lipase activity in this period ranged from 3,300 to 5,000 μmol MUFY h^{-1} g^{-1} dw, with the exception of sludge 25% + PWS 75% (9360 μmol MUFY h^{-1} g^{-1} dw), and decreased to 1,000 μmol MUFY/h/g dw at composting end.

Chitinase activity varied greatly among treatments at the beginning of composting. The values were directly proportional to the proportion of sewage sludge. Poulsen et al. (2008) reported that chitinase activity was much greater in household waste compost (3.97 μmol 4 MU/h × g dry matter) than in garden/park compost (0.46 μmol 4 MU/h × g dry matter). Chitinase genes are found in a range of microorganisms, in particular actinomycetes, and in fungi (Krsek and Wellington, 2001). These organisms were apparently present and active to a greater extent during sludge composting and mixtures with a great proportion of this feedstock.

Cellobiohydrolase activity strongly correlated with β-D-glucosidase because they participated in carbonaceous substance decomposition, especially saccharides such as cellulose and cellobiose. The greatest activity of both enzymes was recorded in sludge 25% + PWS 75%, especially in the first half of the composting. The sludge significantly supported and accelerated microorganism development and thus the decomposition of the above-mentioned substances in straw pellets. The activity of both enzymes was significantly greater compared to the straw pellets itself.

The profile of alanine and leucine aminopeptidase was different from those of other enzymes during composting. After 2 months of composting, there was an increase in enzymatic activity. This increase was greatest at 75 and 100% straw pellets. Thus, enzyme activity must have been related to terminal nitrogen release from alanine and leucine found in straw. For a detailed evaluation, it would be necessary to establish further experiments and also to monitor the amino acid content.

Similar to composting, the greatest enzymatic activity decrease occurred in the first half of vermicomposting. Straw is as an important bulking agent because it matures compost and vermicompost faster as evidenced by decreased enzymatic activities. A great proportion of straw pellets proved successful in vermicomposting coffee grounds (Hanc et al., 2021). The greatest earthworm number and biomass were in the treatment with 75% straw pellets. The experiment was operated in a system with continuous earthworm feeding. The following enzymatic activities were detected in the oldest layer (6 months) of this treatment: β-D-glucosidase—1,194 μmol MUFG/h/g dw, acid phosphatase—1,894 μmol MUFP/h/g dw, arylsulphatase—64 μmol MUFS/h/g dw, lipase—3,924 μmol MUFY/h/g dw, chitinase—207 μmol MUFN/h/g dw, cellobiohydrolase—105 μmol MUFC/h/g dw, alanine aminopeptidase—46 μmol AMCA/h/g dw, and leucine aminopeptidase—55 μmol AMCL/h/g dw. These values were greater than in the current study, which was due to a different processed raw material and technological procedure. During vermicomposting of sewage sludge and coffee grounds, lesser enzymatic activities were found in treatments with earthworms than those without earthworms. Similarly, Domínguez and Gómez-Brandón (2012) reported that earthworm activity greatly reduced protease and cellulase enzyme activities in comparison with the control. These findings are in agreement with the microbial activity data, which reinforces that a greater degree of stability was reached after the active vermicomposting phase. This is also related to the microbiota composition changes in the intestines of earthworms and vermicompost. Domínguez et al. (2021) utilized 16S and ITS rRNA high-throughput sequencing to characterize bacterial and fungal community composition and structure during the gut-associated processes (GAP) and cast-associated processes of sewage sludge vermicomposting. The bacterial and fungal communities of earthworm casts were mainly composed of microbial taxa not found in the sewage sludge. Thus, most of the bacterial (96%) and fungal (91%) taxa in the sewage sludge were eliminated during vermicomposting, mainly through the GAP. Less microbial biomass, mainly fungi, was found in the earthworm treatments, which is consistent with the claim that fungi are a valuable food source

(Zhang et al., 2000). Hřebečková et al. (2019) compared the enzymatic activity in three aged vermicompost types (from household biowaste, malt house sludge mixed with agricultural waste, and grape marc). The vermicomposting was conducted in large-scale heap systems with continuous earthworm feeding, which is applicable in practice. The greatest hydrolytic enzyme activity occurred in the vermicomposting process with household biowaste.

It is necessary to approach enzymatic activity values during composting and vermicomposting with caution. The enzymatic activity amount depends mainly on the feedstock types used as well as the maturity phase and the technological process used. In the case of vermicomposting, earthworm density and activity are important.

Further research should be focused on determining the effectiveness of sewage sludge composting and vermicomposting using other bulking agents. Furthermore, it would be appropriate to verify the materials used from the first half of the composting and vermicomposting period, when enzymatic activity was great. It would be interesting to use straw pellets, which are characterized by purity and whose enzymatic activity remained at greater values for a longer time period, both during composting and vermicomposting compared to other treatments. This could be used as a cheap way, for example, for the acceleration of biowaste decomposition and isolation of enzymes for the production of enzymatic preparations or for the revitalization of biologically inactive or contaminated soil.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AH contributed to conceptualization, methodology, investigation, writing, visualization, funding acquisition, formal analysis, and project administration. BD contributed to experiments, analyses, and data curation. TH contributed to data curation and formal analysis. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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5.3. Dume et al. (2022). Vermicomposting Technology as a Process Able to Reduce the Content of Potentially Toxic Elements in Sewage Sludge.

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Vermicomposting Technology as a Process Able to Reduce the Content of Potentially Toxic Elements in Sewage Sludge

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Abstract: Sewage sludge (SS) contains potential toxic elements (PTEs) that are harmful to the environment, and their bioaccumulation in the food chain is a major environmental health concern. Vermicomposting has been shown to reduce PTEs during composting of sewage sludge. However, the extent of PTE's assimilation into the earthworm tissues during composting is largely unknown. The objectives of this study were to evaluate the potential of vermicomposting to decrease PTEs (As, Cd, Cr, Cu, Pb, and Zn) during composting of SS and whether the bioaccumulation of PTEs in earthworm tissue depends on feed quality. The initial SS was mixed in triplicate with varying proportions of pelletized wheat straw (PWS) (0%, 25%, 50%, and 75% (w/w)) along with a control (100% SS, no earthworms), and the variants were named VC1, VC2, VC3, VC4, and C0 (control), respectively. The experiment was conducted for 120 days using *Eisenia andrei*. In comparison to the control, mixing SS with PWS reduced Arsenic content by 14–67%, Cadmium content by 4–39%, Chromium contents by 24–77%, Copper content by 20–68%, Lead content by 39–75%, and Zinc content by 16–65%. The bioaccumulation factor's (BCF) ranges were 20–80% for Arsenic, 20–60% for Cadmium, 6–16% for Chromium, 32–80% for Copper, and 37–115% for Zinc, demonstrating that the accumulation of PTEs in the earthworm tissues explains the low content of PTEs in the vermicompost. In terms of removal rate, the sludge mixtures with bulking agent can be arranged in the following order: VC4 > VC3 > VC2 > VC1. The total carbon loss showed a significant relationship with BCF_{As} ($r = 0.989$, $p < 0.011$), BCF_{Cd} ($r = 0.996$, $p < 0.004$), BCF_{Cr} ($r = 0.977$, $p < 0.023$), BCF_{Cu} ($r = 0.999$, $p < 0.000$), and BCF_{Zn} ($r = 0.994$, $p < 0.006$). The variant containing 75% PWS (VC4) appeared to be a suitable SS mixture to reduce PTEs. Hence, it is suggested that vermicomposting reduces the content of PTEs in SS.

Keywords: toxic elements; sewage sludge; vermicompost; straw pellets; *Eisenia andrei*

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1. Introduction

The disposal of sewage sludge (SS), a by-product generated in massive amounts in sewage treatment processes, by spreading it on land, causes environmental hazards and poses serious environmental concerns due to the presence of certain soil contaminants such as organic compounds, heavy metals, and human pathogens. Beneficial sewage sludge recycling for land application is usually the most convenient and cost-effective disposal option. Farmers can reduce their use of inorganic fertilizer by recycling sewage sludge for agricultural purposes. However, additional stabilization treatment of sewage sludge is absolutely necessary prior to agronomic use [1].

Sewage sludge (SS) is rich in organic matter and nutrients required by plants [2], and it can help increase crop yield and improve soil quality [3,4]. However, various hazardous contaminants in SS, such as potentially toxic elements (PTEs) (As, Cd, Cr, Cu, Ni, Pb, and Zn), can be harmful to the environment [5,6], and because these metals are not volatile, they are difficult to remove from waste. These elements' toxicity, as well as the risk of bioaccumulation in the food chain, are major environmental health concerns.

Earthworms are an important indicator of ecosystem health, and many studies on their response to toxic metals have been conducted [7]. Earthworms are important process drivers because they improve aeration and fragment the substrate, which leads to increased microbial activity [8]. Earthworms ingest, grind, and digest the organic waste in their gut with the help of aerobic and anaerobic microflora, converting it into much finer, humified, microbially active material [9].

Vermicomposting is the process of earthworms and microorganisms working together to bio-oxidize and stabilize organic materials, although microorganisms are in charge of the biochemical degradation of organic waste. On the other hand, vermicomposting could be viewed as a major environmental sink for the removal of PTEs. Under aerobic conditions, the combined action of earthworms and microorganisms can effectively convert most organic components into valuable products rich in nitrogen, phosphorus, potassium, and humic substances [6]. Many researchers have looked at vermicomposting with various organic wastes, including animal dung [6], plant waste [10], municipal solid waste [11], and sewage sludge [10].

Various bulking agents are used as amendment materials during vermicomposting. Plant wastes such as soybean husk [12] and rice husk [13] can be used in some cases. Organic wastes with low carbon content can be mixed with lignocellulosic materials to improve the C/N ratio [14], and fly ash can be used for further stabilization [15].

Some studies found a decrease in PTEs content after vermicomposting [16–18], which was likely due to PTE bioaccumulation in earthworm tissues during vermicomposting; however, other studies found a clearly higher total content [6,19], which could be due to organic matter decomposition.

The percentage of PTEs that are water-soluble or exchangeable is thought to be the most dangerous to plants and humans. Vermicomposting dramatically reduces the exchangeable proportion of initial raw materials, greatly sequesters the water-soluble ions, and turns them into the residual fraction, as most studies focused on this topic indicate [6] (e.g., Cr was decreased by 35.5% and 22.2% in cow dung and pig manure after vermicomposting respectively, and Cu was decreased by 56.4% after vermicomposting of cow dung for 60 days). The PTEs were sequestered due to earthworms' mineralization and humification effects and microbes, which reduced them to an inert fraction. Notably, the organic wastes used in all PTE vermicomposting were either cow dung or green waste, and the PTE pollution generated by sewage sludge is far worse than that caused by these items [6].

As a result, selecting the appropriate organic waste or additives is critical to the success of the vermicomposting process. Hence, the research presented here is focused on the content of PTEs during sewage sludge vermicomposting at different mass ratios of sewage sludge as the co-substrate in the mixture with the bulking agent (PWS), which could be an important factor that influences the mineralization of bio-waste during the processes [10]. There is very little literature on the reduction of potentially toxic elements during sewage sludge vermicomposting with different proportions of bulking agent (pelletized wheat straw), which is the key factor for using sewage sludge as vermicompost. Therefore, the objectives of this study were to (a) evaluate the content of PTEs (As, Cd, Cr, Cu, Pb, and Zn) during the vermicomposting of sewage sludge in varying proportions with the bulking agent (PWS) and (b) the content of the PTEs in earthworm tissues with the aim of evaluating the ability of earthworms to remove monitored elements from sewage sludge during vermicomposting. It was hypothesized that (i) different mixing mass ratios of bulking agent (PWS) reduced the PTEs among variants, and (ii) earthworms (*Eisenia andrei*) reduced the PTEs among variants during vermicomposting.

2. Materials and Methods

2.1. Initial Raw Materials

For the study, unstabilized sewage sludge and bulking agent (straw pellets) were mixed with water. Sewage sludge from a municipal sewage treatment plant was used. This plant operates with mechanical-biological technology. The mixture of primary and secondary sludge was dewatered by a sludge belt press. Subsequently, half a ton of sludge was taken from different parts of the pile to get a representative sample. It was stored at 4°C for one week and then used for the experiment without any other treatment. A dried pelletized wheat straw (PWS) with a diameter of 10 mm was provided by Granofyt Ltd. Company (Chrášťany, Czechia). Dry straw pellets were mixed with hot water at a rate of 4 L per 1 kg of straw pellets. The wet pellets were added to the sludge after they had been mixed. To achieve good mixing, raw materials were not always mixed in full batches but rather in smaller batches. These were combined, mixed, and then combined again. The experiment was carried out at the Czech University of Agriculture Research Station in Červený Újezd, with samples subsequently analyzed at the Czech University of Life Science laboratories in Prague. The selected physicochemical properties of the initial materials are presented in Table 1.

Table 1. Physico-chemical properties of SS and PWS used in the experiment (±sd).

Parameters	SS	PWS
Dry matter (%)	13.3 ± 0.19	21.2 ± 0.56
pH-H_2O	6.9 ± 0.03	8.3 ± 0.52
EC(mS/cm)	0.6 ± 0.11	0.68 ± 0.07
TC (%)	32.9 ± 0.26	42.6 ± 0.36
TN (%)	5.4 ± 0.03	0.80 ± 0.12
C/N	6.1 ± 0.04	53.7 ± 7.60
PTEs (mg kg^{-1} DW)		
As	12.52 ± 1.20	(<0.03)
Cd	0.38 ± 0.02	(<0.001)
Cr	47.80 ± 6.45	0.70 ± 0.15
Cu	95.33 ± 8.12	1.49 ± 0.14
Pb	9.76 ± 1.04	(<0.02)
Zn	506.1 ± 42.90	8.05 ± 1.51

SS = Sewage sludge, PWS = Pelletized wheat straw.

2.2. Experimental Setup

Vermicomposting

All mixtures were pre-composted in 70-L laboratory reactors and kept in a room at 25 °C for 14 days. An active aeration device was used to push air through the composted materials from the bottom. The mixtures were discontinuously aerated for 5 min every half hour at a rate of 4 L of air per minute. On the basis of their previous experience, Hanc et al. [20] found that this aeration level was usually sufficient to achieve the optimal parameters of the composting process. Pre-composting was performed before vermicomposting to stabilize the material, inactivate pathogenic microorganisms, and decrease the high NH_3 content that was toxic to the worms. The initial SS was mixed in triplicate with varying proportions of pelletized wheat straw (0%, 25%, 50%, and 75% (*w*/*w*)) along with a control (100% SS, no earthworms), and the variants were named VC1, VC2, VC3, VC4, and C0 (control), respectively, after being pre-composted for 14 days. To avoid earthworm mortality and to allow earthworms to return to suitable conditions, the substrate (3L grape marc) containing earthworms was placed into the tray from the side. The earthworms were bought from a culture bank at the Filip and Filip farm, Czech Republic. Grape marc was used as feed for earthworms. The variants (VC1, VC2, VC3, VC4, and C0) were transferred to worm bins (40 × 40 × 15 cm) for vermicomposting in a specially adapted laboratory with controlled conditions (temperature 23 °C, relative humidity 80%) and the moisture level of the material was maintained at about 70–80% of the wet mass throughout the vermicomposting stage by spraying the surface with water at two-day intervals for 120 days. Except for control, each worm-bin received 377 pieces of adult earthworms, *Eisenia andrei* (57.4 g) per variant, with the initial average weight and number of earthworms being 19.13 g/L and 126 pieces/L of the substrate, respectively. The moisture level of the material was maintained at about 70–80% of the wet mass throughout the vermicomposting stage by spraying the surface with water at two-day intervals.

On days 0, 30, 60, 90, and 120, representative vermicompost samples of about 150 g wet basis per variant were taken and then freeze-dried at −25 °C, subsequent lyophilization, and ground for the analysis of the content of PTEs (As, Cd, Cr, Cu, Pb, and Zn), total nitrogen (TN), and total carbon (TC), whereas a sample of 200 g was collected from each worm-bin for dry matter determination, and 30 g of sample was cooled at 4 °C for pH and EC determination. The selected chemical properties and contents of PTEs of variants on the initial day (day 0) are listed in Table 2.

Table 2. Contents of PTEs and selected chemical properties of variants on the initial day (day 0).

Variants	mg kg^{-1}					
	As	Cd	Cr	Cu	Pb	Zn
VC1	12.5 ± 1.20	0.38 ± 0.02	47.8 ± 6.45	95.3 ± 8.12	9.8 ± 1.04	506.1 ± 42.9
VC2	9.9 ± 0.90	0.30 ± 0.01	36.0 ± 4.86	71.9 ± 6.06	7.7 ± 0.70	381.6 ± 32.27
VC3	7.2 ± 0.60	0.22 ± 0.01	24.3 ± 3.26	48.4 ± 3.99	5.7 ± 0.35	257.1 ± 21.65
VC4	4.5 ± 0.30	0.14 ± 0.00	12.5 ± 1.67	24.9 ± 1.93	3.7 ± 0.07	132.6 ± 11.05
Variants	pH-H_2O	EC (mS/cm)	TC (%)	TN (%)	C/N ratio	
VC1	6.9 ± 0.03	0.617 ± 0.11	32.9 ± 0.26	5.36 ± 0.03	6.14 ± 0.04	
VC2	7.3 ± 0.11	0.633 ± 0.08	35.36 ± 0.23	1.98 ± 0.21	18.03 ± 1.92	
VC3	7.6 ± 0.25	0.649 ± 0.06	37.77 ± 0.24	1.34 ± 0.07	28.17 ± 1.43	
VC4	7.9 ± 0.38	0.664 ± 0.05	40.18 ± 0.29	1.05 ± 0.05	38.36 ± 2.03	

VC1 = (100% SS, VC2 = (75% SS + 25% PWS), VC3 = (50% SS + 50% PWS), and VC4 = (25% SS + 75% PWS; w/w), (n = 3). ***NB***: Control (C0) produced the same results as VC1 because it was tested before being mixed with earthworms.

2.3. Analysis of Selected Chemical Properties and Content of PTEs

The following chemical parameters and content of PTEs were analyzed: pH, electrical conductivity (EC), total nitrogen (TN), total carbon (TC), and content of PTEs (As, Cd, Cr, Cu, Pb, and Zn). The pH-H_2O and the electrical conductivity (EC) were analyzed using a WTW pH 340i and a WTW cond 730 (1:5 w/v dry basis), according to BSI EN 15933 [21]. Decomposition using wet digestion, HNO_3 (65%) w/v) + H_2O_2 (30% w/v) (Suprapure, Merck), was used to determine the content of PTEs (As, Cd, Cr, Cu, Pb, and Zn) in the vermicompost and earthworms. After being separated from the samples, earthworms were manually counted. They were then washed and weighed to determine their biomass and the contents of PTEs by decomposition using wet digestion (65% HNO_3 + 30% H_2O_2). An Ethos 1 system was used in a closed system with microwave heating (MLS GmbH, Germany). The content of PTEs was determined using inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia) with an axial plasma configuration (As, Cd, Cr, Cu, Pb, and Zn). After being separated from the samples, earthworms were manually counted. They were then washed and tested for PTE content by decomposition using wet digestion.

The reduction or increase in percentage(Y) of each variant was calculated for the content of all PTEs using the following equation [22].

$$Y(\%) = \frac{Ci - Cf}{Ci} \times 100 \quad (1)$$

where Ci is the content of PTEs in the initial variants (mg kg^{-1}), and Cf denotes the same for the final content of PTEs after 120 days of vermicomposting.

Due to PTE bioavailability, the bio-concentration process results in high concentrations of the corresponding PTEs in earthworms. Bioconcentration refers to the process by which a chemical species accumulates in earthworms from its surrounding phases. Mountouris et al. [23] estimated PTE accumulation in earthworm tissues using the bioconcentration factor (BCF), as shown in [24].

$$\mathrm{BCF} = \frac{C(earthworm)}{C(substrate)} \quad (2)$$

where C (*earthworm*) and C (*substrate*) were the total contents of PTEs in earthworms and the substrate used for vermicomposting experiments, respectively, in mg kg^{-1}. Certified reference materials (CRMs) of Standard Reference Material® 1570a trace elements in spinach leaves were digested in replications alongside samples and blanks for quality assurance (QA) and quality control (QC) [25].

2.4. Statistical Analyses

The statistical analyses were performed with the R statistical package, version 4.0.2. An ANOVA was used to test the significant sources of variation, and the Shapiro–Wilk test was used to compare the variant's means if the factors' effect was significant at $p < 0.05$. The data distributions were examined, and the data were found to be normally distributed. Pearson correlation coefficients were used to analyze the relationships between variables.

3. Results and Discussion

3.1. Contents of Potentially Toxic Elements (PTEs) in Vermicompost

The vermicomposting processes had a significant impact on the PTE content of each variant. As shown in Figure 1, there were statistically significant differences in the contents of PTEs (As, Cd, Cr, Cu, Pb, and Zn) among the variants (C0, VC1, VC2, VC3, and VC4). As ($F = 35.05$, $p < 0.001$), Cd ($F = 11.04$, $p < 0.01$), Cu ($F = 26.8$, $p < 0.001$), Pb ($F = 18.7$, $p < 0.001$), Zn ($F = 34.7$, $p < 0.001$), Cr ($F = 6.05$, $p < 0.05$), and the PTEs of final vermicompost material ranges were: As (7.7–20.2 mg kg^{-1}), Cd (0.44–0.69 mg kg^{-1}), Cr (17.40–104.2 mg kg^{-1}), Cu (49.06–124.2 mg kg^{-1}), Pb (4.13–10.02 mg kg^{-1}), Zn (313.4–738.5 mg kg^{-1}) (dry basis) (Table 3).

Metals in the feed materials were directly and indirectly incorporated with earthworm gut enzymes during vermicomposting, which could explain the increase in PTEs. Metals are liberated in free form as a result of the enzymatic action in earthworm guts [26]. Metal-binding to organic matter is more tightly bound, according to Lukkari et al. [27], which reduces metal availability for earthworms. As a result, the PTE content in vermicompost was higher than the initial contents (Figure 1, Table 3). The PTE content of variants was lower than that of control (C0) (Figure 1), and the percentage of reduction with respect to C0 were: As (14–67%), Cd (4–39%), Cu (20–68%), Cr (24–77%), Pb (39–75%), and Zn (16–65%) (Table 3). In terms of removal rate versus C0, the sludge mixtures with bulking agent PWS (i.e., the variants) can be arranged in the following order: VC4 > VC3 > VC2 > VC1. The two pathways that may influence PTE content during the vermicomposting process are earthworm bioaccumulation and volume reduction caused by organic decomposition. During vermicomposting, mineralization and organic matter decomposition may concentrate and increase the PTE content [28,29]. Because vermicomposting had a higher organic degradation rate than the control in this study, it should contain fewer PTEs.

Furthermore, earthworms accumulate metals in their tissues, which reduce the PTE content of vermicompost [30,31]. All PTEs in this study had higher content than the initial contents of vermicompost. However, all vermicompost produced met European Union (EU) compost quality standards ranges [32,33], and this implies that these materials are suitable for agricultural use (Table 4). Our results were relatively low when compared to [32,34,35]. It has been proposed that the decrease in PTE is due to earthworm bioaccumulation within their tissue via gut/skin absorption [36], and with overloaded metal burdens, the earthworm tissues tend to decompose, making these elements even more available [37].

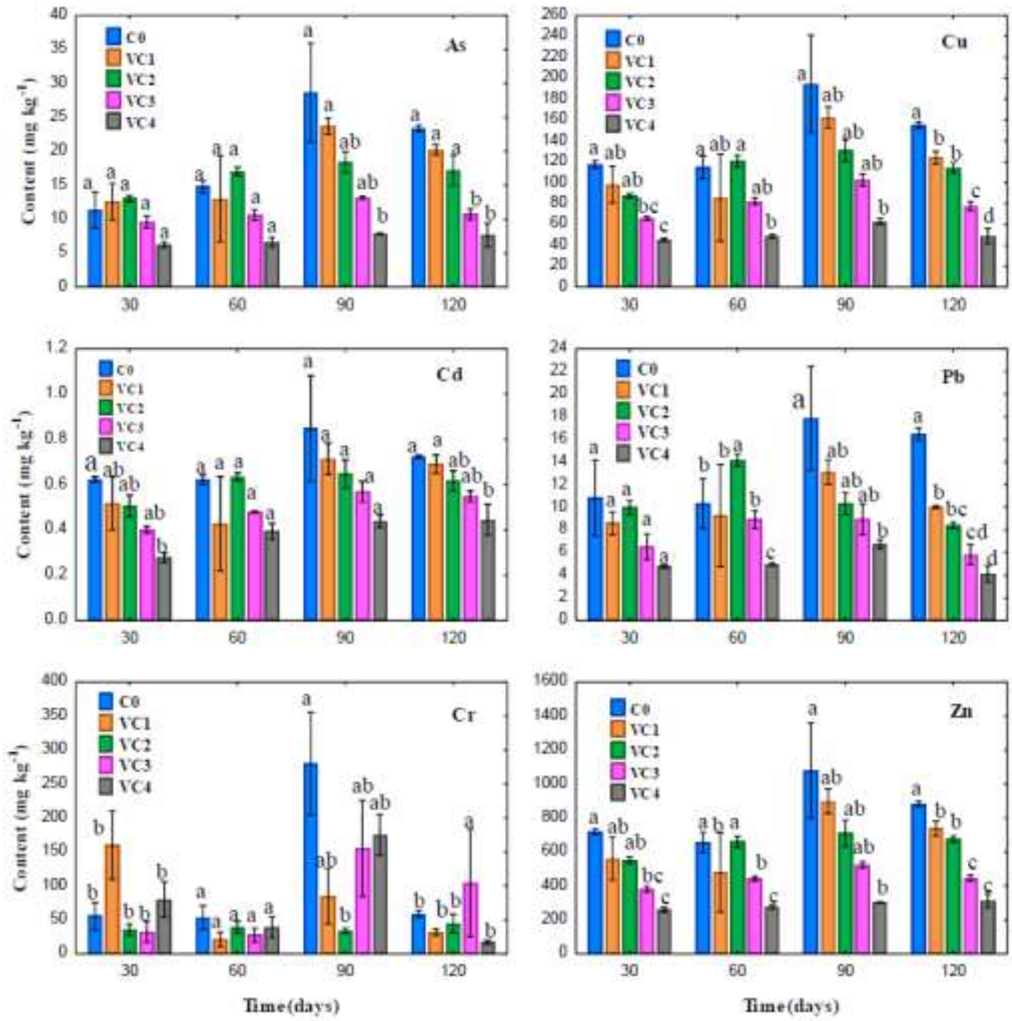

Figure 1. Variations of PTE content during vermicomposting. The bars indicate the standard deviation of the mean (n = 3). Different letters indicate significant differences among the variants ($p < 0.05$).

3.2. Contents of PTEs in Earthworm Tissues and Bio-Concentration Factor (BCF)

As shown in Figure 2, the content of PTEs (As, Cd, Cr, Cu, Pb, and Zn) in earthworm tissues increases with vermicomposting time. The variation of all PTE contents in earthworm tissues was significantly increased: As (F = 24.5, $p < 0.001$), Cd (F = 8.6, $p < 0.0$), Cr (F = 83.3, $p < 0.001$), Cu (F = 8.1, $p < 0.01$), Zn (F = 5.46, $p < 0.05$). However, Pb was recorded below the detection limit (<0.02 mg kg^{-1}), which indicated the bioaccumulation of PTEs by worms. At the end of vermicomposting, the PTE levels in worm tissues were: As (25.9–47.9 mg kg^{-1}), Cd (0.65–0.86 mg kg^{-1}), Cr (1.9–3.2 mg kg^{-1}), Cu (20–29.9 mg kg^{-1}), and Zn (151.4–187.3 mg kg^{-1}). Significantly, the highest contents of As (47.91 mg kg^{-1}) and Cd (0.854 mg kg^{-1}) were found in variant VC3 at the end of vermicomposting (120 days), whereas Cr (3.42 mg kg^{-1}), Cu (29.95 mg kg^{-1}), and Zn (196.93 mg kg^{-1}) were found in variant VC1 at 30, 120, and 90 days, respectively. At 30 days of vermicomposting, variant VC4 had the lowest PTE content (except for Cd): As (6.09 mg kg^{-1}), Cr (0.54 mg kg^{-1}), Cu (9.04 mg kg^{-1}), and Zn (109.99 mg kg^{-1}).

Table 3. Heavy metal content and heavy metal mass balance over 120 days.

Variants	Initial (0 Day)	Final (120 Days)	Increment/Removal (%)	Reduction with Respect to Control (%)
		As (mg kg^{-1})		
C0	12.5 ± 1.20	23.38 ± 0.77a	87	-
VC1	12.5 ± 1.20	20.22 ± 1.31a	62	−14
VC2	9.9 ± 0.90	17.16 ± 3.81a	73	−27
VC3	7.2 ± 0.60	10.72 ± 1.36b	49	−54
VC4	4.5 ± 0.30	7.72 ± 3.03b	72	−67
		Cd (mg kg^{-1})		
C0	0.38 ± 0.02	0.72 ± 0.01a	89	-
VC1	0.38 ± 0.02	0.69 ± 0.07a	82	−4
VC2	0.30 ± 0.01	0.62 ± 0.08ab	107	−14
VC3	0.22 ± 0.01	0.55 ± 0.04ab	150	−24
VC4	0.14 ± 0.00	0.44 ± 0.12b	214	−39
		Cr (mg kg^{-1})		
C0	47.8 ± 6.45	58.82 ± 8.60b	23	-
VC1	47.8 ± 6.45	32.13 ± 8.65b	−33	−45
VC2	36.0 ± 4.86	44.91 ± 23.90b	25	−24
VC3	24.3 ± 3.26	104.22 ± 79.15a	329	77
VC4	12.5 ± 1.67	17.40 ± 5.32b	39	−70
		Cu (mg kg^{-1})		
C0	95.3 ± 8.12	154.88 ± 5.71a	63	-
VC1	95.3 ± 8.12	124.20 ± 10.54b	30	−20
VC2	71.9 ± 6.06	114.28 ± 8.52b	59	−26
VC3	48.4 ± 3.99	77.68 ± 7.61c	60	−50
VC4	24.9 ± 1.93	49.06 ± 12.38d	97	−68
		Pb (mg kg^{-1})		
C0	9.8 ± 1.04	16.46 ± 1.00a	68	-
VC1	9.8 ± 1.04	10.02 ± 0.20b	2	−39
VC2	7.7 ± 0.70	8.40 ± 0.50bc	9	−49
VC3	5.7 ± 0.35	5.83 ± 1.55bc	2	−65
VC4	3.7 ± 0.07	4.13 ± 1.26bc	12	−75
		Zn (mg kg^{-1})		
C0	506.1 ± 42.9	883.13 ± 28.33a	74	-
VC1	506.1 ± 42.9	738.47 ± 74.64b	46	−16
VC2	381.6 ± 32.27	676.11 ± 31.99b	77	−23
VC3	257.1 ± 21.65	446.08 ± 30.00c	74	−49
VC4	132.6 ± 11.05	313.42 ± 72.23c	136	−65

C0 = control (100% SS, no earthworms) VC1 = (100% SS, VC2 = (75% SS + 25% PWS), VC3 = (50% SS + 50% PWS), and VC4 = (25% SS + 75% PWS; *w*/*w*).

Table 4. PTEs in vermicompost (final day—120) compared to EU compost limits.

PTEs	PTE Content in Vermicompost (mg kg^{-1} DW)	* EU Range (mg kg^{-1} DW)
As	7.7(VC4)–20.2(VC1)	5–50
Cd	0.44(VC4)–0.7(VC1)	0.7–10
Cr	17.4(VC4)–104.2(VC3)	70–200
Cu	49.1(VC4)–124.2(VC1)	70–600
Pb	4.1(VC4)–10.0(VC1)	70–1000
Zn	313.4(VC4)–738.5(VC1)	210–4000

* Potentially toxic elements standards for EU: Source [32,33]. VC1 = (100% SS, VC2 = (75% SS + 25% PWS), VC3 = (50% SS + 50% PWS), and VC4 = (25% SS + 75% PWS; *w*/*w*).

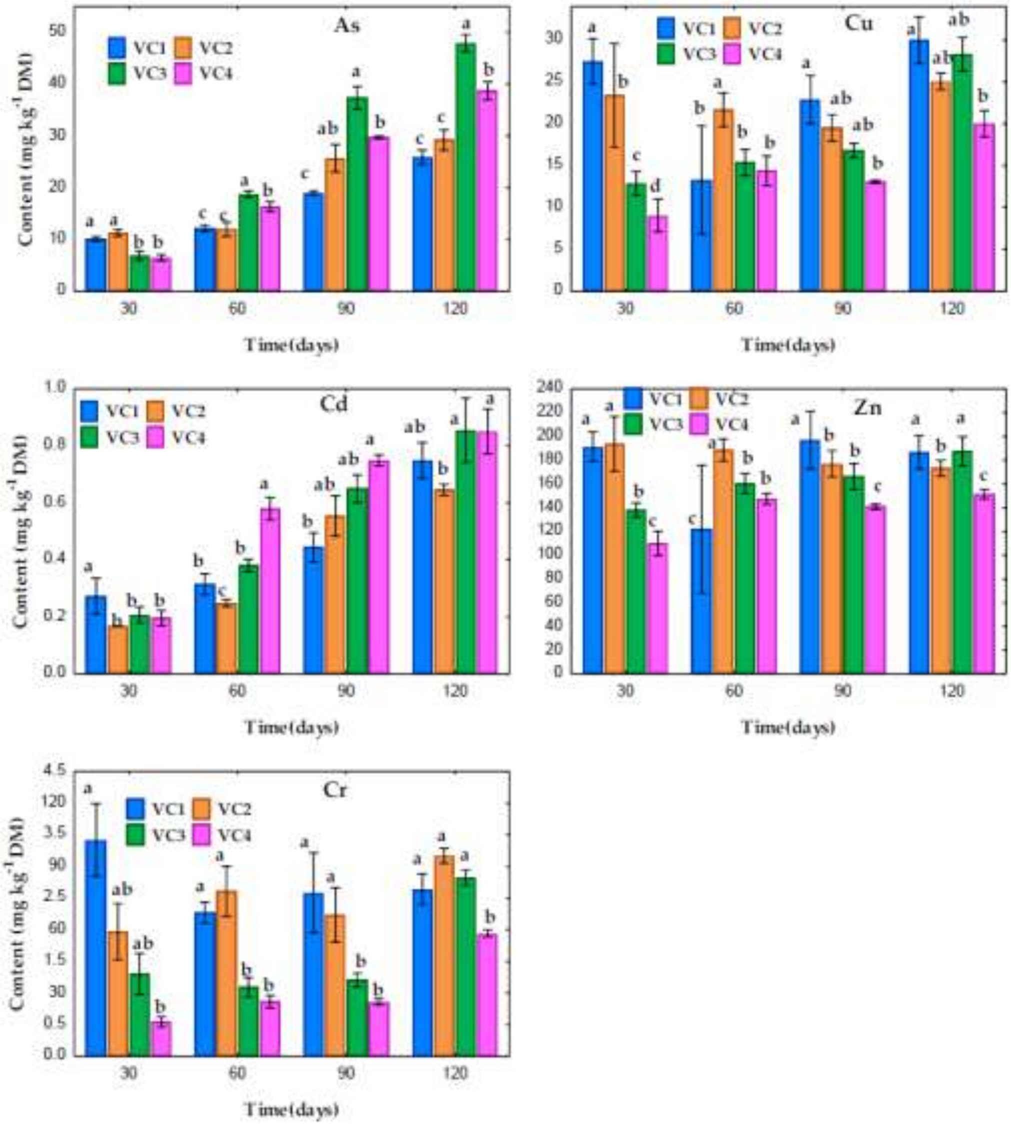

Figure 2. Variations of PTEs content in earthworm tissues. The bars indicate the standard deviation of the mean ($n = 3$). Different letters indicate significant differences among the variants ($p < 0.05$).

The higher PTE content in earthworm tissues clearly indicates that PTEs have accumulated in earthworms from their inhabiting substrate. However, there was a consistent trend of higher metals in the tissues of earthworms, those collected from variants with a higher proportion of sewage sludge, e.g., variant VC1 and variant VC2 for Cr and Zn, whereas in variant VC3 and VC4 for As, Cd, and Cu with a higher proportion of additive material PWS (Figure 2). To obtain adequate nutrition, earthworms consume a large amount of organic waste, and PTEs are liberated in free forms during this process as a result of enzymatic actions in their gut [26]. Additionally, PTEs are absorbed by the gut epithelial layer during waste transit [38]. According to Suthar et al. [39], earthworms accumulate a significant amount of PTEs in their tissues and may be a useful biological indicator of contamination due to fairly consistent relationships between the contents of certain contaminants in earthworms. However, several studies suggest that the earthworm's interaction with local edaphic factors such as pH, organic matter content, and so on is largely responsible for PTE accumulation [39,40]. Lukkari et al. [27] stated that the binding of metals to organic matter partly reduces the availability of PTEs for earthworms.

According to Nahmani et al. [7], the rate of accumulation and excretion varies by metal, with As and Cd demonstrating rapid uptake and equilibration but little uptake for Cr, Cu, Pb, and Zn. Pb is below the detection limit (<0.02 mg kg^{-1}) in all variants. It has been suggested that part of the reason for the increase in As and Cd content and mobility is due to the bioaccumulation of earthworms within their tissue through gut/skin absorption [36] and that with overloaded metal burdens, the earthworm tissues tend to decompose, rendering these elements with an even higher availability [37]. The PTE contents in vermicompost and tissue in this study are essentially consistent with the total content in the feeding mixtures. It demonstrates that, for a limited time, earthworms do not pose an ecological risk of higher food chain contamination, as previous work by [41] on *Eisenia fetida* in municipal sewage sludge vermicomposting demonstrated. This study suggests that inoculating the substrate with *Eisenia andrei* reduced the PTEs in the substrate during vermicomposting, but ecologically, a longer period of vermicomposting should be considered to eliminate the roles of earthworms as PTEs transfer mediators to possible higher food chain contamination due to the earthworms' PTE excretion period. Despite this, different species have different excretion periods, metabolic physiology, and palatability, indicating that more research is required.

Other biochemical parameters that may be relevant include enzymatic action, a mechanism for mobility and availability of PTEs concerning the content of pore water (moisture content), and microbial colonization to determine the PTE content incorporated into the process. This, however, requires further experimental confirmation. Thus, it is concluded that during the vermicomposting period, the earthworms reached the excretion period when the accumulated PTEs were ingested by the earthworms' bodies.

The assimilation of PTEs into earthworm tissues can be quantified using the bio-concentration factor (BCF) [23]. There were statistically significant variations among variants for BCF calculated for As ($F = 22.29$, $p < 0.000$), Cd ($F = 24.21$, $p < 0.0002$), Cr ($F = 16.19$, $p < 0.0009$), Cu ($F = 25.32$, $p < 0.0002$), and Zn ($F = 54.40$, $p < 0.001$). The BCFs varied from 2.09 (VC1) to 8.60 (VC4) for As, 1.97 (VC1) to 5.95 (VC4) for Cd, 0.06 (VC1) to 0.16 (VC4) for Cr, 0.32 (VC1) to 0.80 (VC4) for Cu, and 0.37 (VC1) to 1.15 (VC4) for Zn (Table 5). The accumulation rate (BCF) was calculated as follows: As > Cd > Zn > Cu > Cr > Pb. In terms of removal rate, the sludge mixtures with bulking agent PWS (i.e., the variants) can be arranged in the following order: VC4 > VC3 > VC2 > VC1 (Table 5). PTE accumulation in earthworms is aided by metallothioneins (MTs), which are protein–metal complexes with a low molecular weight. Hopkin [42] proposed that earthworms have a unique ability to regulate metals, particularly PTEs, and that metal-specific accumulation and regulation mechanisms exist. The results indicate that carbon mineralization in the sludge mixture during the vermicomposting system improves PTE bioavailability in sludge.

Table 5. Bio-concentration factors (BCF) for PTEs after 120 days of vermicomposting.

	Bio-Concentration Factors (BCF) for PTEs					
Variants	BCF_{As}	BCF_{Cd}	BCF_{Cr}	BCF_{Cu}	BCF_{Pb}	BCF_{Zn}
VC1	2.09 ± 0.41c	1.97 ± 0.37c	0.06 ± 0.01c	0.32 ± 0.07c	-	0.37 ± 0.07c
VC2	2.97 ± 0.32c	2.14 ± 0.19c	0.09 ± 0.01bc	0.35 ± 0.03c	-	0.46 ± 0.04c
VC3	6.69 ± 0.17b	3.85 ± 0.95b	0.12 ± 0.02ab	0.59 ± 0.11b	-	0.74 ± 0.14b
VC4	8.60 ± 0.46a	5.95 ± 0.79a	0.16 ± 0.03a	0.80 ± 0.09a	-	1.15 ± 0.05a

VC1 = (100% SS, VC2 = (75% SS + 25% PWS), VC3 = (50% SS + 50% PWS), and VC4 = (25% SS + 75% PWS; *w*/*w*). Mean values followed by different letters are statistically different (ANOVA; Tukey's test, $p < 0.05$), and the values indicate the mean ± standard deviation ($n = 3$).

The findings are consistent with [15,28], which reported that organic matter content has a direct role in metal mobility and availability in end material during the vermicomposting/composting process. The reduction of TC causes the formation of intermediate metabolites and acids (humic acids), which lowers the pH of the sludge mixtures. In general, metal accumulation in tissues is a metal-specific phenomenon, with each metal having its own physiological mechanism of assimilation and/or excretion during its metabolism in the earthworm's gut.

3.3. Earthworm Evolution (Biomass, Number, Growth, and Survival) during Vermicomposting

After 30 days, earthworm biomass (g) ($F = 15.03$, $p = 0.0012$) and the number of earthworms ($F = 24.3$, $p = 0.0002$; Table 6) showed significant differences among variants. However, earthworm biomass (g) ($F = 0.448$, $p = 0.73$) and the final number of earthworms ($F = 0.448$, $p = 0.73$) were not significantly different after 120 days.

Table 6. Earthworm evolution (biomass, number, growth, and survival) during vermicomposting.

		Biomass of Earthworm (g/Variants)				Biomass Gain/Loss (%)			
Variants	**Initial**	**Day 30**	**Day 60**	**Day 90**	**Day 120**	**Day 30**	**Day 60**	**Day 90**	**Day 120**
VC1	57	7 ± 1.23b	35 ± 12.08a	18 ± 3.74a	18 ± 2.11a	−88	−39	−69	−69
VC2	57	5 ± 3.91b	39 ± 10.33a	24 ± 7.66a	14 ± 2.51a	−91	−32	−58	−75
VC3	57	20 ± 3.76ab	30 ± 1.25a	21 ± 1.29a	14 ± 3.70a	−65	−48	−63	−75
VC4	57	35 ± 4.59a	26 ± 1.14a	24 ± 0.90a	13 ± 4.79a	−39	−55	−58	−77
		Number of earthworms				Earthworm number gain/loss (%)			
Variants	Initial	Day 30	Day 60	Day 90	Day 120	Day 30	Day 60	Day 90	Day 120
VC1	377	21 ± 4.37c	101 ± 42.18c	51 ± 9.33c	61 ± 6.96a	−94	−73	−86	−84
VC2	377	29 ± 10.91bc	165 ± 48.20a	91 ± 26.69b	74 ± 12.49a	−92	−56	−76	−80
VC3	377	80 ± 16.17b	159 ± 11.10a	110 ± 18.00b	69 ± 17.02a	−79	−58	−71	−82
VC4	377	143 ± 10.98a	143 ± 21.74b	131 ± 20.83a	80 ± 21.20a	−62	−62	−65	−79

VC1 = (100% SS, VC2 = (75% SS + 25% PWS), VC3 = (50% SS + 50% PWS), and VC4 = (25% SS + 75% PWS; *w*/*w*). Mean values followed by different letters are statistically different (ANOVA; Tukey's test, $p < 0.05$), and the values indicate the mean ± standard deviation ($n = 3$).

Other growth parameters, such as biomass gain and loss (percent; $F = 15.0$, $p = 0.0012$) and number gain and loss (percent; $F = 24.3$, $p = 0.0003$), also revealed statistical differences on day 30. However, there were no significant differences in biomass gain and loss (percent; $F = 0.45$, $p = 0.72$) or number gain and loss (percent; $F = 0.43$, $p = 0.74$) on the final day. The highest earthworms' rate of change in biomass (g) was observed on day 60 in the following order: VC2 > VC1 > VC3> VC4, whereas the highest earthworms' number was recorded on day 60 in the order VC2 > VC3 > VC4 > VC1, and VC1 recorded a mortality rate of 94% on day 30 of vermicomposting, whereas the highest percentage of 91% loss of biomass was recorded in variant VC2 on day 30 (Table 6).

The increasing percentage of SS in the variants resulted in a decrease in biomass and the number of earthworms, which was consistent with previous work on municipal sewage sludge vermistabilization amended with sugarcane trash using *Eisenia foetida* [41]. This finding is consistent with the findings of Gupta and Garg [43], who used primary SS in vermicomposting with *Eisenia foetida* and observed a decrease in biomass gain with higher primary SS composition. Furthermore, previous research found that increasing the percentage of SS promoted a decrease in the biomass and number of *L. rubellus* [44]. Yadav and Garg [45] concluded that the rate of food consumption during worm acclimatization in waste mixtures affects the survival rate of earthworms.

Changes in the chemical composition of feed, changes in the pH of the substrate, a higher C:N ratio of the initial substrate, and the production of toxic or foul-smelling gases (ammonia, carbon dioxide, nitrogen oxides, and so on) may all be factors in earthworm mortality [46]. Increases in earthworm multiplication and growth may have resulted from increased consumption and an abundance of food in the vermibeds (biomass gain). This also implies that the palatability and quality of food (in terms of its chemistry) have a direct impact on earthworm survival, growth rate, and reproduction potential [47,48].

Earthworm growth and reproduction are used to assess the suitability of a substrate as feed in the vermicomposting process. Earthworms survived less in the variant containing 100% SS in this study as compared to the other variants. Some worms died during the first days of the variant containing 25% SS + 75% PWS mixture. According to Flegel and

Schreder [46], earthworm survival is also dependent on food availability and the production of odorous gases such as ammonia and carbon dioxide during initial degradation.

3.4. Change in Selected Chemical Properties (pH, EC, TC, TN, C/N ratios) during Vermicomposting

Table 7 shows the pH and EC variations of variants during vermicomposting. The pH of all variants (VC1, VC2, VC3, and VC4) decreased significantly during the vermicomposting period (F = 19.28, $p < 0.001$). A similar decrease in pH behavior was observed during the vermicomposting of sewage sludge, crop straw, municipal solid waste, and livestock manure [49–51]. The release of low molecular weight organic acids from organic decomposition, as well as an increase in nitrification, may cause vermicomposting pH to fall [52,53]. The pH difference between variants, according to Singh and Suthar [49], may reflect the degree of organic mineralization.

Table 7. Selected chemical properties of the end-product vermicompost (day 120).

Variants	pH-H_2O	EC (mS/cm	TC (%)	TN (%)	C/N Ratio
VC1	5.7 ± 0.49a	2.16 ± 0.26a	28.32 ± 2.20b	3.19 ± 0.89a	8.11 ± 1.64b
VC2	5.2 ± 0.11a	2.10 ± 0.11a	28.85 ± 0.39ab	2.89 ± 0.07a	9.08 ± 0.35ab
VC3	6.0 ± 0.45a	2.25 ± 0.14a	31.86 ± 0.63ab	2.82 ± 0.12a	10.5 ± 0.96ab
VC4	5.8 ± 0.18a	2.28 ± 0.18a	34.64 ± 0.17a	3.05 ± 0.09a	11.17 ± 0.15a

VC1 = (100% SS, VC2 = (75% SS + 25% PWS), VC3 = (50% SS + 50% PWS), and VC4 = (25% SS + 75% PWS; *w*/*w*). Mean values followed by different letters are statistically different (ANOVA; Tukey's test, $p < 0.05$), and the values indicate the mean ± standard deviation ($n = 3$).

During vermicomposting, the EC of all variants increased significantly (F = 0.36, $p < 0.05$), as shown in Table 7. The increase in EC in vermicompost may be due to the release of inorganic ions and soluble salts, such as phosphate, ammonium, and nitrate [52,54], and this phenomenon suggests that vermicomposting could speed up the mineralization of organic matter, causing insoluble particles to become soluble. The end-of-vermicomposting EC values ranged from 2.10 to 2.28 mS/cm, indicating that all variants (VC1, VC2, VC3, and VC4) had EC levels below the recommended limit of 4 mS/cm [51] in vermicomposts and were safe for agriculture.

The total carbon (TC), total nitrogen (TN), and C/N ratios in variants are shown in Table 7. TC decreased in all variants during vermicomposting when compared to the initial results. After 120 days of vermicomposting, the reduction in TC in VC1, VC2, VC3, and VC4 was 13.9%, 18.4%, 15.6%, and 13.8%, respectively. Based on this discovery, the greatest reduction in TC was observed during vermicomposting in variant VC1. The reduction in TC was caused by microbe respiration and earthworm stabilization of organic matter [55]. Except for variant VC1, the results of TN increased during vermicomposting in all variants when compared to the initial results. The increase in TN in VC2, VC3, and VC4 after 120 days of vermicomposting was 31.5%, 52.5%, and 65.6%, respectively. However, TN was reduced by 68% in VC1 after 120 days of vermicomposting. During vermicomposting, all variants differed significantly (F = 35.72, $p < 0.001$ for TN, F = 11.93, $p < 0.001$ for TN, F = 55.40, $p < 0.001$). Pigatin et al. [56] discovered that during vermicomposting of various agricultural residues, TN increased by 19.5 to 150%, tea prunings by 30.5–51.29% [57], and vermicomposts made from textile mill sludge mixed with cow dung and agricultural residues contained 2–3.2 times more nitrogen than initial feedstocks, according to Kaushik and Garg [58]. According to Sudkolai and Nourbakhsh [59], cow dung vermicompost had 1.6 times the TN content of feedstocks, while wheat residue vermicompost had 3.2 times the TN content of feedstocks. Higher nitrogen levels in vermicompost are most likely caused by organic carbon in the form of carbon dioxide, as well as nitrogen addition by earthworms in the form of mucus, nitrogenous excretory substances, and growth-stimulating substances. Except for variant VC1, the C/N ratio decreased during vermicomposting in all variants when compared to the initial results. After 120 days of vermicomposting, the C/N ratio in

VC2, VC3, and VC4 was reduced by 49.6%, 62.9%, and 70.9%, respectively. However, after 120 days of vermicomposting, the C/N ratio in VC1 increased by 24.3%.

Because it reflects stabilization and mineralization rates during vermicomposting [60,61], the C/N ratio indicates vermicompost maturity. The decrease in the C/N ratio over time is also due to the enhanced nitrogen content and organic matter degradation [62]. Our results are supported by previous studies [63,64], which reported up to a 50.86% and a 48.8% reduction in the C/N ratio during vermicomposting of cow dung and cow dung with vegetable waste, respectively. The final C/N ratio was calculated for all variants that had a C/N ratio less than the recommended value of 20 for soil applications [65].

3.5. Pearson Correlation Coefficient for the PTEs, BCFs, and the pH, EC, TC, TN, and C/N Ratio

A Pearson correlation coefficient (r) was calculated in order to see the effect of pH, EC, TC, TN, and C/N ratio of variants on PTE removal and BCFs (Table 8). The pH and EC had a significant relationship with Cd reduction in variants. TC had a significant relationship with BCF_{As} (r = 0.99, $p < 0.011$), BCF_{Cd} (r = 0.996, $p < 0.004$), BCF_{Cr} (r = 0.98, $p < 0.023$), BCF_{Cu} (r = 0.9998, $p < 0.000$), BCF_{Zn} (r = 0.994, $p < 0.006$), and the C/N ratio had also a significant relationship with BCF_{As} (r = 0.984, $p < 0.016$), BCF_{Cr} (r = 0.981, $p < 0.019$), and BCF_{Cu} (r = 0.942, $p < 0.044$), as shown in Table 8.

Table 8. Pearson correlation coefficient for the PTEs, BCFs, pH, EC, TC, TN, and C/N ratio.

Variable	pH	EC	TC	TN	C/N Ratio
As	0.7081	0.7674	0.7050	−0.6495	0.8222
Cd	0.9526 *	0.9744 *	0.7887	−0.0495	0.7247
Cr	−0.5475	−0.7867	−0.7745	−0.4895	−0.5643
Cu	0.1236	−0.3943	−0.7378	0.0632	−0.6978
Zn	0.1291	−0.3741	−0.6951	−0.1200	−0.6065
BCF_{As}	0.6021	0.9089	0.9893 *	−0.2938	0.9837 *
BCF_{Cd}	0.5429	0.8932	0.9960 *	−0.0897	0.9327
BCF_{Cr}	0.4069	0.8005	0.9765 *	−0.3165	0.9813 *
BCF_{Cu}	0.5807	0.9112	0.9998 *	−0.1621	0.9562 *
BCF_{Zn}	0.4887	0.8629	0.9944 *	−0.1261	0.9424

* significant at $p < 0.05$, BCF = Bio-concentration factor.

The results indicate that carbon mineralization in the sludge mixture during the vermicomposting system improves the bioavailability of PTEs in the sludge. The TC reduction leads to the formation of intermediate metabolites and acids (humic acids), which, as a result, reduce the pH of the sludge mixtures. Metal accumulation in tissues is, in general, a metal-specific phenomenon, with each metal having a distinct physiological mechanism of assimilation and/or excretion during its metabolism in the earthworm's gut.

4. Conclusions

The mixing ratio of SS and bulking agent (PWS) significantly increased the content of PTEs, according to the results. The PTEs content in earthworm tissues was also significantly increased, but the Pb content was less than the detection limit (0.02 mg kg^{-1}). There were also statistically significant differences between variants for BCF calculation. The PTE content in vermicompost was higher than the initial content. However, the PTE content of variants was lower than that of control (C0), and the percentages of reduction with respect to C0 were: As (14–67%), Cd (4–39%), Cr(24–70%), Cu (20–68%), Pb (39–75%), and Zn (16–65%). Bioaccumulation was in the order As > Cd > Zn > Cu > Cr > Pb, as calculated by BCF. In terms of removal rate, the sludge mixtures with bulking agent PWS (i.e., the variants) can be arranged in the following order: VC4 > VC3 > VC2 > VC1. The high content of PTEs in worm tissues suggests that metals are transferred from the substrate to the tissues of inoculated earthworms. The findings suggest that vermicomposting could be an appropriate technology for reducing PTEs in sewage sludge. The SS mixture with 75% of PWS (VC4) showed a better reduction of PTEs. All PTEs in this study had higher content than the initial contents of vermicompost and less than the C0. However, vermicomposts produced met European Union (EU) compost quality standards ranges, and this implies that these materials are suitable for agricultural use. Hence, it is suggested that vermicomposting reduces the content of PTEs in sewage sludge. The results suggested that vermicomposting could be an appropriate technology for the reduction of PTEs in sewage sludge.

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5.4. **Dume et al. (2023**). Influence of earthworms on the behaviour of organic micropollutants in sewage sludge.

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Influence of earthworms on the behaviour of organic micropollutants in sewage sludge

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ABSTRACT

The objective of this study was to evaluate the concentration of pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals during vermicomposting of sewage sludge using *Eisenia andrei* and in earthworm tissues with the aim of evaluating the effectiveness of earthworms to remove these substances. The experiment was carried out for 120 days with and without earthworms in varying proportions of sewage sludge in a mixture with dried straw pellets at 100, 75, 50, and 25% (w/w) of sludge. The results revealed that the earthworms had the most significant removal efficiencies on triclosan (37%) and mirtazapine (14%). Venlafaxine (193%), triclosan (43%), and citalopram (37%), had the most earthworm influence efficiency of degradation. The maximum vermiaccumulation of caffeine (72%), carbamazepine (65%), cetirizine (32%), citalopram (16%), diclofenac (183%), and triclosan (118%) was obtained. Based on these findings, earthworms show great promise in removing monitored compounds from sewage sludge during vermicomposting. However, further research is needed to optimize the process for maximum removal efficiency and confirm this approach's effectiveness.

1. Introduction

Organic micropollutants, including pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs), pose significant threats to ecosystems and human health (Thomas et al., 2020). PPCPs comprehend a wide range of substances, such as antibiotics, hormones, fungicides, disinfectants, antidepressants, and non-steroidal anti-inflammatory drugs (Jiang et al., 2023). EDCs include detergents, plasticizers, personal care products, and biocides, which can potentially interfere with hormonal systems, causing various developmental, reproductive, and behavioural disturbances (Schug et al., 2016). PPCPs and EDCs have become widespread in the aquatic environment, including surface water, sediments, and soils, where the most important primary source of these compounds usually represents wastewater (Nunes et al., 2021). PPCPs and EDCs represent bioactive substances that provide additional concern due to their hazardous bioactivity, even at very low concentrations (Hu et al., 2021).

In a wastewater treatment system, organic micropollutants are typically removed from wastewater through microbial degradation and sorption on sludge (Menon et al., 2020). For this reason, the content of PPCPs and EDCs in sewage sludge could be significant (Nunes et al., 2021). Considering the significant production of sewage sludge and its potential use as a fertilizer or soil amendment, addressing the issue of organic micropollutants in this waste material is crucial (Mazzeo et al., 2023). In the EU27, nearly 10 million tonnes of dry sludge are produced annually, with more than half of this amount applied to farmland for agricultural uses (Samaras et al., 2014). However, caution is necessary for other aspects due to the presence of PPCPs and EDCs (Buta et al., 2021) in sewage sludge. Although there are no current legislations regarding the levels of organic micropollutants in sewage sludge for agricultural use, it is essential to conduct studies related to minimizing the potential environmental and agriculture hazards, including the problems related to PPCPs and EDCs (Petrie et al., 2014).

Vermicomposting represents an environmentally friendly waste management approach that utilizes earthworms and microorganisms to convert biodegradable organic waste into valuable bio-fertilizers under

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aerobic conditions (Soobhany, 2019). At the same time, sewage sludge could be used as one of a suitable substrate for the vermicomposting process (Rorat et al., 2019). Due to its high tolerance for environmental variables such as toxic substances contained in sewage sludge, pH, moisture, and temperature, as well as its acceptance of a wide variety of feeds, a high growth rate, and the capability of converting biomass into stable products, the *epigeic* earthworm specie *Eisenia andrei* seems to be optimal for a vermicomposting process (Yadav and Garg, 2016). However, if we would like to apply vermicomposting for sewage sludge treatment, it is necessary to search for suitable co-substrates with which it will be optimal to mix the sewage sludge before the starting vermicomposting process. The straw pellets provide a favourable environment for earthworms by increasing the porosity of the composted material and allowing for better aeration and moisture retention. Suthar (2009) compared wheat straw, cow dung, and digested slurry as bulking agents in the vermicomposting of vegetable-market solid waste and found that earthworms preferred wheat straw over other materials.

Previous studies have primarily focused on measuring the concentrations of PPCPs and EDCs in the influent and effluent of wastewater treatment plants (WWTPs) (Gago-Ferrero et al., 2015). Some studies have also explored the occurrence and distribution of PPCPs and EDCs in sewage sludge (Sun et al., 2016). Selected specific previous researches have also investigated the removal efficiency of selected organic micropollutants by earthworms in soil (Shi et al., 2020). However, there is a lack of comprehensive research on the influence of earthworms on the behaviour of PPCPs and EDCs and on the potential ability of earthworms to support the removal of these pollutants during vermicomposting of sewage sludge. This study's novelty lies in its focus on the evaluation of the potential participation of earthworms in removing PPCPs and EDCs from sewage sludge during vermicomposting. This way, this study could fill the existing gap in knowledge regarding the advanced processes applicable for the minimizing of PPCPs and EDCs introduction into the environment. Consequently, reasonable development of more sustainable and effective sewage sludge management strategies could be supported. Based on the available data analysed, 12 selected concrete pollutants belonging to PPCPs and EDCs were monitored during vermicomposting process of the substrate containing the sewage sludge in different mixtures with straw pellets using *Eisenia andrei*. The targeted PPCPs and EDCs concentration was monitored in the treated substrate during the treatment process. Moreover, it was also measured in earthworm tissues to enhance the objectivity of the research.

2. Materials and methods

2.1. Initial raw materials and earthworms

The experiments used freshly deposited sewage sludge collected from a WWTP in a small town in the Czech Republic (3,500 population equivalents). The WWTP was operated on the mechanical-biological principle with an activation process applied for biological (secondary) treatment. The sludge was digested under aerobic conditions. Before being used in the experiment, it was kept at 4 °C for one week. Dried straw pellets were provided by Granofyt Ltd. Company (Chrášťany, Czechia) with a diameter of 10 mm. Because of the low moisture content of the straw pellet, it was mixed with hot water (60 °C) at a 1:4 (w/v) ratio before experimental use. Earthworms were collected from a private vermiculture stock in the Czech Republic with grape marc substrate as survival media. Of the determined organic micropollutants, only 6.05 ng g^{-1} of caffiene (CAF) and 2.24 ng g^{-1} of telmisartan (TE) were detected in earthworm tissues. The selected physicochemical properties of the initial materials and organic micropollutants before vermicomposting are presented in Table 1.

Table 1
Physicochemical properties of the initial materials and organic micropollutants before vermicomposting.

Parameters	Sewage sludge	Straw pellet	Earthworm
Dry matter (%)	13.3 ± 0.19	21.2 ± 0.56	
pH-H_2O	6.9 ± 0.03	8.3 ± 0.52	
Electrical conductivity (mS/cm)	0.617 ± 0.11	0.60 ± 0.07	
Total carbon (%)	32.9 ± 0.26	42.59 ± 0.36	
Total nitrogen (%)	5.36 ± 0.03	0.80 ± 0.12	
C/N ratio	6.1 ± 0.04	53.67 ± 7.60	
Micropollutants (ng g^{-1})			
Bisphenol A	88.78 ± 10	nd	nd
Caffeine	141.81 ± 12.5	10.23 ± 2.56	6.05 ± 0.69
Carbamazepine	38.51 ± 0.73	nd	nd
Cetirizine	78.95 ± 0.69	nd	nd
Citalopram	440 ± 2.84	nd	nd
Diclofenac	284.22 ± 9.8	nd	nd
Ibuprofen	87.33 ± 6.68	nd	nd
Mirtazapine	63.26 ± 2.83	1.75 ± 0.07	nd
Sulfapyridine	15.22 ± 1.03	1.69 ± 0.04	nd
Telmisartan	10,161.60 ± 226	3.74 ± 0.20	2.24 ± 0.11
Triclosan	543.24 ± 36	nd	nd
Venlafaxine	33.97 ± 3.74	1.67 ± 0.03	nd

nd = not detected, values indicate mean ± standard error (n = 3).

2.2. Experimental set-up

The experiment included eight variants with three replications at different mixing proportions of sewage sludge (SS) and straw pellet (SP) with (+EW) and without earthworms (-EW): (T1) 100% SS (+EW); (T2) 100% SS (-EW); (T3) 75% SS + 25% SP (+EW); (T4) 75% SS + 25% SP (-EW); (T5) 50% SS + 50% SP (+EW); (T6) 50% SS + 50% SP (-EW); (T7) 25% SS + 75% SP (+EW); (T8) 25% SS + 75% SP (-EW). Table S1 shows the composition of vermicomposting/composting materials in various proportions. In all variants, the additive material was homogenized and transferred to worm bins (40 × 40 × 15 cm) for 120 days of vermicomposting/composting. The substrate (3 L grape marc) containing earthworm was placed into the tray from the side to avoid earthworm mortality and to allow earthworms to return to optimum conditions (Hanc et al., 2022). The average density of earthworm (*E. andrei*) in the substrate was 126 pieces per litter, with each piece weighing 0.2g. The vermicomposting/composting process was carried out at a constant temperature of 22 °C. The moisture level of the material was maintained at around 70%–80% of the wet mass during vermicomposting/composting by spraying the surface with water every two days. The experiment was carried out at the Faculty of Agrobiology, Food, and Natural Resources experimental station in Cerveny Ujezd, Czech University of Life Sciences Prague.

2.3. Laboratory analysis

2.3.1. Analysis of selected chemical properties

Representative samples of about 150 g wet weight per variant were taken on days 0 and 120, freeze-dried at −25 °C, lyophilised, and ground to analyse selected chemical properties. Another 30 g sample was taken from each variant and kept at 4 °C to determine pH and electrical conductivity (EC). According to BSI EN 15933(2012), the pH-H_2O and EC were determined using a WTW pH 340i and WTW cond 730 (1:5 w/v dry basis). Total carbon (TC) and total nitrogen (TN) were determined using an elemental analyser (CHNS Vario MACRO cube, Elementar Analysensysteme V3.1.1, Hanau, Germany).

2.3.2. Extraction and analysis of PPCPs and EDCs

The PPCPs and EDCs in the samples were analysed using LC-MS/MS after they had been homogenized. Subsequently, 1–2 g samples were moved to an extraction cell and placed in an accelerated solvent extractor (ASE, Dionex). The extraction process included preheating the methanol solvent and the cell to 80 °C and performing three cycles with 5-min fixed intervals between each cycle. The evaporated extracts were spun in a centrifuge at 6000g for 10 min, and the supernatants were collected and transferred to 2 mL vials for further analysis. The Agilent 1260 infinity liquid chromatography system and Agilent 6470 LC/TQ triple quadrupole mass detector were used to examine the samples. Separation was carried out using a Poroshell 120 EC-C18 column (2.7 m, 3 mm × 100 mm, Agilent) and a Poroshell 120 EC-C18 pre-column (2.7 m, 3 mm × 5 mm, Agilent), both of which were heated to 40 °C. The mobile phase was made up of phase A (0.5 mM ammonium fluoride in MQ water plus 0.01% formic acid, LC-MS grade) and phase B (100% methanol, LC-MS grade). The elution schedule of the gradient was such that the % phase B was as follows (time [min]): 0, 5; 4, 50; 6, 50; 18, 100; 21, 100; 22, 5, and 23, 5. The mobile phase had a flow rate of 0.4 mL/min, the duration of the run was 23.50 min, and the amount injected was 2 L. The matrix effect was diminished by the use of automatic standard additions of 1, 5, and 25 ng/mL to measure the samples. Innemanová et al. (2022) utilized MassHunter Source Optimizer and Workstation Optimizer (Versions 10.0, SR1, Agilent) to optimize the mass spectrometric parameters. After the experiment, the analyses were carried out at the Institute of Microbiology of the Czech Academy of Sciences. The analysis was done as part of a planned procedure called "scheduled analysis". 32 organic micropollutants were identified in SS. However, only 12 organic micropollutants, 11 of which were PPCPs: caffeine (CAF), carbamazepine (CBZ), cetirizine (CETI), citalopram (CITA), diclofenac (DCF), ibuprofen (IBF), mirtazapine (MIRT), sulfapyridine (SPD), telmisartan (TE), triclosan (TCS), venlafaxine (VEN), and one was an EDC: bisphenol A (BPA). The reduction percentage (R %) of each variant was calculated for the concentrations of all PPCPs and EDCs using the following equation (Biel-Maeso et al., 2019).

$$R(\%) = \frac{Xi - Xf}{Xi} \tag{1}$$

Where Xi is the concentration of organic micropollutants on the initial (day 0) variants (ng g^{-1}), and Xf denotes the same for the final concentration of organic micropollutants after 120 days of vermicomposting/composting.

2.3.3. Vermidegradation and vermiaccumulation of PPCPs and EDCs

The influence of earthworms on degradation was tested by developing evaluation parameters. The influence of earthworms (vermidegradation (VD)) was determined by calculating the percentage difference between the degradation efficiency (DE) with earthworms (+EW) and the degradation efficiency without earthworms (-EW) (Zeb et al., 2020).

$$VD = (DE(+EW) - DE(-EW)) * 100 \tag{2}$$

$$DE\ (+EW) = 1 - \frac{(+EW)}{\text{Input raw material}} \tag{3}$$

$$DE\ (-EW) = 1 - \frac{(-EW)}{\text{Input raw material}} \tag{4}$$

The influence value indicates how much more significant the reduction in micropollutant concentration was with the use of earthworms compared to the variant without earthworms. The bioconcentration factor (BCF) was calculated by dividing the average concentration of micropollutants in earthworms by the average concentration of micropollutants in vermicomposted material to determine vermiaccumulation (Suthar and Gairola, 2014).

$$BCF = \frac{\textit{Concentrations in earthworms}}{\textit{Concentrations in the substrate}} \tag{5}$$

2.4. Statistical analyses

To ensure that the data were normally distributed and homogeneous, the Shapiro-Wilk and Bartlett tests were used. A one-way variance analysis (ANOVA) was used to determine whether earthworms significantly influenced the concentrations of PPCPs and EDCs during SS vermicomposting. A Tukey test based on the mean differences was applied in a post-hoc analysis to identify the significant variations. The principal component analysis (PCA) was employed to evaluate the relations between the organic micropollutants and specific chemical parameters. The PCA was applied to the variables eigenvalues, variance (%), and cumulative (%) were used to measure the correlation between the variables. The Pearson correlation coefficient (r) was used to analyse the relationships between organic micropollutants and chemical characteristics. The statistical analyses used R version 4.0.2 and STATISTICA 12 software (StatSoft, Tulsa, USA). The significance level of statistical test was set at $p < 0.05$.

3. Results and discussion

3.1. Selected chemical characteristics of vermicomposted sewage sludge

Table 2 presents the initial and final properties of eight different variants. When compared to the initial, the pH of all variants decreased

Table 2
Initial and final selected chemical characterization of different variants.

Variants	pH-H_2O		
	Initial (day-0)	(+EW) (day-120)	(-EW) (day-120)
100% SS	6.9 ± 0.03	5.26 ± 0.49^{a}	5.62 ± 0.31^{a}
75% SS + 25% SP	7.3 ± 0.11	5.61 ± 0.51^{a}	4.99 ± 0.04^{a}
50% SS + 50% SP	7.6 ± 0.25	5.25 ± 0.60^{a}	4.95 ± 0.32^{a}
25% SS + 75% SP	7.9 ± 0.11	5.03 ± 0.46^{a}	5.00 ± 0.20^{a}
Variants	**EC (mS/cm)**		
	Initial (day-0)	(+EW) (day-120)	(-EW) (day-120)
100% SS	0.617 ± 0.11	3.01 ± 0.56^{a}	2.30 ± 0.35^{a}
75% SS + 25% SP	0.633 ± 0.08	2.77 ± 0.60^{a}	2.09 ± 0.44^{a}
50% SS + 50% SP	0.649 ± 0.06	2.31 ± 0.63^{a}	2.65 ± 0.07^{a}
25% SS + 75% SP	0.664 ± 0.05	2.19 ± 0.41^{a}	2.55 ± 0.10^{a}
Variants	**%TC**		
	Initial (day-0)	(+EW) (day-120)	(-EW) (day-120)
100% SS	32.9 ± 0.26	28.96 ± 1.37^{c}	25.40 ± 0.03^{b}
75% SS + 25% SP	35.36 ± 0.23	30.55 ± 0.65^{bc}	29.17 ± 0.71^{c}
50% SS + 50% SP	37.77 ± 0.24	32.66 ± 0.32^{ab}	30.87 ± 0.17^{c}
25% SS + 75% SP	40.18 ± 0.29	34.74 ± 0.44^{a}	35.12 ± 0.20^{a}
Variants	**%TN**		
	Initial (day-0)	(+EW) (day-120)	(-EW) (day-120)
100% SS	5.36 ± 0.03	3.30 ± 0.25^{a}	3.36 ± 0.15^{a}
75% SS + 25% SP	1.98 ± 0.21	3.18 ± 0.25^{a}	2.98 ± 0.14^{a}
50% SS + 50% SP	1.34 ± 0.07	2.92 ± 0.07^{a}	3.16 ± 0.10^{a}
25% SS + 75% SP	1.05 ± 0.05	2.98 ± 0.14^{a}	3.07 ± 0.00^{a}
Variants	**C/N ratios**		
	Initial (day-0)	(+EW) (day-120)	(-EW) (day-120)
100% SS	6.14 ± 0.04	8.88 ± 0.81^{a}	7.61 ± 0.33^{c}
75% SS + 25% SP	18.03 ± 1.92	9.74 ± 0.06^{a}	9.83 ± 0.21^{b}
50% SS + 50% SP	28.17 ± 1.43	11.20 ± 0.20^{a}	9.00 ± 0.27^{b}
25% SS + 75% SP	38.36 ± 2.03	11.71 ± 0.60^{a}	11.45 ± 0.09^{a}

Mean value followed by different letters is statistically different at ($p < 0.05$). Values indicate mean ± standard error (n = 3). (+EW) = vermicompost with earthworms, (-EW) = compost without earthworms, SS = sewage sludge, SP = straw pellet.

significantly ($F = 4.12$, $p < 0.05$); however, the reduction in pH value after 120 days of processing was statistically not significantly different ($p > 0.05$) among the variants, both with (+EW) and without earthworms (-EW) (Table 2).

The pH change during vermicomposting/composting may be attributed to some different processes, including the conversion of organic nitrogen into nitrites and nitrates via mineralization and nitrification (Sharma and Garg, 2019), the conversion of organic phosphorus into orthophosphates, and the bioconversion of organic material into intermediate species such as low-molecular-weight organic acids and humic acids (Karmegam et al., 2019). Similar pH reductions were found when composting and vermicomposting sewage sludge, crop straw, municipal solid waste, and livestock manure (Singh and Suthar, 2012). The initial EC value was significantly increased ($F = 3.80$, $p < 0.05$); however, there was no significant ($p > 0.05$) difference in the reduction of EC value after 120 days of vermicomposting among the variants with and without earthworms (Table 2). The gradual increase in EC could be attributed to the release of minerals in the form of cations and anions during substrate decomposition within vermicomposting processes (Ramnarain et al., 2019). The breakdown of organic matter in the vermicompost, which released minerals such as exchangeable Ca, Mg, K, and P in their accessible forms as cations, likely caused the increased EC in this study during vermicomposting, supporting the findings of (Dume et al., 2022).

TC was significantly reduced ($p < 0.05$) in the earthworm variants, with reductions of 12%, 14%, 14%, and 14%; however, in non-earthworm variants, TC was reduced by 23%, 18%, 18%, and 13% for 100% SS, 75% SS +25% SP, 50% SS + 50% SP, and 25% SS +75% SP, respectively (Table 2). In their study, Rini et al. (2020) observed a decrease in TC after 45 and 90 days of vermicomposting of solid waste from indigenous and exotic cow breeds using *epigeic* earthworms (*Perionyx excavatus and Eudrilus eugeniae*). Esmaeili et al. (2020) reported a reduction in TC after 45 days of combined composting and vermicomposting of pistachio waste (PW) mixed with cow dung (CD) in various ratios. Dume et al. (2022) also reported a reduction in TC during the vermicomposting of hydrolysed chicken feather residues for 120 days using *Eisenia andrei*. Microbial activity releases CO_2 due to a decreased TC, indicating that organic compounds are being biodegraded and mineralized in the variants (Ravindran et al., 2015). Microorganisms consume carbon to generate energy for their activities (Khatua et al., 2018). TN decreased by 38% in the 100% SS variant with earthworms and 37% in the no-earthworm variant, whereas TN increased by 61%, 118%, and 184% in earthworm variants and 51%, 136%, and 192% in non-earthworm variants for 75% SS + 25% SP, 50% SS + 50% SP, and 25% SS + 75% SP, respectively (Table 2). However, greater values were recorded in the earthworm-free variants than in the earthworm-containing variants, possibly due to organic carbon loss during composting (Huang et al., 2004). During vermicomposting of agricultural residues using *E. fetida* for 60 days, TN increased by 19.5%-152% (Jadia and Fulekar, 2008). TN content increased in tea prunings by 30.5%–51.3% after 30 days of vermicomposting with *Eudrilus euginae* (Pramanik et al., 2016). According to Dume et al. (2022), vermicomposting with *Eisenia andrei* earthworms increased TN by 42.3%-56.9% for 120 days. In comparison, vermicomposting hydrolysed chicken feather residues (HCFR) without the presence of earthworms increased TN by 56.4%–61.4% (Dume et al., 2022). Kaushik and Garg (2004) reported that vermicomposting of textile mill sludge combined with cow dung and agricultural residues using *E. fetida* for 11 weeks resulted in vermicompost with 2–3 times more TN than initial feedstocks. After 60 days of vermicomposting, Sudkolai and Nourbakhsh (2017) discovered that TN was 1.6 times greater in cow dung vermicompost and three times greater in wheat residue vermicompost than the feedstocks using *E. fetida*. A decrease in organic C in the form of CO_2 and the addition of N by earthworms in the form of mucus, nitrogenous excretory substances, and growth-stimulating hormones could be responsible for greater N levels in vermicompost.

The C/N ratio decreased in both earthworm and non-earthworm variants, with an overall reduction of 46%, 60%, and 69% in earthworm variants and 45%, 65%, and 70% in non-earthworm variants for 75% SS + 25% SP, 50% SS + 50% SP, and 25% SS + 75% SP, respectively. In contrast, the C/N ratio increased in both earthworm and non-earthworm variants for the 100% SS variant. This could be due to the lesser TN in this 100% SS variant (Table 2). The C/N ratio indicates compost maturity because it reflects stability and mineralization rates during the processes (Arumugam et al., 2018).

Increasing TN content and organic matter degradation also contribute to the decreased C/N ratio (Devi and Khwairakpam, 2020). Zhi-wei et al. (2019) found that using *Eisenia fetida* for 45 days reduced the C/N ratio of rice straw and kitchen waste vermicompost by 58.5–71.9%. Soobhany et al. (2015) found that vermicomposting organic solid wastes with *Eudrilus eugeniae* for 10 weeks reduced the C/N ratio by 41.5–48.4%. Boruah et al. (2019) observed that using *E. fetida* for 45 days reduced the C/N ratio by 91.1% in citronella bagasse and paper mill sludge vermicomposting. Biruntha et al. (2020) also found that the C/N ratio was reduced by 48.8%, during vermicomposting of different organic materials (seaweed, sugarcane trash, coir pith, and vegetable waste) with cow dung using *Eudrilus eugeniae* for 50 days. Vermicomposting with *E. andrei* earthworms decreased the C/N ratio by 65.8%-67.2% over 120 days, while vermicomposting HCFR without earthworms increased the C/N ratio by 61.7%–67.9% (Dume et al., 2022).

3.2. PPCPs and EDCs concentration in vermicomposted sewage sludge

The concentrations of PPCPs and EDCs, including bisphenol A (BPA), caffeine (CAF), carbamazepine (CBZ), cetirizine (CETI), citalopram (CITA), diclofenac (DCF), ibuprofen (IBF), mirtazapine (MIRT), sulfapyridine (SPD), telmisartan (TE), triclosan (TCS), and venlafaxine (VEN), are presented in Fig. 1. The concentrations of PPCPs and EDCs decreased from the initial concentration (day 0) to the final concentration after 120 days in the final products (vermicomposts/composts). The concentrations varied significantly among the variants ($F = 9.64$, $p < 0.001$ for CAF, $F = 12.50$, $p < 0.001$ for CBZ, $F = 4.53$, $p < 0.05$ for CETI, $F = 4.17$, $p < 0.05$ for DCF, $F = 6.21$, $p < 0.01$ for CITA, $F = 5.97$, $p < 0.01$ for MIRT, $F = 4.07$, $p < 0.05$ for SPD, $F = p < 0.01$ for TCS). Some PPCP and ED concentrations; however, did not differ significantly among the variants ($F = 1.67$, $p > 0.05$ for BPA, $F = 1.91$, $p > 0.05$ for IBF, $F = 1.50$, $p > 0.05$ for TE, $F = 2.06$, $p > 0.05$ for VEN). In the variants that included earthworms (+EW), the concentrations of PPCPs and EDCs varied as follows: BPA (16–59 ng g^{-1}), CAF (25–48 ng g^{-1}), CBZ (16–33 ng g^{-1}), CETI (20–60 ng g^{-1}), CITA (127–388 ng g^{-1}), DCF (3.0–11 ng g^{-1}), IBF (0–7.8 ng g^{-1}), MIRT (4.8–29 ng g^{-1}), SPD (1.6–2.7 ng g^{-1}), TE (4,099–8,257 ng g^{-1}), TCS (9.6–227 ng g^{-1}), and VEN (11–32 ng g^{-1}). In the variants without earthworms (-EW), the concentrations ranged from BPA (18–71 ng g^{-1}), CAF (25–53 ng g^{-1}), CBZ (19–38 ng g^{-1}), CETI (20–56 ng g^{-1}), CITA (146–444 ng g^{-1}), DCF (3.9–12 ng g^{-1}), IBF (2.0–8.8 ng g^{-1}), MIRT (7.38–32 ng g^{-1}), SPD (1.8–3.9 ng g^{-1}), TE (3,211–9,130 ng g^{-1}), TCS (47–454 ng g^{-1}), and VEN (12–48 ng g^{-1}) (dw) (Fig. 1, Table S2). CAF, DCF, IBF, MIRT, SPD, and TCS concentrations were reduced from their initial concentration in all variants, and the reduction percentages with respect to the initial variants (+EW) were: CAF (25–66%), DCF (94–97%), IBF (83–100%), MIRT (42–61%), SPD (61–84%), and TCS (58–90%), and in variants (-EW) were: CAF (25–62%), DCF (92–97%), IBF (79–89%), MIRT (29–49%), SPD (55–78%), and TCS (17–51%). However, BPA (53%), CBZ (14%), CETI (38%), CITA (12%), and VEN (7%) showed reductions only in the 100% SS variant (+EW) and increased in the remaining variants (BPA: 1–4%, CBZ: 29–144%, CETI: 17–46%, CITA: 17–68%, and VEN: 33–46%). In the 100% SS variant (-EW), BPA (45%), CBZ (1%), CETI (42%), CITA (25%), and VEN (20%) showed reductions and increased in the other variants (BPA: 17–23%, CBZ: 45–183%, CETI: 9–48%, CITA: 55–93%, and VEN: 9–241%). Additionally, the 50% SS +

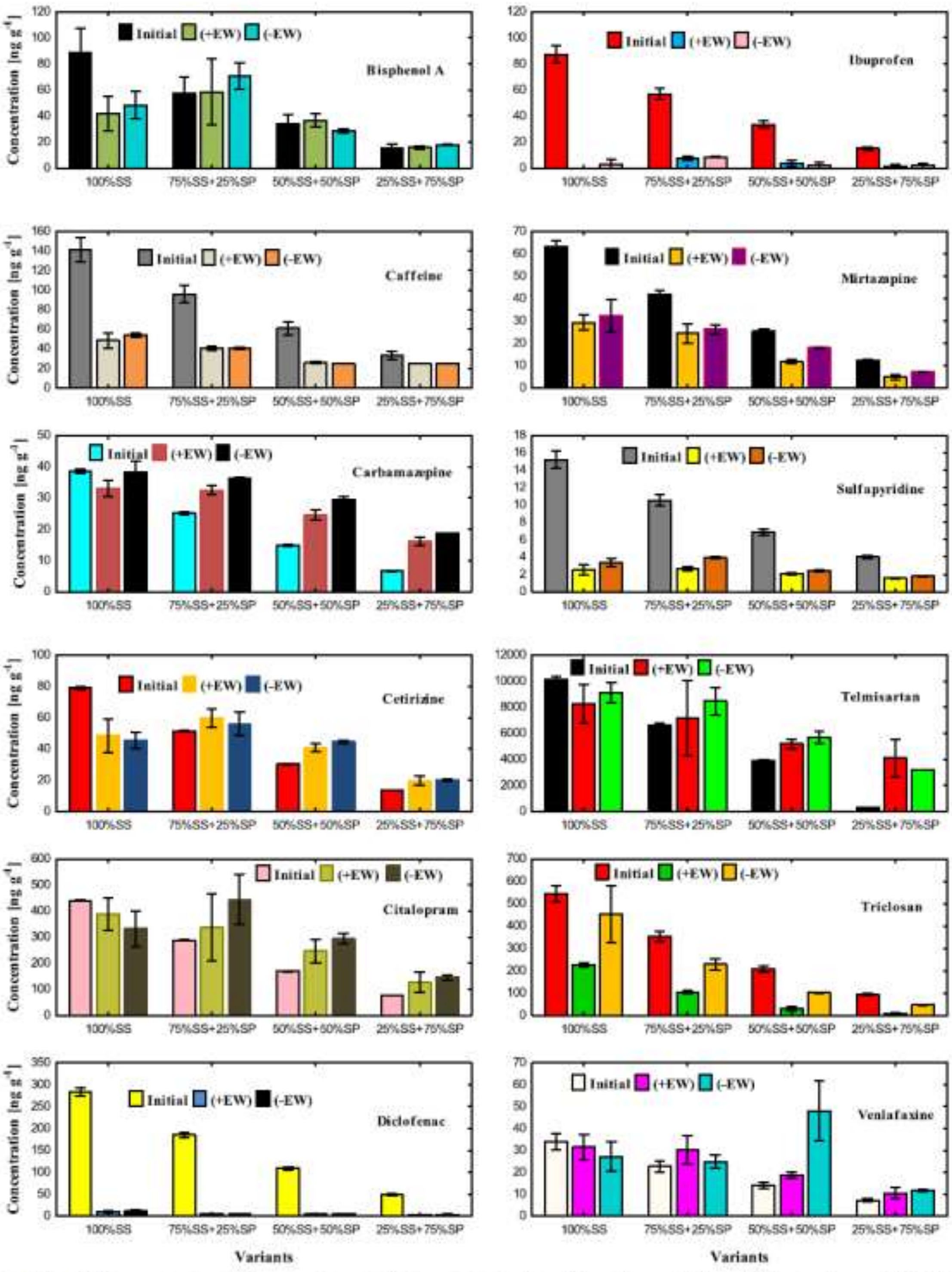

Fig. 1. PPCP and EDC concentrations of different variants at initial input, in final products with earthworms (+EW), and without earthworms (-EW). The bars indicate the standard error of the mean (n = 3).

50% SP variant (+EW) showed a 16% reduction in BPA (Table 3). The decrease in PPCP and EDC concentrations was thought to be caused by bioaccumulation in earthworm tissue during vermicomposting, in their intestine, or by skin absorption. However, a decrease in vermicompost's weight and volume may increase in PPCP and EDC concentration (Mazzeo et al., 2023). According to a report by Hammer and Palmowski (2021), the efficiency of micropollutant removal can differ based on the particular substances and research conducted. The range of removal can fall anywhere between nearly complete to no or insignificant removal. To categorize this range, Hammer and Palmowski (2021) have divided it into five groups: insignificant removal (0–20%), low removal (20–40%), medium removal (40–60%), high removal (60–80%), and very high removal (80%). A medium (53%) removal efficiency of BPA was achieved in 100% SS variant (+EW); however, insignificant to medium (16–45%) removal efficiency was achieved in variant (-EW). From (25–66%), removal efficiency of CAF was achieved in the variant (+EW) and (-EW) (25–62%). The maximum removal efficiency for CBZ in the 100% SS variant (+EW) was 14%, and 1% in the variant (-EW), which is in the range of insignificant removal. A low (38%) removal efficiency of CETI was achieved in 100% SS variant (+EW); however, a medium (42%) removal efficiency was achieved in 100% SS variant (-EW) variant, and an insignificant (12%) and low (25%) of CITA was achieved respectively in variants (+EW) and (-EW). Very high removal efficiency (≥80%) for DCF (94–96%) in the variants (+EW); however, (92–97%) in the variants (-EW), and IBP (83–100%) in the variants (+EW); however, (79–89%) in variants (-EW) was achieved. A medium (42–61%) removal efficiency of MIRT was achieved in variants (+EW); however, low to medium (29–49%) removal efficiency was achieved the variants (-EW). High to very high (61–84%) of SPD in variants (+EW) and medium to high (55–78%) in the variants (-EW) were achieved. 100% SS variants (+EW) (19%) and (-EW) (10%) achieved insignificant removal efficiency of TE, and VEN also achieved insignificant removal efficiency in these variants (+EW) (7%) and (-EW) (20%). Medium to high (58–90%) of TCS in variants (+EW); however, insignificant to medium (17–51%) in variants (-EW) removal efficiency was recorded (Table 3). These present findings, show some inconsistency regarding the removal efficiency of certain PPCPs and EDCs during vermicomposting of SS. It is important to note that the removal efficiency of PPCPs and EDCs could also be influenced by factors such as the type and amount of microorganisms present, the organic loading rate, the retention time, and the system's temperature (Shi et al., 2020). Therefore, it is necessary to conduct more studies under different experimental conditions to understand better the fate and behaviour of organic micropollutants during vermicomposting of SS. No clear and sufficient similar studies that have been published to date. However, Innemanová et al. (2022) conducted a study on the removal efficiency of PPCP and EDC during vermicomposting of dewatered SS under outdoor conditions for one year. Nevertheless, the behaviour of these compounds was not extensively elaborated upon. Moreover, the experiment was conducted outdoors, which could have been impacted by various external factors such as temperature and humidity. According to findings reported by Hammer and Palmowski (2021), CBZ removal efficiency was insignificant during anaerobic sludge digestion. Furthermore, Taboada-Santos et al. (2019) reported a high removal during anaerobic digestion of SS for 115 days. In contrast, other studies (Samaras et al., 2014) achieved very high removal (≥80%) for DCF and IBF during anaerobic digestion of SS for 113 days. Phan et al. (2018) reported that TCS removal efficiencies varied from no removal to high removal during anaerobic digestion. Currently, no environmental legislation limits exist for CAF, CETI, CITA, MIRT, SPD, TE, and VEN. However, the EU has established a legislation limit in SS for CBZ (100 ng g^{-1}), DCF (1,000 ng g^{-1}), IBF (10,000 ng g^{-1}), TCS (1,000 ng g^{-1}), and (20,000 ng g^{-1}) dw (European Union, 2019). It should be noted that these restrictions are subject to change and may differ based on the regulatory body and country in issue. It is also crucial to remember that some organic micropollutants may not have legal limitations but may still have negatively impact on human health and the environment.

As shown in Table 3, some PPCP and EDC concentrations were reduced in the final products of particular variants. The average negative reduction percentages (R%) of CBZ, CETI, CITA, TE, and VEN showed that the concentrations of PPCP and EDC had increased in both variants (+EW) and (-EW). The increase in some concentrations of PPCP during vermicomposting/composting could be due to the transformation of these compounds into other forms that were not measured in the study. Additionally, some compounds could have been released from the sewage sludge due to the breakdown of organic matter during the processes. Furthermore, the presence of earthworms during vermicomposting could have also contributed to the increased concentration of some compounds by altering the microbial activity and organic matter decomposition rate (Mazzeo et al., 2023), resulting in the formation of new compounds or the release of previously bound compounds (Innemanová et al., 2022). The total average concentrations of BPA, CAF, DCF, IBF, MIRT, SPD, and TCS were reduced by an average of 10, 52, 96, 90, 53, 72, and 76%, respectively and the reduction percentage (R%) value ranged from 10% for BPA to 96% for DCF in variants (+EW); however, 5, 51, 95, 86, 39, 65, and 39%, respectively and the average (R%) value ranged from 5% for BPA to 95% for DCF in variants (-EW). BPA, CAF, DCF, IBF, MIRT, SPD, and TCS reductions were higher in variants (+EW) than in variants (-EW) by 5, 2, 1, 4, 14, 7, and 37%, respectively (Table 3). In general, the reduction in PPCPs and EDCs revealed that their absorption/accumulation in earthworms outweighed the volume reduction effect during processes, and the additive materials

Table 3
PPCP and EDCs reduction percentage in the final products after 120 days of processing (n = 3).

Variants (+EW)	Reduction percentage (R %)											
	BPA	CAF	CBZ	CETI	CITA	DCF	IBF	MIRT	SPD	TE	TCS	VEN
100%SS	53	66	14	38	12	96	100	54	84	19	58	7
75%SS+25%SP	−1	58	−29	−17	−17	97	86	42	74	−6	71	−33
50%SS+50%SP	−8	57	−66	−34	−46	96	83	53	69	−32	86	−33
25%SS+75%SP	−4	25	−144	−46	−68	94	91	61	61	−1150	90	−46
Average	10	52	−56	−15	−30	96	90	53	72	−292	76	−26
Variants (-EW)	**BPA**	**CAF**	**CBZ**	**CETI**	**CITA**	**DCF**	**IBF**	**MIRT**	**SPD**	**TE**	**TCS**	**VEN**
100%SS	45	62	1	42	25	96	89	49	78	10	17	20
75%SS+25%SP	−23	58	−45	−9	−55	97	85	37	63	−28	36	−9
50%SS+50%SP	16	59	−99	−47	−74	95	89	29	65	−46	51	−241
25%SS+75%SP	−17	25	−103	−48	−93	92	79	41	55	−888	50	−63
Average	5	51	−82	−16	−49	95	86	39	65	−238	39	−73

BPA = bisphenol A, CAF = caffeine, CBZ = carbamazepine, CETI = cetirizine, CITA = citalopram, DCF = diclofenac, IBF = ibuprofen, MIRT = mirtazapine, SPD = sulfapyridine, TE = telmisartan, TCS = triclosan, VEN = venlafaxine, (+EW) = variants with earthworms, (-EW) = variants without earthworms, SS = sewage sludge, SP = straw pellet.

enhanced the PPCP and EDC removal efficiency even further (Zeb et al., 2020), and also due to microbial degradations and adsorption of these chemical substances onto organic matter of compost (Dubey et al., 2022).

3.3. PPCP and EDC concentrations in earthworm tissues

Earthworm tissues initially contained only 6.05 ng g^{-1} of CAF and 2.24 ng g^{-1} of TE. However, at the end of vermicomposting, the following seven PPCPs were detected at higher concentrations in the final earthworm tissues: CBZ, CETI, DCF, CAF, CITA, TCS, and TE (Fig. 2). CAF concentration at the end vermicomposting was not detected in the variant of 100% SS, while it increased from 6.05 ng g^{-1} to 23.58 ng g^{-1} (74%) for the 75% SS + 25% SP variant, from 6.05 ng g^{-1} to 11.33 ng g^{-1} (47%) for the 50% SS + 50% SP variant, and from 6.05 ng g^{-1} to 17.78 ng g^{-1} (66%) for the 25% SS + 75% SP variant. The variants with 100% SS, 75% SS + 25% SP, 50% SS + 50% SP, and 25% SS + 75% SP showed a significant increase in TE concentration, ranging from 2.24 to 373.9 ng g^{-1} (99%), 2.24–104.7 ng g^{-1} (98%), 2.24–266.3 ng g^{-1} (99%), and 2.24–116.8 ng g^{-1} (98%), respectively. Other PPCPs that increased were CBZ (5.5–15.9 ng g^{-1}), CETI (6.3–10.9 ng g^{-1}), DCF (5.5–15.5 ng g^{-1}), CITA (16.9–38.2 ng g^{-1}), TCS (8.4–42 ng g^{-1}), and TE (104.7–373.9 ng g^{-1}) (Table S2). The maximum reductions in PPCPs were observed in the 100% SS variant. The highest concentration of PPCP in earthworm tissue was TE; however, BPA, IBF, MIRT, SPD, and VEN were not found in earthworm tissues for all variants. It is therefore concluded that the earthworms had reached the excretion period during vermicomposting, which saw the egestion of accumulated PPCPs and EDCs from their bodies (Zeb et al., 2020). Additionally, the results of PPCPs and EDCs pointed to the possibility of PPCPs and EDCs, essential

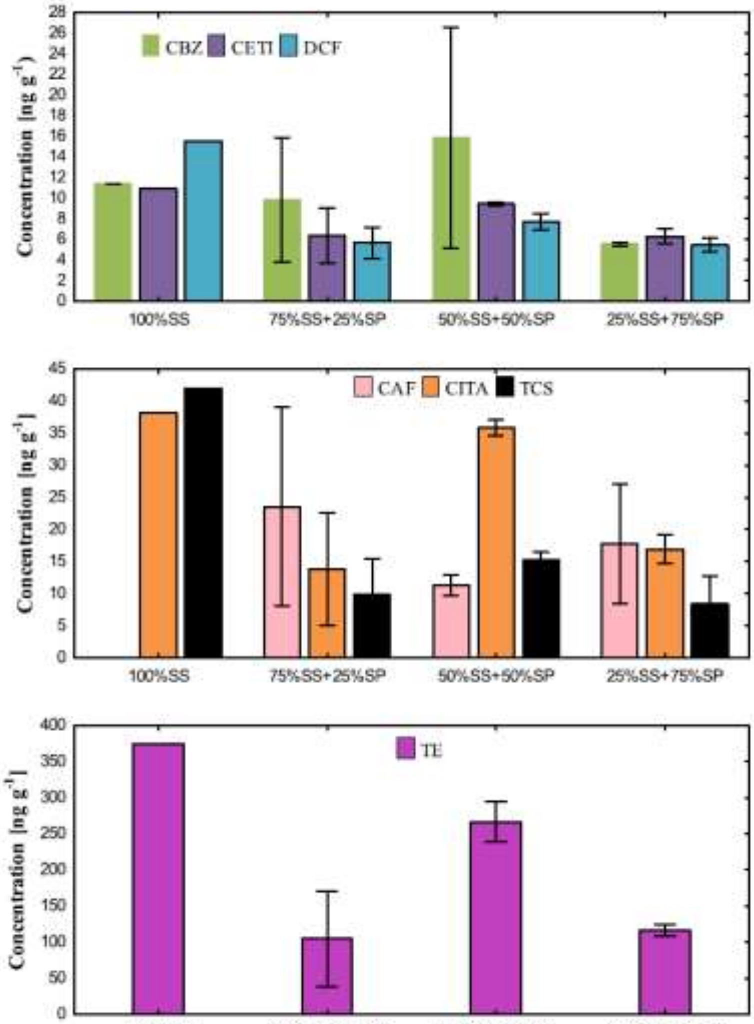

Fig. 2. Concentrations of PPCP found in earthworm tissues. The bars indicate the standard error of the mean (n = 3). A high standard error of the mean indicates that there was some amount of variability in the data.

components of bio-fertilizers, being detoxified by earthworms through metabolisation (Table S2); however, and further histological analysis is needed to validate this hypothesis (Zeb et al., 2020). Because of the reasonably consistent relationships between the concentrations of certain pollutants in earthworms, earthworms accumulate a significant amount of PPCPs and EDCs in their tissues and may be a useful biological indicator of contamination. The earthworm's interaction with local edaphic factors such as pH, organic matter content, enzyme activities and are mainly responsible for the accumulating PPCPs and EDCs (Zeb et al., 2020). TC reduction also results in the formation of intermediate metabolites and acids (humic acids), which lower the pH of the sludge mixtures (Zziwa et al., 2021).

3.4. The influence of earthworms on degradation of PPCPs and EDCs

Vermidegradation is the process by which various pollutants in earthworms are degraded using enzymes such as CYP450 and peroxidase or by gut microbes, also known as 'vermin-endophytes' which are microbes, bacteria, or fungi that live within earthworm tissues without causing any disease. It is one of the pathways of vermicomposting (Zeb et al., 2020). Vermidegradation is primarily concerns removing of organic micro-pollutant compounds such as PPCPs and EDCs (Bhat et al., 2018). Fig. 3 indicates the vermidegradation of some PPCP and EDC. The 100% SS variant had the most earthworms influence (efficiency of degradation) for TCS, with (43%). BPA was second, at (15%), followed by CBZ (14%), TE (8.7%), SPD (6.4%), IBF (5.4%), CAF (4.2%), MIRT (4.3%), and DCF (0.5%). Conversely, three PPCPs (CITA, CETI, and VEN) had negative vermidegradation percentages, with (−13%), (−4%), and (−12%), respectively. CITA (37%) had the most earthworms influence (efficiency of degradation) in the 75% SS + 25% SP variant, followed by TCS (36%), TE (22%), CBZ (16%), SPD (12%), MIRT (4%), IBF (2%), and DCF (0.3%). However, CETI (−8%), VEN (−21%), BPA (−3%), and TE (−262%) had negative vermidegradation percentages, indicating that these PPCPs and EDCs are resistant to vermidegradation (Haiba et al., 2018). Overall, the negative vermidegradation of PPCPs and EDCs highlights the complexity of the environmental fate and impact of these emerging pollutants. Further research is needed to fully understand the factors that influence the effectiveness of earthworms in degrading PPCPs and EDCs and to develop effective strategies for their removal and degradation in the environment.

The variant of 50% SS + 50% SP exhibited the most significant percentage of vermidegradation of VEN (193%), followed TCS (35%), CBZ (34%), CITA (28%), MIRT (24%), TE (13%), CETI (13%), SPD (4.5%), and DCF (1.2%), whereas, BPA (−23%), CAF (−2.4%), and IBF (−1.6%) showed negative values (Table S4). The variant with 25% SS + 75% SP had percentage of vermidegradation of TCS (40%), CBZ (38%), CITA (25%), VEN (20%), MIRT (19%), BPA (16%), IBF (11%), SPD (6%), CETI (3%), and DCF (2%) (Fig. 3). The negative percentage of vermidegradation for some PPCPs and EDCs implies that the final concentrations of PPCPs and EDCs found in vermicompost were more significant than the initial input materials, which implied that the earthworms did not influence on the degradation of PPCPs and EDCs during vermicomposting and this difference might be due to the extremely high concentration in the variant without earthworms (Shi et al., 2020). Table 4 summarizes the sum of PPCP and EDC concentrations in the initial variant, as well as the sum of these concentrations in the variant at the end of processing for both the (+EW) and (-EW) variants.

The summarised data shows how the concentrations of all determined substances changed during the experiment. The variants with 75% SS + 25% SP had the most earthworm influence on the degradation of targeted organic micropollutants (20.3%), followed by the variant with 50% SS + 50% SP (14.2%) (Table 4). These findings suggest that more research is needed to assess the influence of earthworms on organic micropollutants including PPCPs and EDCs.

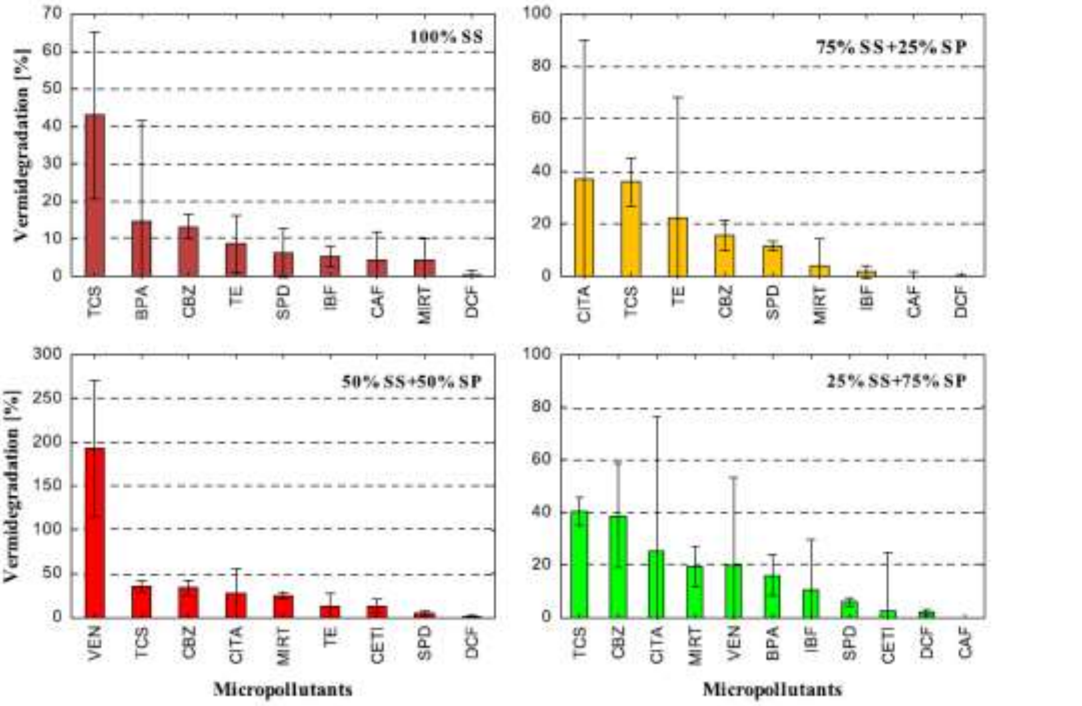

Fig. 3. Influence of earthworms on the degradation of PPCPs and EDCs in different variants. The bars indicate the standard error of the mean (n = 3). A high standard error of the mean indicates that there was some amount of variability in the data.

Table 4
A summary of the degradation efficiencies of PPCPs and EDCs.

Variants	Concentration [ng g^{-1} dw]			Efficiency of degradation [%]		
	Σ Initial	Σ (+EW)	Σ(-EW)	(+EW)	(-EW)	Influence of earthworms (%)
100% SS	11977	9119	10179	24	15	8.9
75% SS + 25% SP	7817	7839	9423	−0.28	21	20.3
50% SS + 50% SP	4617	5637	6293	−22	−36	14.2
25% SS + 75% SP	651	4333	3512	−566	−439	−126.1

Σ Initial = summation of all PPCP concentrations in the initial input materials, Σ (+EW) = summation of all PPCP concentrations in the final vermicompost (with earthworms), Σ (-EW) = summation of all PPCP concentrations in the final product (without earthworms), SS = sewage sludge, SP = straw pellet. dw = dry weight.

3.5. Vermiaccumulation of PPCP and EDC

Shi et al. (2020) explain that vermiaccumulation is the process by which earthworms absorb and retain pollutants, leading to a decrease in the concentration of substances like PPCPs and EDCs in SS. To quantify this assimilation of PPCPs and EDCs into earthworm tissues, the bio-concentration factor (BCF) can be used. The concentrations of PPCPs and EDCs in earthworm tissues were recorded by examining earthworm samples before and after vermicomposting. The vermiaccumulation percentage varied for all PPCPs among the variants; however, maximum vermiaccumulation of caffeine was CAF (72%), CBZ (65%), CETI (32%), CITA (16%), DCF (183%), TE (5%), and TCS (118%) (Fig. 4).

The presence of organic micropollutants in SS is proportional to the level of organic micropollutants in wastewater. The BCF was indicated in the following manner: DCF > TCS > CAF > CBZ > CETI > CITA > TE (Table S3). *Eisenia andrei*, a species of earthworm, has the ability to ingest and process pollutants during vermicomposting. This includes the process of grinding and digestion, allowing for the absorption of these pollutants through the intestinal tract into the worm tissues. This process is known as nutrient uptake and is further facilitated by epidermal uptake, both of which allow for earthworms to acquire organic micropollutants. Vermicomposting has been found to be effective in reducing the concentration of organic micropollutants in SS, thus addressing the issue. However, a new question arises about how to handle the earthworms that have accumulated organic micropollutants in their bodies, as highlighted by Shi et al. (2020). This is troubling for two reasons. Separation of vermicomposting earthworms is not difficult; this is often accomplished simply by adding fresh material where earthworms naturally crawl. However, earthworms can still be found in vermicompost or other materials; they are simply separated from the final product. When careful separation of earthworms from impurities and matrixes becomes economically viable, a problem arises because these methods are typically time-consuming. This represents the second issue in dealing with earthworms. The spectrum of use for uncontaminated earthworms is broad; however, there is currently no use for earthworms with high PPCP and EDC bioaccumulation. One option is not to separate the earthworms but to leave the earthworm population in vermicompost. However, this option has limitations in terms of earthworm bioaccumulation limits. These organisms will vermiaccumulate PPCP and EDC to a certain level, after which the concentration of pollutants inside the organism will either stop increasing or the organism will die. In both cases, this means that earthworms' ability to degrade PPCP and EDC is reduced. Earthworms' ability to degrade PPCP and EDC is reduced in both cases. Measurable influence earthworms may be possible only if new earthworms are used in each situation (Zeb et al., 2020).

3.6. Worm reproduction and growth

Growth rate, earthworm number (*E. andrei*), and cocoons in the vermicompost process in different variants are shown in Table S5. *E. andrei* exhibited significant ($p < 0.05$) variations in the number of earthworm pieces/kg in the vermicomposted material and also the number of cocoons/kg in the vermicomposted material (Table S5). The initial weight and amount of earthworms were 0.2 g/piece and 125 pieces/kg of vermicomposted material, respectively. The initial earthworms weighed 25 g per kilogram of vermicomposted material. After 120 days, the variant with 25% SS + 75% SP contained the maximum number of cocoons (178 pieces/kg), and the 50% SS + 50% SP variant contained the minimum (59 pieces/kg). The results indicate that, despite some mortality, there was an increase in the number of earthworms in some variants. This increase was more significant in the variant with 50% SS + 50% SP than in the other variants, and worm mass was also more significant in this variant. This could be due to the presence of nutrients for worm growth in the additive material, which makes this variant (50% SS + 50% SP) a favourite feed for earthworms (Pérez-Godínez et al., 2017). *E. andrei* produced more cocoons in the variant with 25% SS + 75% SP than in the other variants. The additive material is a carbon source, a vital determinant of earthworm production initiation, and might explain differences in cocoon production levels among the variants. A higher carbon content additive material promotes growth and reproduction by providing earthworms with an adequate amount of organic matter. Higher carbon source of additive material appears to a significant impact cocoon production (Sonnenzlag et al., 2017).

3.7. Principal component analysis (PCA)

Fig. 5 shows the principal component analysis of 12 organic micropollutants and the correlation between organic micropollutants and select chemical parameters. Principal component analysis (PCA) was used to evaluate the relationships between the PPCP and EDC (BPA, CAF, CBZ, CETI, CITA, DCF, IBF, MIRT, SPD, TE, TCS, and VEN) and selected chemical parameters (pH, EC, TC, TN, and C/N ratio), and plotted PC1 with PC2. The PCA analysis was designed to compare all of the investigated parameters, focusing on exciting relationships. The relationship between the variables was determined by analysing their eigenvalues, variance (%), and cumulative (%) criteria. The principal component (PC) accounted for 60.11% of the variance, 7.98 of the eigenvalue and was dominant for the variables pH, TC, and C/N ratio. All 12 PPCP and EDC were negatively correlated with pH, TC, and C/N ratios and positively correlated with EC and TN. PC2 accounted for 14.07% of the variance and 2.8 of the eigenvalue. All PPCPs and EDCs dominated this component and were positively correlated with TN, apart from IBF, which was negatively correlated with TN. VEN also had a significantly positive correlation with EC ($r = 0.4177$, $p < 0.05$) except for SPD, IBF, DCF and TCS, which negatively correlated with EC. TE was significantly correlated with EC ($r = 0.4696$, $p < 0.05$) and TN ($r = 0.7057$, $p < 0.001$), whereas CITA was significantly correlated with EC ($r = 0.5751$, $p < 0.01$), and TN ($r = 0.6514$, $p < 0.01$); however, CITA ($r = -0.4115$, $p < 0.05$) and TE ($r = -0.5228$, $p < 0.01$) had a significantly negative correlation with pH (Table S6).

As a result of TC reduction, the formation of intermediate metabolites and acids (humic acids) reduces the pH of the sludge mixtures. PPCP and EDC accumulation in tissues is a distinct phenomenon. Each PPCP and EDC exhibits a distinct physiological mechanism of assimilation and excretion during its metabolism in the earthworm's gut. As a result, higher TC and C/N values result in better PPCP and EDC degradation. The degradation of PPCP and EDC is not affected by pH. According to Dubey et al. (2022), the degradation of PPCP and EDC is not influenced by pH. Bacteria tend to favour high carbon and C/N ratios for breaking down PPCPs and EDCs, whereas fungi prefer environments with high pH and nitrogen levels.

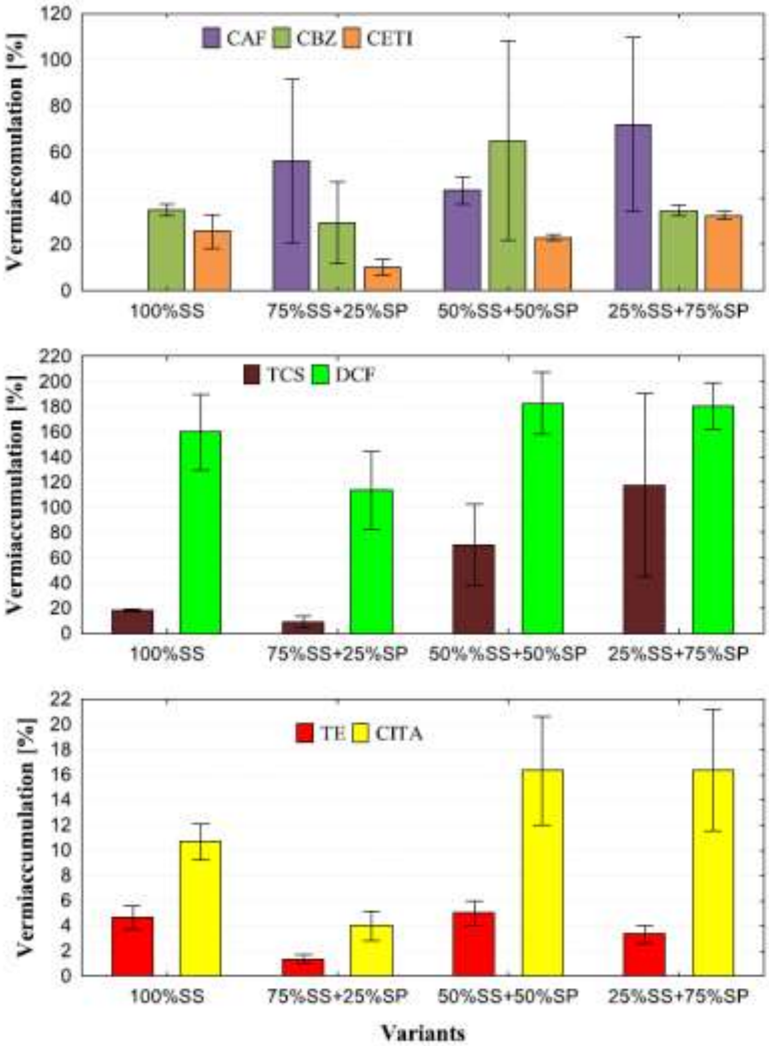

Fig. 4. Vermiaccumulation of some PPCPs during SS vermicomposting in different variants. The bars indicate the standard error of the mean (n = 3). A high standard error of the mean indicates that there was some amount of variability in the data.

3.8. Current research challenges and future perspectives

Although earthworms are known to contribute to the degradation of PPCPs and EDCs in SS, the specific enzymes and metabolic pathways involved in this process are not fully understood (Shi et al., 2020). Identifying these mechanisms is crucial for optimising earthworm-based treatment and developing more effective methods for removing PPCP and EDC levels in SS. Identifying these pathways to optimize earthworm-based treatment systems and developing more effective strategies for lowering PPCP and EDC levels in SS is critical. Temperature, moisture content, and other organic matter can all impact activity and the rate of PPCP and EDC degradation (Zeb et al., 2020). Understanding these impacts is critical for optimising earthworm-based treatment systems and forecasting their efficacy in various environmental situations. Although earthworm-based treatment systems have shown promise in the laboratory, assessing their viability at an industrial or municipal scale is critical. This includes determining the economic viability of large-scale earthworm culture and the possibility of combining earthworm-based treatment systems with existing wastewater treatment infrastructure. Therefore, the following concerns must be addressed.

1. Earthworms employed for SS vermicomposting may accumulate specific organic micropollutants from the SS, such as PPCPs and EDCs. If these earthworms are utilized in soil or other applications, these micropollutants may be transferred to the new environment. As a result, it is critical to appropriately manage or treat earthworms to remove toxins before employing them in other applications. One method is to submit the earthworms to a procedure known as "phytoremediation," in which they are fed plants capable of absorbing and breaking down toxins in their bodies (Zheng et al., 2022).
2. Developing earthworm-based treatment systems for wider usage: While earthworm-based treatment systems have shown promise in

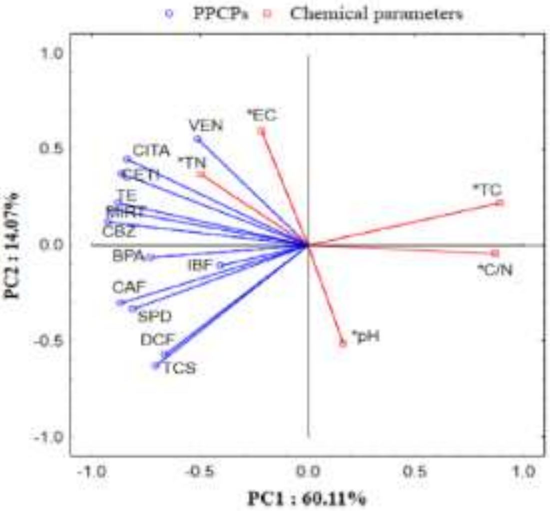

Fig. 5. Principal components (PC) of PPCP and EDC and along with their correlation with selected chemical parameters (pH, EC, TC, TN, and C/N ratio). BPA = bisphenol A, CAF = caffeine, CBZ = carbamazepine, CETI = cetirizine, CITA = citalopram, DCF = diclofenac, IBF = ibuprofen, MIRT = mirtazapine, SPD = sulfapyridine, TE = telmisartan, TCS = triclosan, VEN = venlafaxine, TC = total carbon; TN = total nitrogen, C/N = carbon to nitrogen ratio.

lowering PPCP and EDC levels in SS, more effective and scalable methods are needed for general application. Optimising the conditions for earthworm activity, developing new strains of earthworms that are more efficient at degrading pollutants, and integrating earthworm-based treatment systems with existing wastewater treatment infrastructure could all be part of this.

3. Developing new analytical methods for monitoring pollutant degradation in SS: This can help to make monitoring more efficient and cost-effective. Future studies could focus on developing new real-time methods for monitoring pollution levels, such as employing nanoparticles or sophisticated imaging techniques.
4. Evaluating the long-term viability of earthworm-based treatment systems: While earthworms can effectively reduce pollutant levels in SS, it is critical to evaluate the long-term viability of these systems. This involves comprehending the effects of repeated cycles of earthworm digestion and the potential accumulation of pollutants in earthworm tissues.

4. Conclusion

It was hypothesised that earthworms could remove the PPCPs and EDCs due to bioaccumulation of these chemicals in earthworm tissue during vermicomposting. According to this assumption, variants with earthworms reduced some PPCPs and EDCs such as BPA, CAF, DCF, IBF, MIRT, SPD, and TCS more effectively than variants without earthworms. However, the concentrations of CBZ, CETI, CITA, TE, and VEN increased in both variants with and without earthworms. Furthermore, the reduction in the weight and volume of end product (vermicompost/compost) may result in an increase in the concentration of these selected organic micropollutants. In all variants with and without earthworms, a very high removal efficiency of DCF and IBF was achieved. Therefore, from this finding, earthworms have shown great promise in removing selected PPCP and EDC from sewage sludge. Simultaneously, it is strongly suggested to perform further research oriented to the development of more effective and sustainable methods for removing organic micropollutants from sewage sludge.

CRediT authorship contribution statement

Bayu Dume: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization. **Aleš Hanč:** Conceptualization, Formal analysis, Resources, Data curation, Writing – original draft, Methodology, Supervision, Project administration, Funding acquisition. **Pavel Švehla:** Conceptualization, Methodology, Supervision, Formal analysis, Resources, Data curation, Writing – original draft. **Pavel Michal:** Sample and data collection. **Vojtěch Pospíšil:** Formal analysis. **Alena Grasserová:** Formal analysis. **Tomáš Cajthaml:** Project administration, Review, Editing. **Abraham Demelash Chane:** Sample and data collection. **Abebe Nigussie:** Review, Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2023.137869.

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5.5. **Dume et al. (2023**). Composting and vermicomposting of sewage sludge at various C/N ratios: Technological feasibility and evaluation of end-product quality

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Composting and vermicomposting of sewage sludge at various C/N ratios: Technological feasibility and end-product quality

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ABSTRACT

Even though sewage sludge (SS) contains a high level of pollutants, it is rich in essential plant nutrients and has the potential to enhance soil fertility. However, the SS must be further treated through pre-composting plus vermicomposting to make it safe for use on food crops. More research and data are needed to determine how different carbon-to-nitrogen ratios (C/N) affect the feasibility and quality of composting vs vermicomposting of SS. Therefore, in this study we comprehensively evaluated the feasibility and end-product quality of compost and vermicompost produced from SS under different C/N ratios. SS was mixed with pelletized wheat straw (PWS) at various proportions to produce C/N ratios of 6:1, 18:1, 28:1, and 38:1, then pre-composted for 14 days followed by vermicomposting using the earthworm *Eisenia andrei* for 120 days. Agrochemical properties were measured at 0, 30, 60, 90, and 120 days. Results revealed significantly higher levels of agrochemicals in vermicompost compared to compost, including total potassium (37–88%) and magnesium (4.3–12%), nitrate nitrogen (71–98%), available potassium (53–88%), available phosphorus (79%), available magnesium (54–453%), available boron (48–303%), and available copper (2.5–82%). However, lower levels of ammonium nitrogen by (59–85%), available iron (2.3–51.3%), available manganese (29.7–52.2%), available zinc (10.5–29.8%), total carbon (0.75–4.5%), and total nitrogen (1.6–22.2%) were measured. Comparison of the various C/N ratios, showed that vermicompost with an 18:1 C/N ratio outperformed compost and demonstrated the highest earthworm population (165 pieces/kg). Thus, vermicomposting SS at an 18:1 C/N ratio is strongly recommended as a sustainable technology for producing high-quality vermicompost from SS.

1. Introduction

Sewage sludge (SS) is a by-product produced in large quantities during wastewater treatment processes. Because of the presence of pollutants such as organic chemicals and toxic heavy metals, and potential pathogens, raw SS is hazardous and raises serious environmental concerns about its use as a soil amendment (Hait and Tare, 2012). The amount of SS produced yearly rises in lockstep with the global urban population. Because of this increase and the problems associated with SS disposal, this product poses significant challenges in several regions of the world (Mousavi, 2022). Mateo-Sagasta et al. (2015) estimated that the total SS volume produced in Europe (2010), China (2006), and the United States (2004) was 9, 3, and 6.5 million tons of dry matter per year, respectively. Composting and vermicomposting of SS for soil amendment is typically the most efficient and cost-effective treatment method and allows farmers to utilize less chemical fertilizer. Since it has a high content of organic matter and essential plant nutrients, SS is best suited as a bio-fertilizer (Guilayn et al., 2019). It has been widely recognized that SS helps to improve the soil's physical, chemical, and biological characteristics (Alvarenga et al., 2015). Because of the occurrence of specific pollutants, including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), endocrine disruptors (EDs), pharmaceutical residues (PRs), toxic heavy metals (e.g., Pb, Cd, Ni, Co, and Cr), and potentially pathogenic organisms (bacteria, viruses, and parasites), it is necessary to treat the SS prior to agricultural use to remove or sequester toxic substances and kill pathogens (Lillenberg et al., 2010). Some studies have been conducted to assess the use of aerobic composting and vermicomposting to transform the sludge into a

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safe product for agricultural application (Kinney et al., 2012; García-Gómez et al., 2014; Singh et al., 2020).

Composting and vermicomposting are two common bioorganic methods for transforming organic waste materials into valuable bio-fertilizers. In composting, environmentally friendly bacteria and fungi break down the waste matter and produce stable organic material with enhanced biochemical and physical properties (Hait and Tare, 2012). In vermicomposting, earthworms ingest the organic material, converting it into nutrient-rich castings that can be used as a valuable soil amendment (Liu et al., 2012). As a bio-oxidative process that involves earthworms and microorganisms, vermicomposting is gaining popularity due to its versatility (Soobhany, 2019; Lv et al., 2018; Nigussie et al., 2016). Composting is also recognized as an environmentally friendly process in which microorganisms degrade organic matter and turn it into a sanitized agricultural soil amendment. The sanitization process is associated with the early aerobic phase of composting when the temperature in the composter increases to 45–70 °C (Bernal et al., 2009). This elevated temperature creates conditions inhospitable to many pathogens and helps eliminate potential health risks associated with composting some organic materials like sludge.

Fresh sewage sludge should not be used for vermicomposting since it is anaerobic, contains substances toxic to earthworms, and also has excessively high concentrations of ammonia (NH_3) and methane (CH_4) (Awiszus et al., 2018). SS pre-composting at high temperatures is needed to avoid harm to earthworms and to remove excess ammonia (Kaushik and Garg, 2003). The material should be aerated and stabilized during the pre-composting process, reducing moisture content and inactivating pathogens (Yadav et al., 2012; Yadav and Garg, 2016; Malinska et al., 2016).

Studies have been conducted comparing the results of composting and vermicomposting (Lazcano et al., 2008; Rékási et al., 2019; Tognetti et al., 2007) and vermicompost has been shown to have a greater market acceptance than compost because of its better performance and the significantly greater availability of organic matter and plant nutrients (Tognetti et al., 2005). Vermicompost contains more humic substances and stable organic compounds than traditional compost, and also has a more comprehensive impact on nutrient management because of the slower nutrient release and the presence of higher levels of plant hormones that encourage growth (Rékási et al., 2019). Researchers have compared compost with vermicompost by using the same raw materials and conditions (Fornes et al., 2012; Hanc and Dreslova, 2016). Thus, for this study, we used the same raw materials, sewage sludge combined with pelletized wheat straw, to compare the quality of the final products.

Because of their importance as primary nutrients needed for microbial activity, the carbon and nitrogen levels, and especially the ratio of carbon to nitrogen (C/N) are regarded as crucial factors influencing compost quality (LV et al., 2018; Sánchez-Monedero et al., 2010; Zhang et al., 2016) and how long it takes for the compost to reach maturity (Tripetchkul et al., 2012; Guo et al., 2012). Evidence has shown that a C/N ratio of 25–35 is optimal for composting microbes to remain stable and active (Akratos et al., 2017). According to Kumar et al. (2010), the ideal starting C/N ratio range for composting is 25–30; however, Vochozka et al. (2017) argued that improved global standards necessitated a C/N range of 20–30. A high C/N ratio causes the process to start slowly and take longer to produce finished compost, whereas a low initial C/N ratio results in high ammonia (NH_3) emissions and increased nitrogen loss (Oudart, 2013). A too low initial C/N of 15 significantly negatively affected several agrochemical properties during the process (Huang et al., 2004). El-mrini et al. (2022) reported that a C/N ratio of 25 during composting decreased copper (Cu) and zinc (Zn) mobility, but increased total metal ion content, which could alter urease enzyme activity (Wu et al., 2017). Other researchers found significant effects of C/N on pathogen reduction (Macias-Corral et al., 2019). The C/N ratio can be adjusted by choosing the appropriate combination of compost materials and adding co-substrates to attain the desired final ratio (Akratos et al., 2017). However, it is still unclear how different C/N ratios affect compost quality and the time needed to produce it, and more studies are needed to determine how different C/N ratios affect the quality of compost vs vermicompost and the feasibility of producing a good product from a given substrate. Therefore, this study comprehensively evaluated the feasibility and end-product quality of compost and vermicompost produced under different C/N ratios.

2. Materials and methods

2.1. *Initial raw materials and earthworms*

The unstabilized, freshly collected sewage sludge used in this experiment was obtained from a wastewater treatment plant in a small town in the Czech Republic. It had a dry matter content of 13.3%, a pH-H_2O of 6.9, and electrical conductivity (EC) of 0.6 mS/cm. The SS material was 32.9% C and 5.4% N (C/N = 6.1), and per kg of dry weight contained, 5002 mg of potassium (K), 4809 mg of magnesium (Mg), and 15,996 mg of phosphorus (P). Dried pelletized wheat straw (PWS) was obtained from the Granofyt Co., Ltd.(Chrášťany, Czechia). It had a diameter of 10 mm and a dry matter content of 21.2%, a pH-H_2O of 8.3, and an EC of 0.68 mS/cm, with 42.6% C and 0.8% N (C/N = 53.2). Per kg of dry weight, the PWS contained 5953 mg of K, 935 mg of Mg, and 704 mg of P. Because of the low moisture content of the PWS, it was mixed with hot water (60 °C) at a 1:4 (w/v) ratio before use. Earthworms were collected from a private vermiculture stock in the Czech Republic with apple pomace as survival medium. The epigeic earthworm species, *Eisenia andrei*, was used in the experiments because of its high tolerance for toxic substances in SS, adaptability to a relatively wide range of pH, moisture, and temperature levels, a high growth rate, and ability to convert semi-composted biomass into stable products, (Gupta and Garg, 2011; Yadav and Garg, 2016).

2.2. *Experimental set-up*

2.2.1. *Pre-composting*

The experiment included four different initial C/N ratios (1) 6:1, (2) 18:1, (3) 28:1, and (4) 38:1 achieved by mixing SS with PWS in different proportions: (1) 100% SS, (2) 75% SS + 25% PWS, (3) 50% SS + 50% PWS, and (4) 25% SS + 75% PWS. On a dry-weight basis, this results in different ratios of SS to PWS: Mix1 (4:0), Mix2 (3:1), Mix3 (2:2), and Mix4 (1:3). Three replicates were run for each condition (n = 3). Before vermicomposting, all mixtures were pre-composted for 14 days in 70-L laboratory reactors with 56-cm diameters. The pre-composting phase is crucial since this breaks down highly unstable materials, decreases the concentration of volatile acids, and stabilizes the temperature conditions for the earthworms (Zziwa et al., 2021; Karwal and Kaushik, 2020; Mainoo et al., 2009).

2.2.2. *Vermicomposting*

The vermicomposting method used was a technique which had been proved to give the optimal environmental and technical conditions for the process (Hanc et al., 2022). After pre-composting for 14 days, the variants VC1, VC2, VC3 and VC4 for Mix1 (4:0), Mix2 (3:1), Mix3 (2:2) and Mix4 (1:3) SS:PWS, respectively, were transferred to worm bins (40 ×40×15 cm) for vermicomposting in a specially adapted laboratory under controlled conditions of temperature (22 °C) and relative humidity (80%) and vermicomposted for 120 days. Each worm bin received adult *Eisenia andrei* earthworms with an average weight of 0.2 g/piece and number of earthworms at 125 pieces/L of substrate. The earthworms weighed 25 g per kilogram of substrate. The substrate (3 L grape marc) containing the earthworms were put inside the plastic container from the side to ensure earthworm survival and quickly return them to favourable conditions. A 6-mm mesh separated the materials, which were sprayed with water every two days to keep the moisture content of the material at 70–80%.

2.2.3. Composting

The same vermicomposting mixtures were used for the composting experiment, which was also run for 120 days. The various SS:PWS formulations, Mix1 (4:0), Mix2 (3:1), Mix3 (2:2), and Mix4 (1:3), labelled C1, C2, C3, and C4, respectively, were transferred to aerobic composters (fermenter barrels) with a working volume of 70 L and a diameter of 56 cm, which were constructed with the aim to ensure optimal conditions for composting (Hanc et al., 2022). For optimal composting, aeration was provided to promote the growth and activity of aerobic microorganisms, which require oxygen to carry out the decomposition process efficiently (Wang et al., 2021). In vermicomposting, aeration is not required since earthworms burrow through the organic materials and create tunnels that provide oxygen to the microorganisms involved in the decomposition process. Earthworms can tolerate lower oxygen levels than aerobic microorganisms, so aeration is not as critical in vermicomposting as it is in composting (Pathma et al., 2012). Air was pumped from the bottom through the composted materials using an air compressor and active aeration device. Batch aeration was performed on the mixtures for 5 min every half hour at a rate of 4 L of air per minute, followed by 3 min every half hour. The temperature probe was inserted from the composter's top to half the material height, and the temperature was recorded and stored in a data logger every hour. The leachate was collected and poured back into the composted material before sampling at the end of each month to achieve a closed loop of substances produced in the pile. Based on their experiments, Hanc et al. (2012) affirmed that these aeration conditions were optimal for good composting. Some studies compared the effectiveness of composting and vermicomposting without regard for container volume (Lazcano et al., 2008; Rékási et al., 2019). However, it is important to note that the results of composting and vermicomposting can vary depending on a number of factors, including the starting material, moisture content, temperature, and other environmental conditions (FAO, 2017). Thus, our study was performed with controlled temperature and humidity. The composting and vermicomposting experiments were conducted at the Czech University of Agricultural Research Station in Červený Újezd. The samples were then collected and analysed at the Czech University of Life Science's, Prague laboratories in the Faculty of Agrobiology, Food and Natural Resources, department of Agro-environmental Chemistry and Plant nutrition. The experimental study design is shown in Fig. 1.

2.3. Sampling and analysis of agrochemical properties

Representative composite samples (-150 g wet basis for every variant) were collected on days 0, 30, 60, 90, and 120 and then freeze-dried (-25 °C) for agrochemical analysis. In addition, 30 g samples were collected from each variant and frozen at 4 °C for later measurement of pH and electrical conductivity (EC). The following agrochemical parameters were analysed: pH, EC, total and available macronutrients (K, Mg, and P), mineral nitrogen (N-NO_3^-, N-NH_4^+), micronutrients (B, Cu, Fe, Mn and Zn), total nitrogen (TN) and total carbon (TC). The pH-H_2O and EC values were determined using a WTW pH 340i and WTW Cond 730 (1:5 w/v dry basis), following the BSI EN 15933 (2012). Total concentrations of macronutrients, K, Mg, and P, were determined by decomposition in a closed system with microwave heating using an Ethos1 system (MLS GmbH, Germany). The contents of N-NO_3^-, N-NH_4^+, readily available macronutrients (K, Mg, and P), and available micronutrients (B, Cu, Fe, Mn, and Zn) were analysed using CAT solution (0.01 M $CaCl_2$ and 0.002 M diethylenetriamine pentaacetic acid (DTPA) at a ratio of 1:10 (w/v), according to BSI EN 13651 (2001). Optical emission spectrometry using an inductively-coupled plasma detector (ICP-OES, VARIAN VistaPro, Varian, Australia) with axial plasma configuration was used to determine total and available nutrient contents. To determine the C/N ratio, a CHNS Vario MacroCube (Elementar Analysensysteme GmbH, Germany) analyser was used according to Hanc et al. (2017). The CHNS Vario MacroCube analyser is a highly accurate and reliable instrument for determining total carbon and total nitrogen content. Earthworms and cocoons were hand-sorted, separated from the samples, counted, and then washed with water and weighed.

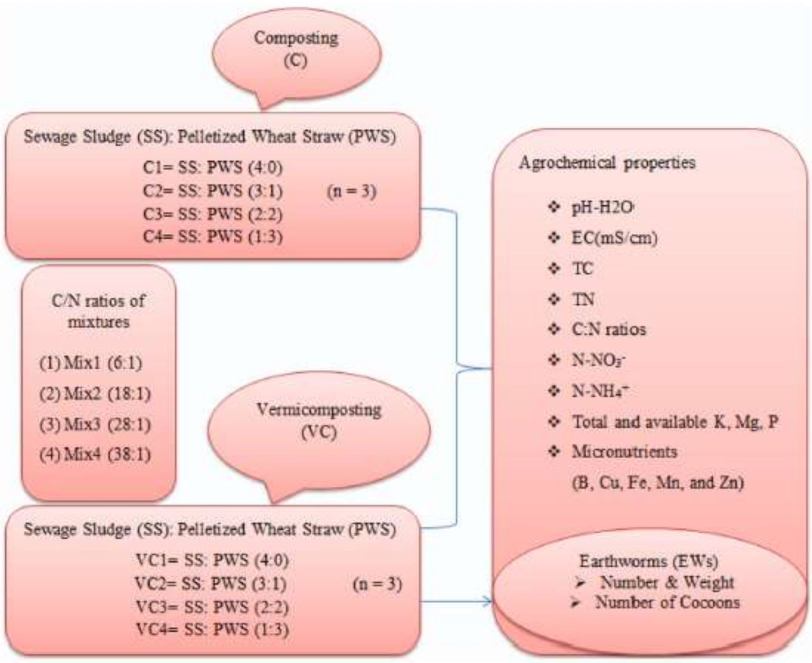

Fig. 1. The experimental study design.

2.4. Statistical analyses

The Shapiro-Wilk and Bartlett tests were used to ensure that the data were normal and homogeneous. The residual plot points were normally distributed around the mean with relatively homogeneous variances matching the variance analysis hypotheses. Two-way ANOVA was used to analyse the variation of agrochemical properties and determine significant differences in the properties of the final product between composting methods and C/N ratios. Post-hoc analysis was performed to determine significant differences using Tukey's test on the mean values. The statistical analyses used R version 4.0.2 and Statistica 12 software (StatSoft, Tulsa, USA). The level of the statistical test for significance was set at $p < 0.05$.

3. Results and discussion

3.1. Changes in temperature during vermicomposting and composting

The temperature during vermicomposting ranged from 19° to 28°C (Fig. 2a), which was lower than during composting and better for earthworms (Sinha et al., 2002). The temperature of the variant with a 38:1 C/N ratio (VC4) increased to 28.6 °C at the start (day one) of vermicomposting. During days three and two, the temperatures of (C3) and (C4) rapidly reached the thermophilic stage (>45 °C) (Fig. 2b). In four days, C4 reached a maximum thermophilic phase reading of 65.5 °C, while C3 reached 57.4 °C. The thermophilic phase lasted 14 days in variant C4 and ten days in C3.

The maximum temperatures lasted seven days for variant C2 (37.6 °C) and eight days for variant C1 (29.6 °C), the temperatures then gradually dropped over the remainder of the experimental period. The highest C/N ratio caused the most rapid thermophilic decomposition during the first 10–14 days. However, because of the depletion of easily degraded organic compounds, the degradation process resulted in less heat in these mixtures during the cooling phase (Wu et al., 2017). The variants with C/N ratios of 6:1(C1) and 18:1 (C2) reached maturity at mesophilic temperatures. These results might have stemmed from the high moisture content (79–84%) in these variants or because the SS: PWS ratio affected microbial activity, which influenced the temperature distribution of the composting process. After 60 days of composting, all variants' temperatures were near ambient and constant (Fig. 2b).

3.2. pH and electrical conductivity (EC)

The changes in pH and EC of the variants during composting and vermicomposting are shown in Fig. 3. Vermicomposting and composting showed significantly different trends in pH among the variants in each time period (Fig. 3). The pH significantly ($p < 0.001$) decreased during vermicomposting. For example, variant VC2 showed a significant reduction in pH from 7.3 to 5.2 (Fig. 3a), most likely caused by the transformation of organic phosphorus into orthophosphates, and the conversion of biomass into organic acids as humic substances during vermicomposting (Lazcano et al., 2008; Sharma and Garg, 2018; Suthar, 2010). During composting, the variants showed significant ($p < 0.05$) differences in pH with the values decreasing up to 90 days and then gradually increasing up to 120 days (Fig. 3b).

The highest pH value occurred in variant C1, which increased from an initial value of 6.9–8.6 during composting. According to Gigliotti et al. (2012), microorganisms break down organic nitrogen-containing compounds such as proteins to ammonia (NH_3). Ammonia is alkaline and elevates the pH of the compost. The final pH value of the compost was significantly higher than that of vermicompost. Several researchers have reported similar pH changes during the composting and vermicomposting of SS, crop straw, municipal solid waste, and livestock manure (Li et al., 2012; Singh and Suthar, 2012; Wang et al., 2014). According to Singh and Suthar (2012), the pH differences among variants could indicate the degree of organic material mineralization.

The EC increased significantly during vermicomposting and composting ($p < 0.001$); however, the final EC of the vermicompost (Fig. 3c) was significantly higher than that of compost (Fig. 3d). The variant with the highest C/N ratio (38:1) exhibited the highest EC value during vermicomposting. The production of inorganic ions and dissolved substances such as phosphate, ammonium, and nitrate could have contributed to the increase in EC in vermicompost (Lazcano et al., 2008; He et al., 2016; Negi and Suthar, 2018), and this occurrence indicated that vermicomposting might increase the mineralization of organic matter by transforming insoluble materials to soluble materials. At the end of composting, the values of EC ranged from 0.68 to 2.14 mS/cm, while for vermicomposting the values varied from 2.10 to 2.28 mS/cm. Thus, in both vermicompost and compost, all variants' EC values were within the recommended limit (4 mS/cm) (Li et al., 2012).

3.3. Total carbon(TC), total nitrogen(TN) and C/N ratio

The TC, TN, and C/N values changed significantly ($p < 0.001$) over the period of vermicomposting and composting (Fig. 4). Compared to the initial level, the TC decreased in all variants during vermicomposting (Fig. 4a) and composting (Fig. 4b), and the final TC in vermicompost was lower than that of compost in all variants.

The TC in vermicompost, VC1, VC2, VC3, and VC4, over the period of 120 days was 27.6%, 22.6%, 18.5%, and 16%, respectively. In compost, C1, C2, C3, and C4, the TC was 24.2%, 17.5%, 17.7%, and 11.9%, respectively. The most significant reduction in TC was recorded in the variant VC1 with a 6:1 C/N ratio during vermicomposting, followed by variant VC2 with an 18:1 C/N ratio. Microbial respiration and earthworm activity during vermicomposting reduce TC (Garg et al., 2006; Hanc et al., 2017). Rini et al. (2020) found a decrease in TC during vermicomposting of solid livestock wastes with earthworms of the species *Perionyx excavatus and Eudrilus eugeniae* for two cycles, 45 days and 90 days. Esmaeili et al. (2020) also showed a decrease in TC during vermicomposting pistachio waste mixed with cow dung in various ratios

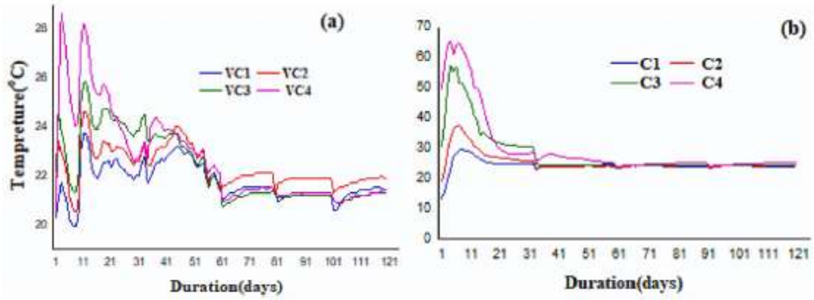

Fig. 2. Temperature variations during (a) vermicomposting and (b) composting.

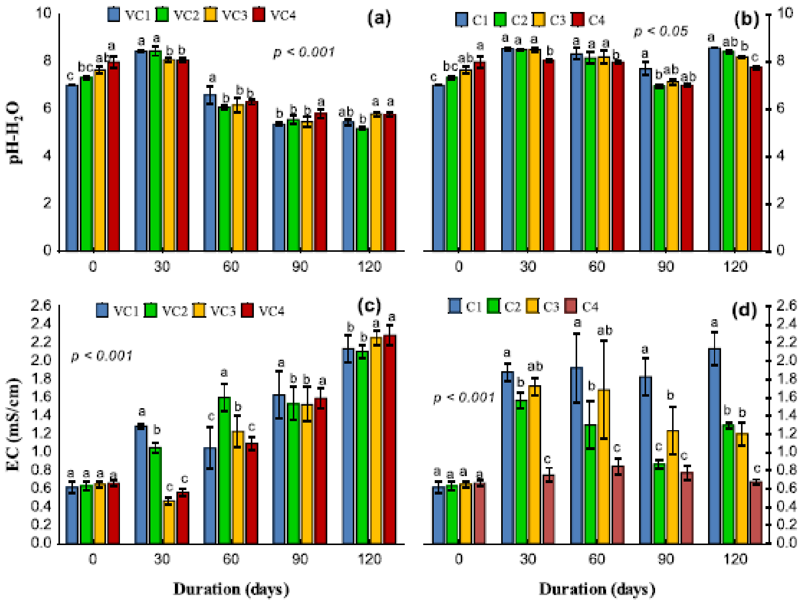

Fig. 3. Changes in pH and EC in variants during vermicomposting and composting. The bars represent the standard error of the mean (n = 3). Different letters indicate significant differences among the variants ($p < 0.05$) in each time period.

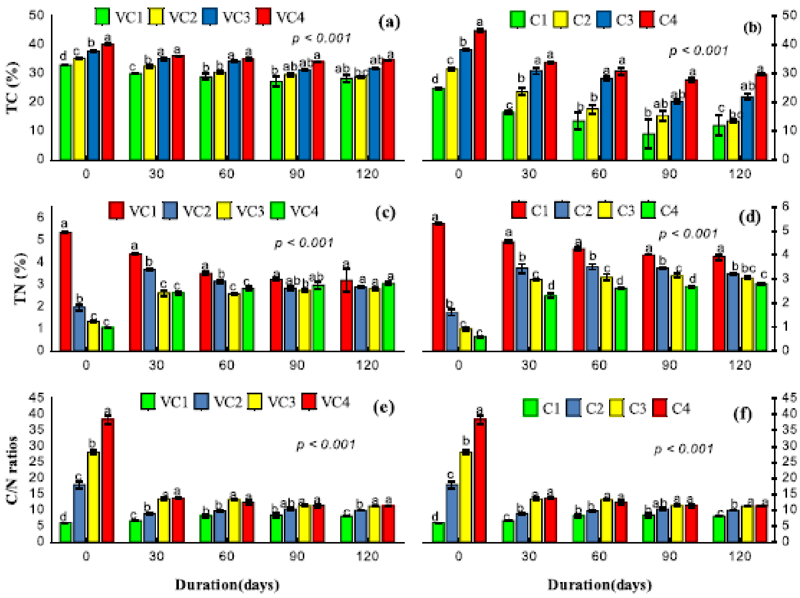

Fig. 4. TC, TN, and C/N in variants during of vermicomposting and composting. Bars indicate standard error of the mean (n = 3). Different letters indicate significant differences among the variants ($p < 0.05$) in each time period.

for 45 days using *Eisenia fetida*. Microbes use carbon to produce energy for metabolism (Esmaeili et al., 2020; Ravindran et al., 2015; Arumugam et al., 2018). The observed reduction in TC revealed that organic compounds were being bio-degraded and mineralized as a result of microbial activity in the variants, resulting in the release of carbon dioxide (Khatua et al., 2018; Yang et al., 2017).

TN increased in all variants during vermicomposting (Fig. 4c) and composting (Fig. 4d) from the initial values, except in the variant VC1 with a 6:1 C/N ratio. However, the final TN in vermicompost was lower than in compost in all variants. In the final (120 days) vermicompost, the increase in TN in variants VC2, VC3, and VC4 reached 31.5%, 52.5%, and 65.6%, respectively, while in C2, C3, and C4 it reached 43.4%, 59.4%, and 66.1%, respectively. The reduction in TN was 68% in VC1 and 30.7% in C1 after 120 days. Pigatin et al. (2016) found that by using *E. foetida* for vermicomposting, the TN in agricultural residues increased from 19.5% to 152% in 60 days. Dume et al. (2022) reported that vermicomposting hydrolysed chicken feather residue for 120 days using *Eisenia andrei* earthworms increased TN content by 42.3–56.86%, compared to 56.4–61.4% during composting (without earthworms). Kaushik and Garg (2004) also reported that 11 weeks of vermicomposting using *E. foetida* with sludge from textile mills mixed with cow dung and agricultural waste increased nitrogen levels 2–3 times over the initial feedstocks. Sudkolai and Nourbakhsh (2017) found that after 60 days of vermicomposting with *E. foetida*, wheat residue vermicompost had 3.2 times the TN content of the initial feedstocks. The increase in TN levels in vermicompost is most likely due to the addition of organic carbon from CO_2 and nitrogen from earthworms' nitrogenous excretory substances in the form of mucus and growth-stimulating hormones.

The C/N ratio decreased during vermicomposting (Fig. 4e) and composting (Fig. 4f). The reduction in C/N ratio in vermicompost, VC2, VC3, and VC4, reached 81%, 148.9%, 238.3%, respectively, and in compost, C2, C3, C4, 107.2%, 190.4%, and 227.9%, respectively. Because it reflected the rates of stabilization and mineralization, the C/N ratio indicated vermicompost maturity (Arumugam et al., 2018; Srivastava et al., 2020; Soobhany et al., 2015). Over time, the decrease in the C/N ratio was correlated with enhanced nitrogen content and organic matter degradation (Devi and Khwairakpam, 2020a). Previous research by Karmegam et al. (2019) and Biruntha et al. (2020) supported these findings, reporting up to a 50.9% and a 48.8% reduction in the C/N ratio during vermicomposting of cow dung and cow dung with vegetable waste, respectively. Zhi-wei et al. (2019) reported that feeding rice straw and kitchen waste to *Eisenia fetida* for 45 days decreased the C/N ratio by 58.55–71.96%. Boruah et al. (2019) also found the C/N ratio was reduced by 91.10% during the vermicomposting of citronella bagasse and paper mill sludge by *E. fetida* for 45 days. The final C/N ratios recorded for all the variants were within the recommended value (<20) for soil applications (Esmaeili et al., 2020).

3.4. Mineral Nitrogen (N-NO_3^- and N-NH_4^+)

Fig. 5 shows the content of nitrate nitrogen (N-NO_3^-) and ammonium nitrogen (N-NH_4^+). The N-NO_3^- content during vermicomposting (Fig. 5a) and composting (Fig. 5b) increased in all variants. The final N-NO_3^- content with respect to the initial value, in vermicompost showed an overall increase of 99.96% (VC1), 99.89% (VC2), 99.80% (VC3), and 99.70% (VC4); in compost, the increase was 98.96% (C1), 97.15% (C2), 91.3% (C3), and 98.97% (C4). The final N-NH_4^+ content in vermicompost decreased in VC1 and VC2, but increased in VC3 and VC4 (Fig. 5c). In compost, the final N-NH_4^+ content increased by 70.4% (C1), 67.2% (C2), 72.4% (C3), and 15% (C4) (Fig. 5d). The increase in N-NO_3^- levels during vermicomposting was consistent with the findings of Hait and Tare (2012), who showed a decrease in N-NH_4^+ and an increase in N-NO_3^- during SS vermicomposting versus composting. During the nitrification process, a significant proportion of N-NH_4^+ can be transformed into N-NO_3^-, and a portion of N-NH_4^+ can also be vaporised as NH_3.

There is also the potential for nitrogen loss due to N-NO_3^- being converted into N_2 during denitrification (Van Vliet et al., 2004). Tognetti et al. (2007) claimed that the decline of N-NH_4^+ implied compost maturity, and Wu et al. (2017) also reported a similar trend of N-NH_4^+ and N-NO_3^- changes that occurred during the composting of pig manure. N-NH_4^+ was reduced during the decomposition of organic matter due to nitrogen fixation, ammonia volatilization, and immobilisation by microbes (Raj and Antil, 2011; Van Vliet et al., 2004; Awasthi et al., 2016). The reduction of NH_4^+ in vermicompost indicated maturity of the final vermicompost product.

3.5. Total and available contents of K, Mg and P macronutrients

The total content of K, Mg and P was significantly ($p < 0.001$) increased during vermicomposting and composting (Fig. 6). The final total K level increased significantly among the variants, with an overall increase of 56%, 57%, 63%, and 73% in vermicompost for VC1, VC2, VC3, and VC4, respectively (Fig. 6a) and 18%, 34%, 49%, and 56% in compost for C1, C2, C3, and C4, respectively (Fig. 6b). The percentage of

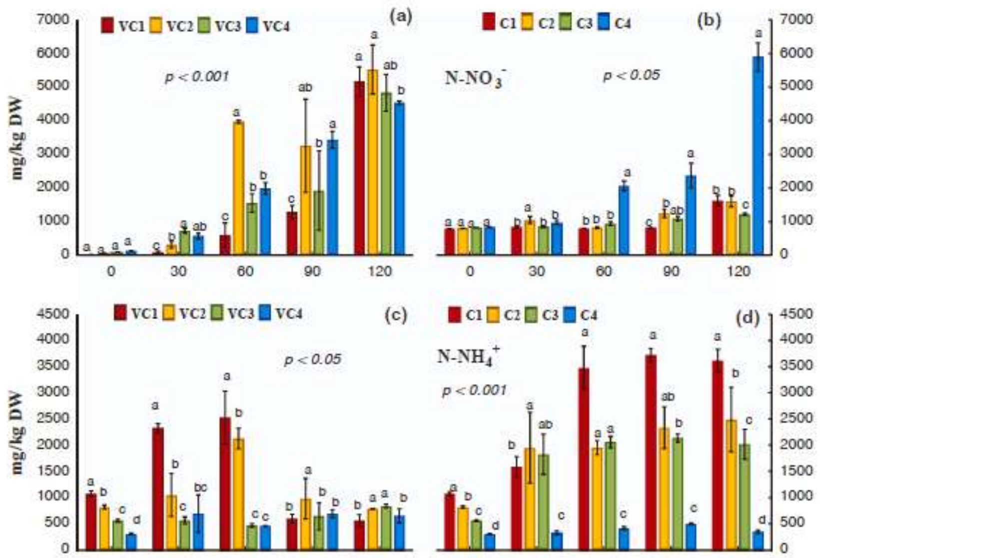

Fig. 5. Mineral nitrogen (N-NO_3^- and N-NH_4^+) in variants during vermicomposting and composting. Bars indicate the standard error of the mean (n = 3). Different letters indicate significant differences among the variants ($p < 0.05$) in each time period.

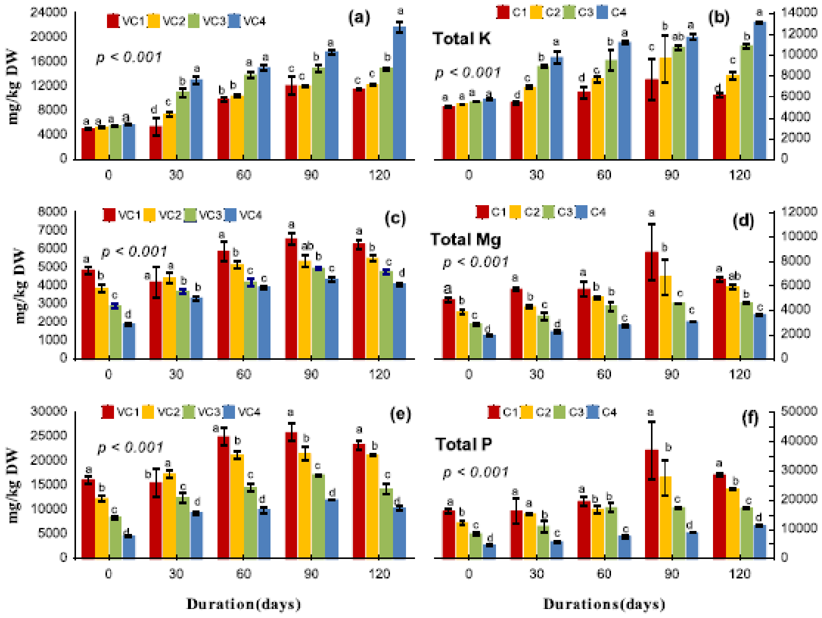

Fig. 6. Total K, Mg, and P macronutrients in variants during vermicomposting and composting. Bars indicate the standard error of the mean (n = 3). Different letters indicate significant differences among the variants ($p < 0.05$) in each time period.

total K in vermicompost was 37–88% higher than in compost. Total Mg significantly increased with overall changes of 23%, 30%, 39%, and 53% in vermicompost (Fig. 6c) for VC1, VC2, VC3, and VC4, respectively, and 26%, 35%, 37%, and 47% in compost (Fig. 6d) for C1, C2, C3, and C4, respectively. However, vermicompost showed significant increases of total Mg in the variants with 28:1 and 38:1 C/N ratios by 4% and 12% over compost. The total P increased significantly with overall increases of 31%, 42%, 41%, and 56% for VC1, VC2, VC3, and VC4, respectively (Fig. 6e), and 43%, 48%, 51%, and 59% in compost C1, C2, C3, and C4, respectively (Fig. 6f).

The concentration of plant-available K, Mg, and P increased during vermicomposting and composting (Fig. 7), with significant ($p < 0.001$)

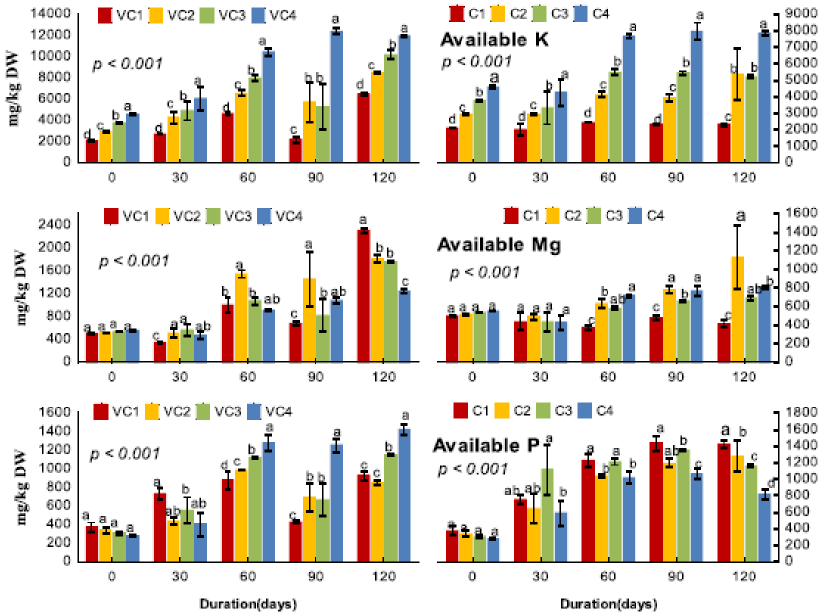

Fig. 7. Available K, Mg, and P macronutrients in variants during vermicomposting and composting. Bars indicate standard error of the mean (n = 3). Different letters indicate significant differences among the variants ($p < 0.05$) in each time period.

differences during vermicomposting. The available K in vermicompost increased significantly among the variants, with overall increases of 68%, 66%, 63%, and 62% in VC1, VC2, VC3, and VC4, respectively (Figs. 7a), and9%, 45%, 28%, and 42% in compost for C1, C2, C3, and C4, respectively (Fig. 7b); the percentage of available K in vermicompost increased by 53–183% over compost.

The overall increase in available Mg in vermicompost was 78%, 71%, 70%, and 56% for VC1, VC2, VC3, and VC4, respectively (Figs. 7c), and 3%, 54%, 23%, and 32% in compost for C1, C2, C3, and C4, respectively (Fig. 7d), and the percentage of available Mg in vermicompost over compost increased from 54% to 453%. Available P rose sharply among the variants overall increases of 60%, 60%, 73%, and 81% in vermicompost for VC1, VC2, VC3, and VC4, respectively (Fig. 7e), and 74%, 73%, 74%, and 66% in compost for C1, C2, C3, and C4, respectively (Fig. 7f). Vermicompost with 38:1 C/N showed a significant increase of 79% in available P over compost.

The changes in K, Mg, and P were probably due to the high mass loss under vermicomposting. There was no additional nutrient input unless the worms died and decomposed during vermicomposting. An increase in these macronutrients has been linked to decrease in weight and organic matter (Wani and Rao, 2013). The increase in potassium might be related to acid production by microbes, which causes the solubilization of organically bound potassium (Garg et al., 2006), and also an earthworm's intestine may aid in the release of K in vermicompost (Khatua et al., 2018; Pramanik et al., 2007). These influences could have resulted in an overall rise in total K in the variants. An earthworm's gut can increase the release of potassium in vermicompost (Pramanik et al., 2007; Khatua et al., 2018). These influences could all have contributed to the variants' overall potassium rise over time. The existence of phosphatase in earthworm intestine that enhance P release in various forms and phosphorus-solubilizing microbes in their casts may explain the rise in phosphorus, (Deka et al., 2011). The mineralization and mobilization of organic matter by earthworms, and the combined effect of microorganisms and phosphate excretion may also have increased P content (Yadav and Garg, 2019). The reduction in pH could also have enhanced the solubilization of phosphorous and the release of organically bound phosphate, thus increasing its concentration in the final product (Devi and Khwairakpam, 2020a; b). Ghosh et al. (2018) found phytase enzymes that enhance phosphorus mineralization.

3.6. *Availability of micronutrients, B, Cu, Fe, Mn, and Zn*

Fig. 8 depicts the available B, Cu, Fe, Mn, and Zn contents during vermicomposting and composting. The availability of these nutrients increased significantly ($p < 0.001$) in all vermicomposting and composting variants compared to initial concentrations. The B content in vermicompost increased significantly among the variants, with overall increases of 80%, 78%, 82%, and 82% in vermicompost for VC1, VC2, VC3, and VC4, respectively (Fig. 8a), and 21%, 67%, 60%, and 65% in compost for C1, C2, C3, and C4, respectively (Fig. 8b), and the available B in vermicompost increased significantly from 48% to 303% over compost. The content of available Cu in vermicompost increased significantly from 2.5% to 82% over compost (Fig. 8c). The available Fe was significantly elevated in vermicompost with overall increases of 8%, 25%, 48%, and 66% for VC1, VC2, VC3, and VC4, respectively (Fig. 8e), and 55%, 59%, 49%, and 75% in compost for C1, C2, C3, and C4, respectively (Fig. 8f). The lower Fe contents found in vermicompost over compost may be due to the bioaccumulation of Fe in earthworm tissues (Suthar and Gairola, 2014). The final results of available Mn in compost showed an overall increase of 41%, 39%, and 49% for C2, C3, and C4, respectively (Fig. 8h), and a 56% decrease in C1, whereas in vermicompost, Mn decreased by 3%, 7%, 11%, and 27% in VC1, VC2, VC3, and VC4, respectively (Fig. 8g). Available Zn increased significantly among the variants with overall increases of 47%, 78%, 78%, and 81% for C1, C2, C3, and C4, respectively (Fig. 8j), and 60%, 68%, 72%, and 79% in vermicompost for VC1, VC2, VC3, and VC4, respectively (Fig. 8i).

The increases in these nutrients are caused by the earthworm's

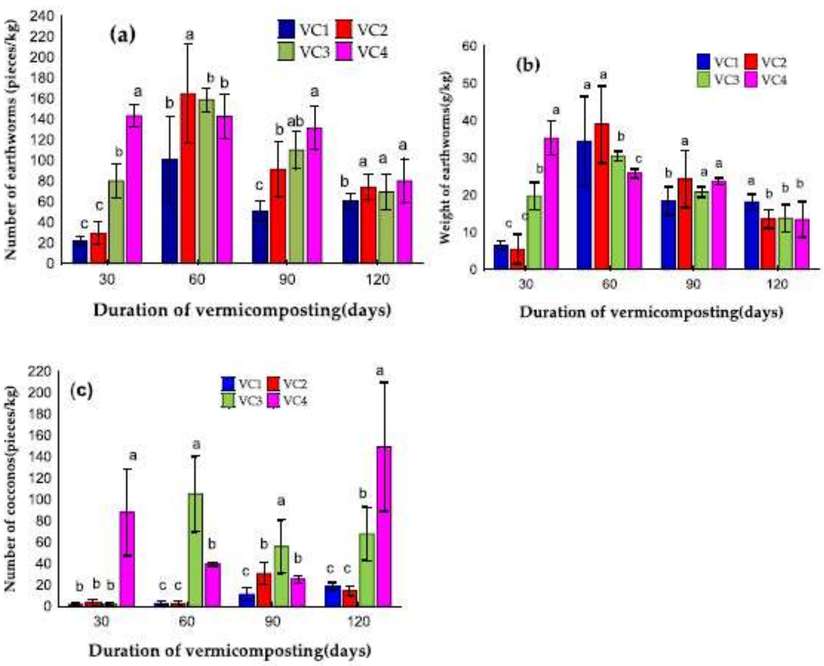

Fig. 9. Number (a) and weight (b) of earthworms and number of cocoons (c) during vermicomposting. Bars indicate standard error of the mean (n = 3). Different letters indicate significant differences among the variants ($p < 0.05$) in each time period.

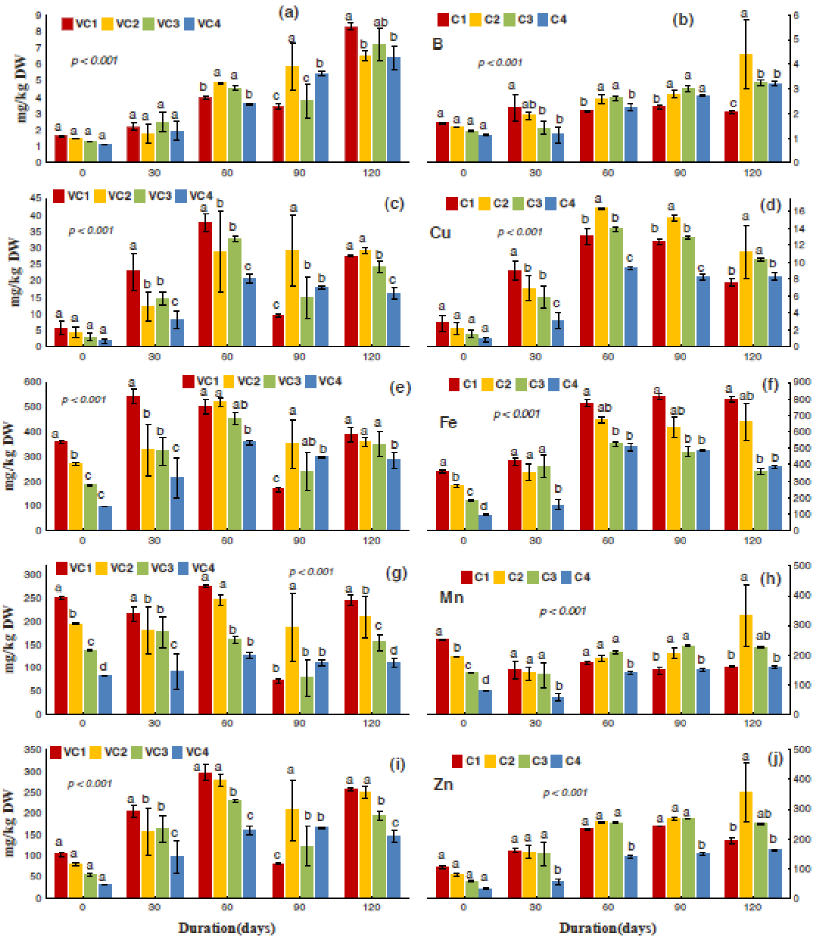

Fig. 8. Available B, Cu, Fe, Mn, Zn micronutrients in variants during vermicomposting and composting. Bars indicate standard error of the mean (n = 3). Different letters indicate significant differences among the variants ($p < 0.05$) in each time period.

catabolic activity on carbonic anhydrase in the calciferous gland and the accumulation of Zn in their tissues during vermicomposting (Gupta and Garg, 2008; Manyuchi and Phiri, 2013; El-Haddad et al., 2014). Our results are consistent with those of Gupta and Garg (2008), who found higher concentrations of micronutrients in sewage sludge vermicompost, and the increase in available micronutrient concentrations could be attributed to the progressive mineralization of organic matter and loss through respiration during the composting process (Amir et al., 2005; Lv et al., 2016). Our increases in B, Cu, Fe, Mn, and Zn followed the same pattern as those of Pattnaik and Reddy (2010) and Dortzbach (2010) reported that pig manure strongly enhanced Mn, Cu, and Zn concentrations.

3.7. Growth and reproduction of earthworms (E. andrei)

Fig. 9 depicts the number and weight of earthworms and the number of cocoons. The average initial earthworm weight and number were 0.2 g/piece and 125 pieces per kg of substrate.

The initial weight of earthworms was 25 g per kg of substrate. Earthworm production was very low during the first 30 days in the variants with 6:1 and 18:1 C/N ratios relative to the variants with 28:1 and 38:1 C/N ratios. The maximum number (Fig. 9a), 165 pieces/kg, and weight (Fig. 9b), 39 g/kg, were recorded in the variant with an 18:1 C/N ratio after 60 days of vermicomposting, while the lowest number, 21pieces/kg, occurred in the 6:1 C/N variant after 30 days of vermicomposting (Fig. 9c). Cocoon production was very low during the first 30 days except in the 38:1 C/N group. After 120 days, the 38:1 C/N variant had the highest number of cocoons (150 pieces/kg), while the variant with a 28:1 C/N ratio had the lowest (15 pieces/kg). Cocoon production fluctuated during the vermicomposting period. The cocoon production rate was initially low, but increased with vermicomposting time. The C/N ratio, a vital determinant of earthworm production, could explain differences in cocoon production among the variants. A high C/N ratio promotes growth and reproduction by providing earthworms with greater amounts of organic matter (Gupta et al., 2007).

4. Conclusions

This research highlights the crucial role of carbon-to-nitrogen (C/N) ratios and the composting and vermicomposting processes in determining the characteristics of the final products. Our findings show that both composting and vermicomposting were feasible at a range of C/N ratios, although variations were observed in the final product quality. However, a comparison between the two end-products derived from the same initial materials (sewage sludge and pelletized wheat straw) revealed that vermicomposting led to increased electrical conductivity (EC), total and available potassium (K), available magnesium (Mg), total Mg, available phosphorus (P), nitrate nitrogen ($N\text{-}NO_3^-$), available boron (B), and copper (Cu). However, vermicomposting resulted in decreases in pH, total carbon (TC) and total nitrogen (TN), available iron (Fe), manganese (Mn), zinc (Zn), and ammonium (NH_4^+). The highest number of earthworms was recorded in the variant with an 18:1 C/N ratio after 60 days of vermicomposting, while the lowest number was observed in the variant with a 6:1 C/N ratio after 30 days of vermicomposting. The agrochemical characteristics of the 18:1 C/N ratio vermicompost significantly outperformed those of compost; therefore, vermicomposting demonstrated superior agrochemical properties compared to composting. This study confirmed that vermicomposting sewage sludge mixed with pelletized wheat straw at an 18:1 C/N ratio yielded the best results, likely due to improved and favourable agrochemical properties.

CRediT authorship contribution statement

Bayu Dume: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization. **Ales Hanc:** Conceptualization, Formal analysis, Resources, Data curation, Writing – original draft, Methodology, Supervision, Project administration, Funding acquisition. **Pavel Svehla:** Conceptualization, Methodology, Supervision, Formal analysis, Resources, Data curation, Writing – original draft. **Pavel Michal, Abraham Demelash Chane:** Sample, Data collection. **Abebe Nigussie:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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6. Summary discussion

6.1. Effects of composting and vermicomposting on carbon dioxide and methane emissions with varying straw pellet ratios

Initially, the emissions of CO_2 and CH_4 were highest during composting/vermicomposting of sewage sludge (SS) and gradually declined over time. The fate of carbon in the waste substrate was closely linked to the release of CH_4 and CO_2 during both composting and vermicomposting processes. Vermicomposting generally results in reduced CH_4 emissions due to the aerobic conditions it creates (Manios et al. 2007).Vermicomposting led to higher CO_2 emissions, indicating further decomposition progress. CH_4 emissions were significantly reduced by 18-38% through vermicomposting, while CO_2 emissions increased by 64-89% compared to composting. The addition of the mixing agent (straw pellet-SP) decreased CO_2 emissions by 60-70% and CH_4 emissions by 30-80% compared to the no addition of SP. Mass balance analysis revealed that composting resulted in a carbon loss of 5.5-10.4%, with CH_4 accounting for 0.3-1.7% and CO_2 accounting for 2.3-8.7% of the emitted gases. In contrast, vermicomposting led to a carbon loss of 8.9-13.7%, with CH_4 representing 0.1-0.6% and CO_2 accounting for 5.0-11.6% of the emitted gases.

6.2. Evaluating enzymatic activities during composting and vermicomposting of sewage sludge at different proportions of straw pellets

The enzymatic activity during composting and vermicomposting of SS and their mixtures exhibited the most significant decrease in the first half of the processes. After four months, the sludge with 50% SP addition showed the least enzymatic activity, indicating the importance of straw in accelerating the production of mature compost from SS. Similarly, lower enzyme activity was observed during vermicomposting of fresh feedstocks compared to pre-composted material due to the digestive processes within the earthworms. The enzymatic activity during composting and vermicomposting of SS and their mixtures stabilized at the following values: β-D-glucosidase 50 mmol MUFG/h/g dw, acid phosphatase 200 mmol MUFP/h/g dw, arylsulphatase 10 mmol MUFS/h/g dw, lipase 1,000 mmol MUFY/h/g dw, chitinase 50 mmol MUFN/h/g dw, cellobiohydrolase 20 mmol MUFC/h/g dw, alanine aminopeptidase 50 mmol AMCA/h/g dw, and leucine aminopeptidase 50 mmol AMCL/h/g dw. These values and lower indicate the maturity and stability of the final products. The contents of microorganisms including fungi, bacteria, actinobacteria, gram positive bacteria (G+ bacteria), gram negative bacteria (G- bacteria), and total microbial biomass (TBM) were analysed. Fungi were significantly decreased in final vermicompost for all variants as compared with initial. Bacteria, actinobacteria, G+ bacteria, G- bacteria and TBM significantly decreased in some variants (100% SS and 75% SS+ 25% SP); however, significantly increased in remaining variants (50% SS + 50% SP and 25% SS + 75% SP). The contents of microorganisms (fungi, bacteria, actinobacteria, G+ bacteria, G- bacteria and TBM) varied significantly ($p < 0.001$) among variants. Bacteria and fungi are frequently employed in the degradation of OMPs present in SS (Semblante et al. 2015).

6.3. Effects of vermicomposting on potentially toxic elements in sewage sludge

The results demonstrated that the mixing ratio of SS and the bulking agent (SP) decreased the content of potentially toxic elements (PTEs) as compared to control (without earthworms and SP). The PTE content in earthworm tissues showed a significant increase; except for lead (Pb) which was below the detection limit (0.02 mg kg^{-1}) in earthworm tissues. The reduction percentages of PTEs with respect to control were as follows: arsenic (As) (14-67%), cadmium (Cd) (4-39%), chromium (Cr) (24-70%), copper (Cu) (20-68%), lead (Pb) (39-75%), and zinc (Zn) (16-65%). In terms of the removal efficiency, the SS mixtures with SP as the bulking agent can be arranged in the following order: 75% SP > 50% SP > 25% SP > 0% SP. The findings indicate that vermicomposting can be an effective technology for reducing PTEs in SS as compared to control. Despite having higher content of potentially toxic elements (PTEs) compared to the initial vermicompost, all PTE levels remained within the acceptable range of compost quality standards defined by the European Union (EU). This suggests that the vermicomposts produced are suitable for agricultural use. Overall, the results indicate that vermicomposting is a suitable technology for PTE reduction in SS.

6.4. Evaluating the earthworms influence on organic micropollutant in sewage sludge

The concentration of some PPCPs and EDCs decreased from the initial concentration (day 0) to the final concentration (day 120) in the final products (vermicomposts/composts). The concentrations varied significantly ($p < 0.05$) among the variants. The decrease in PPCP and EDC concentrations was thought to be caused by bioaccumulation in earthworm tissue during vermicomposting, in their intestine, or by skin absorption. However, a decrease in vermicompost's weight and volume may increase in PPCP and EDC concentration (Mazzeo et al. 2023). According to a report by Hammer & Palmowski (2021), the efficiency of micropollutant removal can differ based on the particular substances and research conducted.

Table 9. OMPs reduction percentage in the final products after 120 days of processing (n =3)

	Reduction percentage R (%)										
Variants (+EW)	AM	APAP	AT	ATN	BPA	CAF	CBZ	CBZE	CETI	CITA	CLM
100%SS	23	100	88	42	53	66	14	100	38	12	89
75%SS+25%SP	-11	100	74	-2	-1	58	-29	100	-17	-17	81
50%SS+50%SP	-32	100	100	-34	-8	57	-66	100	-34	-46	77
25%SS+75%SP	-92	100	100	-134	-4	25	-144	100	-46	-68	69
Average	-28	100	91	-32	10	52	-56	100	-15	-30	79
Variants (-EW)	AM	APAP	AT	ATN	BPA	CAF	CBZ	CBZE	CETI	CITA	CLM
100%SS	9	100	80	36	45	62	1	100	42	25	85
75%SS+25%SP	-38	100	79	-11	-23	58	-45	100	-9	-55	86
50%SS+50%SP	-58	100	72	-27	16	59	-99	100	-47	-74	79
25%SS+75%SP	-124	100	100	-149	-17	25	-183	100	-48	-93	62
Average	-53	100	83	-38	5	51	-82	100	-16	-49	78
Variants (+EW)	DAZ	DCF	E1	EQ	GPN	GNT	HCTZ	IBF	LMT	MET	MIRT
100%SS	73	96	75	100	62	74	77	100	65	71	54
75%SS+25%SP	58	97	50	100	20	48	55	86	59	69	42
50%SS+50%SP	52	96	49	100	55	41	55	83	61	73	53
25%SS+75%SP	23	94	67	100	33	16	71	91	57	69	61
Average	52	96	60	100	43	45	65	90	61	71	53
Variants (-EW)	DAZ	DCF	E1	EQ	GPN	GNT	HCTZ	IBF	LMT	MET	MIRT
100%SS	74	96	79	100	64	78	76	89	51	73	49
75%SS+25%SP	52	97	55	100	65	55	52	85	55	77	37
50%SS+50%SP	47	95	38	100	64	37	36	89	49	75	29
25%SS+75%SP	12	92	-4	100	43	-41	57	79	49	59	41
Average	46	95	42	100	59	32	55	86	51	71	39
Variants (+EW)	OPZ	PXN	SAA	SMX	SPD	TE	TCS	TMD	TMP	VEN	
100%SS	100	68	73	100	84	19	58	29	92	7	
75%SS+25%SP	100	66	56	100	74	-6	71	4	87	-33	
50%SS+50%SP	100	57	1	100	69	-32	86	3	85	-33	
25%SS+75%SP	100	27	19	100	61	-1150	90	-15	76	-46	
Average	100	55	37	100	72	-292	76	5	85	-26	
Variants (-EW)	OPZ	PXN	SAA	SMX	SPD	TE	TCS	TMD	TMP	VEN	
100%SS	100	72	43	100	78	10	17	37	91	20	
75%SS+25%SP	100	60	34	100	63	-28	36	20	86	-9	
50%SS+50%SP	100	59	32	100	65	-46	51	-17	78	-241	
25%SS+75%SP	100	21	5	100	55	-888	50	-14	70	-63	
Average	100	53	29	100	65	-238	39	7	81	-73	

AM = Amitriptyline, APAP = Acetaminophen, AT = Atorvastatin, ATN = Atenolol, BPA = bisphenol A, CAF = caffeine, CBZ = carbamazepine, CBZE = Carbamazepine 10,11-epoxide, CETI = cetirizine, CITA = citalopram, CLM = Clarithromycin, DAZ = Daidzein, DCF = diclofenac, Equol, E1 = Estrone, EQ = Equol, GPN = Gabapentin, GNT = Genistein, HCTZ = Hydrochlorothiazide, IBF = ibuprofen, LMT = Lamotrigine, MET = Metoprolol, MIRT = mirtazapine, OPZ = Omeprazole, PXN = Paraxanthine, SAA = Sulfanilamide, SMX = Sulfamethoxazole, SPD = sulfapyridine, TE = telmisartan, TCS = triclosan, TMD = Tramadol, TMP = Trimethoprim, VEN = venlafaxine. SS = sewage sludge, SP= straw pellet, (+EW) = variants with earthworms, (-EW) = variants without earthworms.

The range of removal can fall anywhere between nearly complete to no/insignificant removal. To categorize this range, Hammer & Palmowski (2021) have divided it into five groups: insignificant removal (0-20%), low removal (20-40%), medium removal (40-60%), high removal (60-80%), and very high removal (80%). Variants with earthworms (+EW) reduced some OMPs (PPCPs and EDCs) than variants without earthworms (-EW) (Table 9). The reduction of these OMPs substances was recorded; however there was an increase of some OMPs. These present findings, show some inconsistency regarding the removal efficiency of certain PPCPs and EDCs during vermicomposting of SS. It is important to note that the removal efficiency of PPCPs and EDCs could also be influenced by factors such as the type and amount of microorganisms present, the organic loading rate, the retention time, and the system's temperature (Shi et al. 2020). Therefore, it is necessary to conduct more studies under different experimental conditions to understand better the fate and behaviour of particular OMPs during vermicomposting of SS. Innemanová et al. (2022) conducted a study on the removal efficiency of PPCP and EDC during vermicomposting of dewatered SS under outdoor conditions for one year. Nevertheless, the behaviour of these compounds was not extensively elaborated upon. Moreover, the experiment was conducted outdoors, which could have been impacted by various external factors such as temperature and humidity. The average negative reduction percentages R (%) of some OMPs showed that the concentrations of PPCP had increased (Table 9). During vermicomposting/composting, specific organic micropollutants (OMPs) might experience an increase due to intricate interactions involving earthworms, microorganisms, and the organic substrate. As earthworms ingest and process the organic material, the vermicomposting system undergoes shifts in microbial communities and metabolic processes. Consequently, certain OMPs could undergo breakdown or transformation into metabolites with heightened stability and concentration, thus raising their levels in the final product. Additionally, earthworms possess the capability to selectively accumulate particular compounds, potentially concentrating OMPs within their tissues, thereby influencing OMP concentrations in the resulting vermicompost. This intricate interplay of biological and chemical processes contributes to the variation in OMP concentrations during vermicomposting. The observed increase in OMP concentrations during vermicomposting or composting might arise from the conversion of these compounds into unmeasured forms. Furthermore, microbial degradation of organic matter during these processes could release certain compounds from the SS. Earthworm presence during vermicomposting could further augment compound concentrations by modifying microbial activity and organic matter decomposition rates, potentially leading to the creation of novel compounds or the liberation of previously bound ones (Mazzeo et al. 2023; Innemanová et al. 2022). In general, the reduction in PPCPs and EDCs revealed that their absorption/accumulation in earthworms outweighed the volume reduction effect during processes, and the additive materials enhanced the PPCP and EDC removal efficiency even further (Zeb et al. 2020), and also due to microbial degradations and adsorption of these chemical substances onto organic matter of compost (Dubey et al. 2022).

Out of the 32 evaluated OMPs, earthworm tissues initially contained only 6.05 ng g^{-1} of CAF and 2.24 ng g^{-1} of TE, while the other 30 OMPs were not detected in the initial analysis of earthworm tissues. However, at the end of vermicomposting, the following seven PPCPs were detected at higher concentrations in the earthworm tissues: CBZ, CETI, DCF, CAF, CITA, TCS, and TE. The highest concentration of PPCP in earthworm tissue was TE. Because of the reasonably consistent relationships between the concentrations of certain pollutants in earthworms, earthworms accumulate a significant amount of PPCPs and EDCs in their tissues and may be a useful biological indicator of contamination. The earthworm's interaction with local edaphic factors such as pH, organic matter content, enzyme activities and are mainly responsible for the accumulating PPCPs and EDCs (Zeb et al. 2020). TC reduction also results in the formation of intermediate metabolites and acids (humic acids), which lower the pH of the sludge mixtures (Zziwa et al. 2021).

Vermidegradation is the process by which various pollutants in earthworms are degraded using enzymes such as CYP450 and peroxidase or by gut microbes, also known as ‘vermin-endophytes’ which are microbes, bacteria, or fungi that live within earthworm tissues without causing any disease (Zeb et al. 2020). Vermidegradation is primarily concerns removing of organic micro-pollutant compounds such as PPCPs and EDCs (Bhat et al. 2018). The negative percentage of vermidegradation for some OMPs (PPCPs and EDCs) implies that the final concentrations of some OMPs found in vermicompost were greater than the initial input materials, which implied that the earthworms had insignificant influence on the degradation of some PPCPs and EDCs during vermicomposting (Shi et al. 2020). This difference might be due to the extremely high concentration in the variant without earthworms (-EW) (control) and indicating that these PPCPs and EDCs are resistant to vermidegradation (Haiba et al. 2018). Shi et al. (2020) explain that vermiaccumulation is the process by which earthworms absorb and retain pollutants, leading to a decrease in the concentration of substances like PPCPs and EDCs in SS. The concentrations of PPCPs and EDCs in earthworm tissues were recorded by examining earthworm samples before and after vermicomposting. *Eisenia andrei*, a species of earthworm, has the ability to ingest and process pollutants during vermicomposting. This includes the process of grinding and digestion, allowing for the absorption of these pollutants through the intestinal tract into the worm tissues. Vermicomposting has been found to be effective in reducing the concentration of OMPs in SS, thus addressing the issue. However, a new question arises about how to handle the earthworms that have accumulated OMPs in their bodies, as highlighted by Shi et al. (2020). The EW will accumulate PPCP and EDC to a certain level, after which the concentration of pollutants inside the organism will either stop increasing or the organism, will die. In both cases, this means that earthworms' ability to degrade PPCP and EDC is reduced. Earthworms' ability to degrade PPCP and EDC is reduced in both cases. Measurable influence of earthworms may be possible only if new earthworms are used in each situation (Zeb et al. 2020).

6.5. Sewage sludge composting and vermicomposting at various C/N ratios: Technological feasibility & product quality

The effects of carbon-to-nitrogen (C/N) ratios, the composting and vermicomposting processes on the final product's properties were emphasized. Both composting and vermicomposting were found to be viable across various C/N ratios, though the quality of the end-products varied. A comparison of the two end-products obtained from the same starting materials (SS and SP) revealed that vermicomposting led to notable increase in electrical conductivity (EC), total and available potassium (K), available magnesium (Mg), total Mg, available phosphorus (P), nitrate nitrogen ($N\text{-}NO_3^-$), available boron (B), and copper (Cu). However, vermicomposting resulted in decreased pH, total carbon (TC), total nitrogen (TN), available iron (Fe), manganese (Mn), zinc (Zn), and ammonium (NH_4^+). In particular, the variant with an 18:1 C/N ratio during the 60-day vermicomposting process recorded the highest number of earthworms, whereas the lowest number of earthworms was observed in the variant with a 6:1 C/N ratio during a 30-day vermicomposting process. The agrochemical characteristics of the 18:1 C/N ratio variant significantly outperformed those of the compost. Overall, the research underscores the importance of C/N ratios and the choice of composting method in determining the final product's properties, with vermicomposting showing distinct advantages in certain areas over composting.

7. Conclusion

Vermicomposting demonstrated a noteworthy decrease in CH_4 emissions, accompanied by a simultaneous increase in CO_2 emissions. The introduction of SP as a mixing agent yielded a significant reduction in both CO_2 and CH_4 emissions. These findings underscore the potential of both composting and vermicomposting, augmented by the incorporation of a mixing agent like SP, as viable strategies for mitigation, particularly concerning the reduction of CH_4 and CO_2 emissions, contingent upon the specific targeted gas.

Earthworms possess a specialized digestive system with enzymes that aid in breaking down organic matter. As they consume the organic material in the compost, the enzymatic processes are initiated within their digestive system to decompose and digest the organic matter. When earthworms are introduced into a system for the purpose of composting or organic matter decomposition, some of the enzymatic activity that would have otherwise occurred externally (compost) is redirected to their internal digestive processes. As a result, the overall enzymatic activity in the surrounding compost may decrease, as the earthworms efficiently digest and process the organic matter within their digestive tract. In essence, the earthworms act as efficient decomposers themselves, utilizing enzymatic processes internally, which can lead to a decrease in enzymatic activity in the external environment or compost where they are present. This phenomenon is a natural consequence of their role in the decomposition process and is often observed when earthworms are introduced into composting systems. Similarly, during vermicomposting of fresh feedstocks, lower enzyme activity was observed compared to pre-composted material, as a result of the digestive processes within the earthworms. Furthermore, the final vermicompost produced from fresh feedstocks exhibited lower microbial biomass, fewer fungi, and G- bacteria in comparison to the pre-composted feedstock.

The addition of a bulking agent (SP), to SS resulted in a reduction in the content of various potentially toxic elements (PTEs). Compared to the control, the mixing of SS with SP led to decreases in PTEs (As, Cd, Cr, Co, Pb, and Zn). The removal efficiency of PTEs varied among the sludge mixtures, with the arrangement being 75% SP > 50% SP > 25% SP > 0% SP. Furthermore, a significant ($p < 0.001$) relationship was observed between the total carbon loss and PTEs. These results suggest that vermicomposting is an effective method for decreasing the content of PTEs in SS.

It was hypothesised that earthworms could remove some OMPs (PPCPs and EDCs) compared to SS/composting due to bioaccumulation of these chemicals in earthworm tissue during vermicomposting. The reduction of these OMPs substances was recorded; however there was an increase of some OMPs. Furthermore, the reduction in the weight and volume of end product (vermicompost/compost) may result in an increase in the concentration of these selected OMPs. Therefore, from this finding, earthworms have shown great promise in removing selected PPCP and EDC from SS. Simultaneously; it is strongly suggested to perform further research oriented to the development of more effective and sustainable methods for removing OMPs from SS.

The study findings indicated that vermicompost exhibited significantly better agrochemical properties compared to compost. Vermicompost showed elevated levels of total potassium, total magnesium, nitrate nitrogen, available potassium, available phosphorus, available magnesium, available boron, and available copper. On the other hand, vermicompost displayed lower levels of ammonium nitrogen, available iron, available manganese, available zinc, total carbon, and total nitrogen. Among the various C/N ratios studied, vermicompost with an 18:1 C/N ratio surpassed compost and showcased the highest earthworm population. Therefore, the research strongly recommends vermicomposting SS with an 18:1 C/N ratio as a sustainable and effective technology for producing top-quality vermicompost from SS.

8. References

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