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

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A R T I C L E I N F O

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A B S T R A C T

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	0.68 ± 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	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).

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

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).

$$VD = (DE(+EW) - DE(-EW)) * 100 \quad (2)$$

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

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

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).

$$BCF = \frac{\text{Concentrations in earthworms}}{\text{Concentrations in the substrate}} \quad (5)$$

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 ± 0.68[a]	4.95 ± 0.32[a]
25% SS + 75% SP	7.9 ± 0.11	5.83 ± 0.46[a]	5.08 ± 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 ± 0.68[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 ± 0.18[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 ± 0.28[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 ± 0.86[a]	9.83 ± 0.21[b]
50% SS + 50% SP	28.17 ± 1.43	11.20 ± 0.20[a]	9.80 ± 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 +

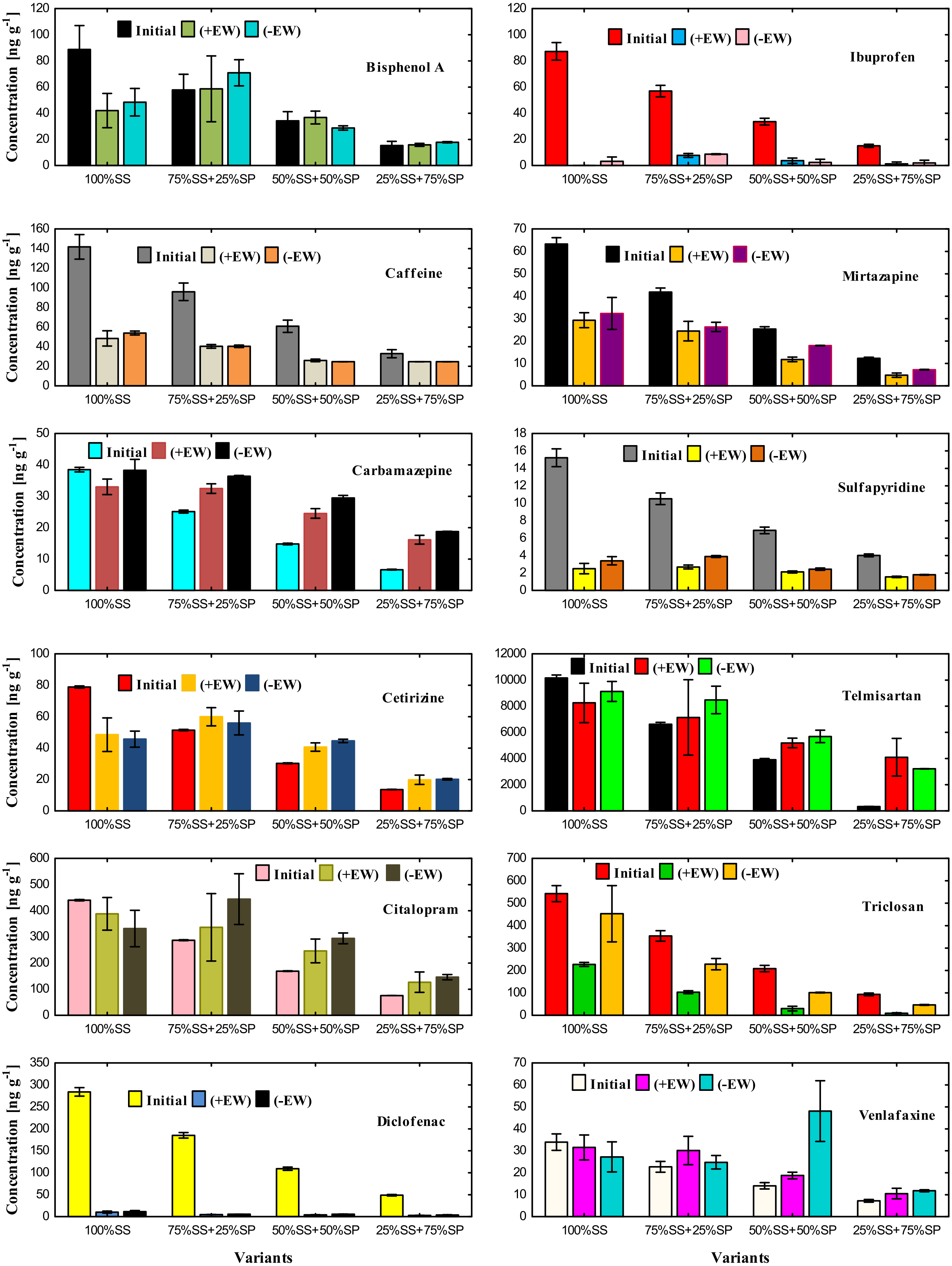

Fig. 1. PPCP and EDC concentrations of different variants at initial input, in final products with earthworms (+EW), and without earthworms (-EW). The bars indicate the standard error of the mean (n = 3).

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	−183	−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

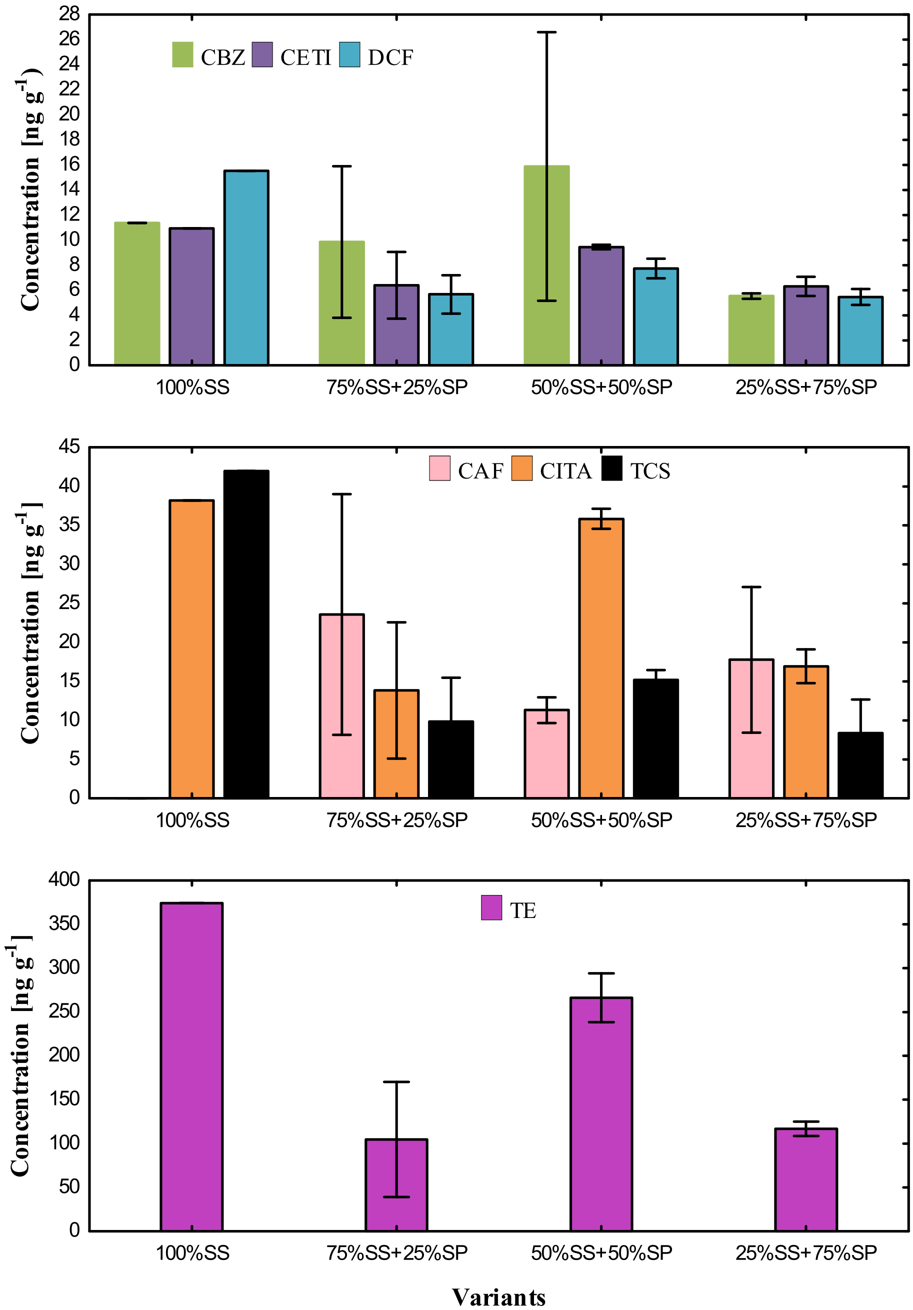

Fig. 2. Concentrations of PPCP found in earthworm tissues. The bars indicate the standard error of the mean (n = 3). A high standard error of the mean indicates that there was some amount of variability in the data.

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.

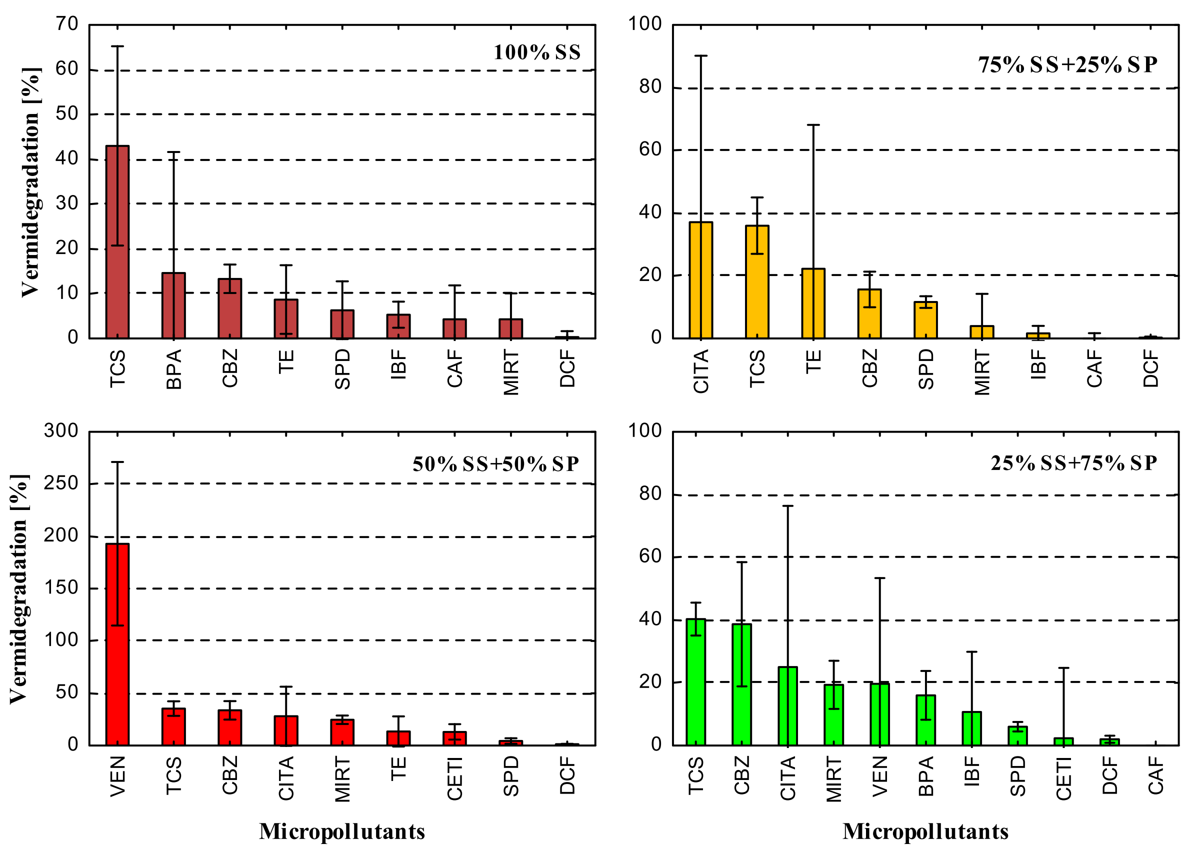

Fig. 3. Influence of earthworms on the degradation of PPCPs and EDCs in different variants. The bars indicate the standard error of the mean (n = 3). A high standard error of the mean indicates that there was some amount of variability in the data.

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 (Sonmezdag 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.

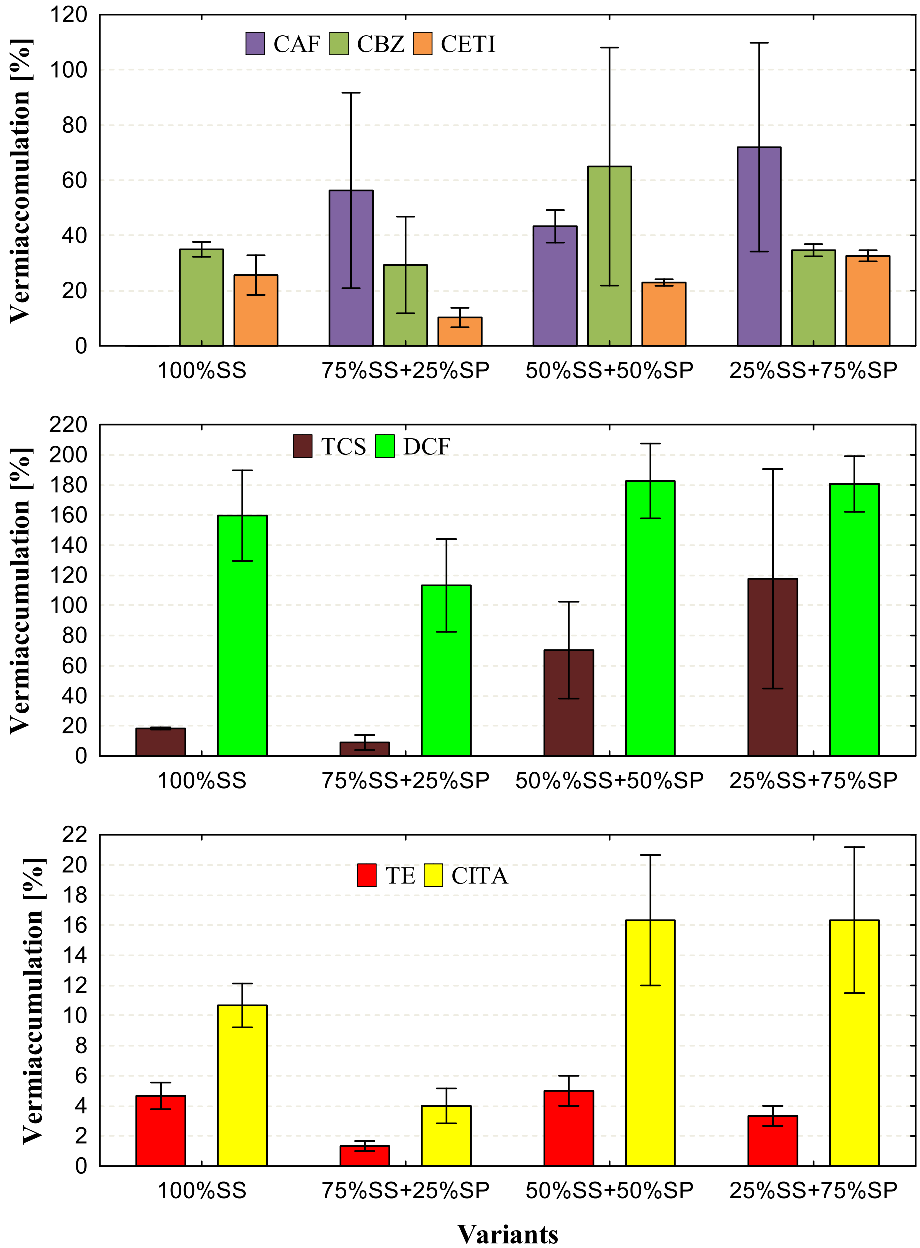

Fig. 4. Vermiaccumulation of some PPCPs during SS vermicomposting in different variants. The bars indicate the standard error of the mean (n = 3). A high standard error of the mean indicates that there was some amount of variability in the data.

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

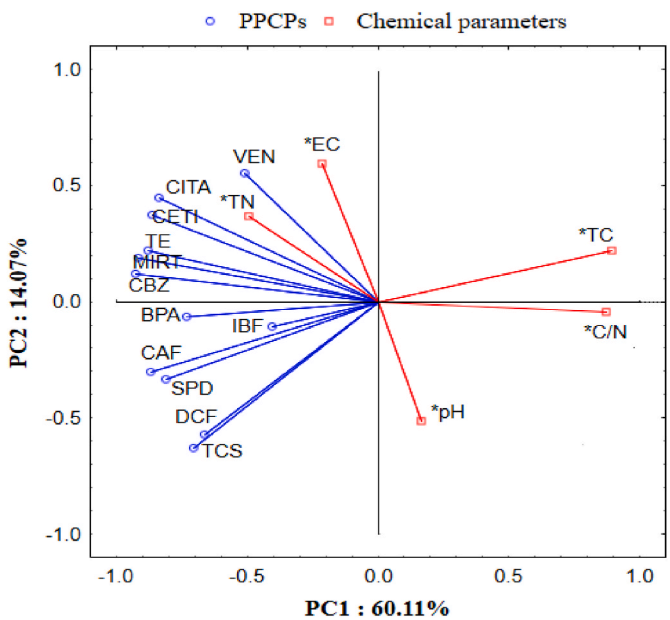

Fig. 5. Principal components (PC) of PPCP and EDC and along with their correlation with selected chemical parameters (pH, EC, TC, TN, and C/N ratio). BPA = bisphenol A, CAF = caffeine, CBZ = carbamazepine, CETI = cetirizine, CITA = citalopram, DCF = diclofenac, IBF = ibuprofen, MIRT = mirtazapine, SPD = 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

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References

Arumugam, K., Renganathan, S., Babalola, O.O., Muthunarayanan, V., 2018. Investigation on paper cup waste degradation by bacterial consortium and *Eudrilus eugeniae* through vermicomposting. Waste Manage. (Tucson, Ariz.) 74, 185–193.
Bhat, S.A., Singh, S., Singh, J., Kumar, S., Vig, A.P., 2018. Bioremediation and detoxification of industrial wastes by earthworms: vermicompost as powerful crop nutrient in sustainable agriculture. Bioresour. Technol. 252, 172–179.
Biel-Maeso, M., Corada-Fernández, C., Lara-Martín, P.A., 2019. Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. Water Res. 150, 129–139.
Biruntha, M., Karmegam, N., Jeyaprakasam, A., Selvi, B.K., Paul, J.A.J., Balamuralikrishnan, B., Chang, S.W., Ravindran, B., 2020. Vermiconversion of biowastes with low-to-high C/N ratio into value added vermicompost. Bioresour. Technol. 297, 122398.
Boruah, T., Barman, A., Kalita, P., Lahkar, J., Deka, H., 2019. Vermicomposting of citronella bagasse and paper mill sludge mixture employing *Eisenia fetida*. Bioresour. Technol. 294, 122147.
British Standards Institution (BSI) European Standard (EN) 15933, 2012. Sludge, Treated Biowaste and Soil Determination of pH.
Buta, M., Hubeny, J., Zieliński, W., Harnisz, M., Korzeniewska, E., 2021. Sewage sludge in agriculture–the effects of selected chemical pollutants and emerging genetic resistance determinants on the quality of soil and crops–a review. Ecotoxicol. Environ. Saf. 214, 112070.
Devi, C., Khwairakpam, M., 2020. Bioconversion of *Lantana camara* by vermicomposting with two different earthworm species in monoculture. Bioresour. Technol. 296, 122308.
Dubey, M., Rajpal, A., Vellanki, B.P., Kazmi, A.A., 2022. Occurrence, removal, and mass balance of contaminants of emerging concern in biological nutrient removal-based sewage treatment plants: role of redox conditions in biotransformation and sorption. Sci. Total Environ. 808, 152131.
Dume, B., Hanc, A., Svehla, P., Michal, P., Solcova, O., Chane, A.D., Nigussie, A., 2022. Nutrient recovery and changes in enzyme activity during vermicomposting of hydrolysed chicken feather residue. Environ. Technol. 43, 1–27.
Esmaeili, A., Khoram, M.R., Gholami, M., Eslami, H., 2020. Pistachio waste management using combined composting-vermicomposting technique: physico-chemical changes and worm growth analysis. J. Clean. Prod. 242, 118523.

European Union, 2019. Regulation (EU) 2019/1021 of the European Parliament and of the Council of 20 June 2019 on Persistent Organic Pollutants (Text with EEA Relevance). http://data.europa.eu/eli/reg/2019/1021/oj.

Gago-Ferrero, P., Borova, V., Dasenaki, M.E., Thomaidis, N.S., 2015. Simultaneous determination of 148 pharmaceuticals and illicit drugs in sewage sludge based on ultrasound-assisted extraction and liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 407, 4287–4297.

Haiba, E., Nei, L., Herodes, K., Ivask, M., Lillenberg, M., 2018. On the degradation of metformin and carbamazepine residues in sewage sludge compost. Agron. Res. 16, 696–707.

Hammer, L., Palmowski, L., 2021. Fate of selected organic micropollutants during anaerobic sludge digestión. Water Environ. Res. 93, 1910–1924.

Hanc, A., Dume, B., Hrebeckova, T., 2022. Differences of enzymatic activity during composting and vermicomposting of sewage sludge mixed with straw pellets. Front. Microbiol. 12, 3892.

Huang, G.F., Wong, J.W.C., Wu, Q.T., Nagar, B.B., 2004. Effect of C/N on composting of pig manure with sawdust. Waste Manage. (Tucson, Ariz.) 24, 805–813.

Hu, X., Shi, W., Wei, S., Zhang, X., Yu, H., 2021. Identification of (anti-)androgenic activities and risks of sludges from industrial and domestic wastewater treatment plants. Environ. Pollut. 268, 115716.

Innemanová, P., Grasserová, A., Cajthaml, T., 2022. Pilot-scale vermicomposting of dewatered sewage sludge from medium-sized wastewater treatment plant (WWTP). Detritus 18, 35.

Jiang, L., Li, Y., Chen, Y., Yao, B., Chen, X., Yu, Y., Yang, J., Zhou, Y., 2023. Pharmaceuticals and personal care products (PPCPs) in the aquatic environment: biotoxicity, determination and electrochemical treatment. J. Clean. Prod. 135923.

Jadia, C.D., Fulekar, M.H., 2008. Vermicomposting of vegetable waste: a biophysicochemical process based on hydro-operating bioreactor. Afr. J. Biotechnol. 7, 3723–3730.

Karmegam, N., Vijayan, P., Prakash, M., John Paul, J.A., 2019. Vermicomposting of paper industry sludge with cow dung and green manure plants using *Eisenia fetida*: a viable option for cleaner and enriched vermicompost production. J. Clean. Prod. 228, 718–728.

Kaushik, P., Garg, V.K., 2004. Dynamics of biological and chemical parameters during vermicomposting of solid textile mill sludge mixed with cow dung and agricultural residues. Bioresour. Technol. 94, 203–209.

Khatua, C., Sengupta, S., Balla, V.K., Kundu, B., Chakraborti, A., Tripathi, S., 2018. Dynamics of organic matter decomposition during vermicomposting of banana stem waste using *Eisenia fetida*. Waste Manage. (Tucson, Ariz.) 79, 287–295.

Mazzeo, D.E.C., Dombrowski, A., Oliveira, F.A., Levy, C.E., Oehlmann, J., Marchi, M.R. R., 2023. Endocrine disrupting activity in sewage sludge: screening method, microbial succession and cost-effective strategy for detoxification. J. Environ. Manag. 330, 117207.

Menon, N.G., Mohapatra, S., Padhye, L.P., Tatiparti, S.S.V., Mukherji, S., 2020. Review on Occurrence and Toxicity of Pharmaceutical Contamination in Southeast Asia. Emerging Issues in the Water Environment during Anthropocene: A South East Asian Perspective, pp. 63–91.

Nunes, N., Ragonezi, C., Gouveia, C.S.S., Pinheiro de Carvalho, M.A.A., 2021. Review of sewage sludge as a soil amendment in relation to current international guidelines: a heavy metal perspective. Sustainability 13, 2317.

Pérez-Godínez, E.A., Lagunes-Zarate, J., Corona-Hernández, J., Barajas-Aceves, M., 2017. Growth and reproductive potential of *Eisenia fetida* (Sav) on various zoo animal dungs after two methods of pre-composting followed by vermicomposting. Waste Manage. (Tucson, Ariz.) 64, 67–78.

Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2014. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. Water Res. 72, 3–27.

Phan, H.V., Wickham, R., Xie, S., McDonald, J.A., Khan, S.J., Ngo, H.H., Guo, W., Nghiem, L.D., 2018. The fate of trace organic contaminants during anaerobic digestion of primary sludge: a pilot scale study. Bioresour. Technol. 256, 384–390.

Pramanik, P., Safique, S., Jahan, A., Bhagat, R.M., 2016. Effect of vermicomposting on treated hard stem leftover wastes from pruning of tea plantation: a novel approach. Ecol. Eng. 97, 410–415.

Ramnarain, Y.I., Ansari, A.A., Ori, L., 2019. Vermicomposting of different organic materials using the *epigeic* earthworm *Eisenia fetida*. Int. J. Recycl. Org. Waste Agric. 8, 23–36.

Ravindran, B., Contreras-Ramos, S.M., Sekaran, G., 2015. Changes in earthworm gut associated enzymes and microbial diversity on the treatment of fermented tannery waste using *epigeic* earthworm *Eudrilus eugeniae*. Ecol. Eng. 74, 394–401.

Rini, J., Deepthi, M.P., Saminathan, K., Narendhirakannan, R.T., Karmegam, N., Kathireswari, P., 2020. Nutrient recovery and vermicompost production from livestock solid wastes with *epigeic* earthworms. Bioresour. Technol. 313, 123690.

Rorat, A., Courtois, P., Vandenbulcke, F., Lemiere, S., 2019. Sanitary and environmental aspects of sewage sludge management. In: Industrial and Municipal Sludge. Butterworth-Heinemann, pp. 155–180.

Samaras, V.G., Stasinakis, A.S., Thomaidis, N.S., Mamais, D., Lekkas, T.D., 2014. Fate of selected emerging micropollutants during mesophilic, thermophilic and temperature co-phased anaerobic digestion of sewage sludge. Bioresour. Technol. 162, 365–372.

Schug, T.T., Johnson, A.F., Birnbaum, L.S., Colborn, T., Guillette Jr., L.J., Crews, D.P., Collins, T., Soto, A.M., Vom Saal, F.S., McLachlan, J.A., Sonnenschein, C., 2016. Mini review: endocrine disruptors: past lessons and future directions. Mol. Endocrinol. 30, 833–847.

Sharma, K., Garg, V.K., 2019. Recycling of lignocellulosic waste as vermicompost using earthworm *Eisenia fetida*. Environ. Sci. Pollut. Res. 26, 14024–14035.

Shi, Z., Liu, J., Tang, Z., Zhao, Y., Wang, C., 2020. Vermiremediation of organically contaminated soils: concepts, current status, and future perspectives. Appl. Soil Ecol. 147, 103377.

Singh, D., Suthar, S., 2012. Vermicomposting of herbal pharmaceutical industry waste: earthworm growth, plant-available nutrient and microbial quality of end materials. Bioresour. Technol. 112, 179–185.

Sonmezdag, A.S., Kelebek, H., Selli, S., 2017. Characterization and comparative evaluation of volatile, phenolic and antioxidant properties of pistachio (*Pistacia vera L.*) hull. J. Essent. Oil Res. 29, 262–270.

Soobhany, N., 2019. Insight into the recovery of nutrients from organic solid waste through biochemical conversion processes for fertilizer production: a review. J. Clean. Prod. 241, 118413.

Soobhany, N., Mohee, R., Garg, V.K., 2015. Experimental process monitoring and potential of *Eudrilus eugeniae* in the vermicomposting of organic solid waste in Mauritius. Ecol. Eng. 84, 149–158.

Sudkolai, S.T., Nourbakhsh, F., 2017. Urease activity as an index for assessing the maturity of cow manure and wheat residue vermicomposts. Waste Manage. (Tucson, Ariz.) 64, 63–66.

Sun, Q., Li, M.Y., Ma, C., Chen, X.Q., Xie, X.Q., Yu, C.P., 2016. Seasonal and spatial variations of PPCP occurrence, removal and mass loading in three wastewater treatment plants located in different urbanization areas in Xiamen, China. Environ. Pollut. 208, 371–381.

Suthar, S., Gairola, S., 2014. Nutrient recovery from urban forest leaf litter waste solids using *Eisenia fetida*. Ecol. Eng. 71, 660–666.

Suthar, S., 2009. Vermicomposting of vegetable-market solid waste using *Eisenia fetida*: impact of bulking material on earthworm growth and decomposition rate. Ecol. Eng. 35, 914–920.

Taboada-santos, A., Braz, G.H.R., Fernandez-gonzalez, N., Carballa, M., Lema, J.M., 2019. Thermal hydrolysis of sewage sludge partially removes organic micropollutants but does not enhance their anaerobic biotransformation. Sci. Total Environ. 690, 534–542.

Thomas, A.R., Kranert, M., Philip, L., 2020. Fate and impact of pharmaceuticals and personal care products during septage co-composting using an in-vessel composter. Waste Manage. (Tucson, Ariz.) 109, 109–118.

Yadav, A., Garg, V.K., 2016. Vermiconversion of biogas plant slurry and parthenium weed mixture to manure. Int. J. Recycl. Org. Waste Agric. 5, 301–309.

Zeb, A., Li, S., Wu, J., Lian, J., Liu, W., Sun, Y., 2020. Insights into the mechanisms underlying the remediation potential of earthworms in contaminated soil: a critical review of research progress and prospects. Sci. Total Environ. 740, 140145.

Zhi-Wei, S., Tao, S., Wen-Jing, D., Jing, W., 2019. Investigation of rice straw and kitchen waste degradation through vermicomposting. J. Environ. Manag. 243, 269–272.

Zheng, Y., Sun, Z., Liu, Y., Cao, T., Zhang, H., Hao, M., Chen, R., Dzakpasu, M., Wang, X. C., 2022. Phytoremediation mechanisms and plant eco-physiological response to micro-organic contaminants in integrated vertical-flow constructed wetlands. J. Hazard Mater. 424, 127611.

Zziwa, A., Jjagwe, J., Kizito, S., Kabenge, I., Komakech, A.J., Kayondo, H., 2021. Nutrient recovery from pineapple waste through controlled batch and continuous vermicomposting systems. J. Environ. Manag. 279, 111784.