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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 (Chrášt'any, 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].

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

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].

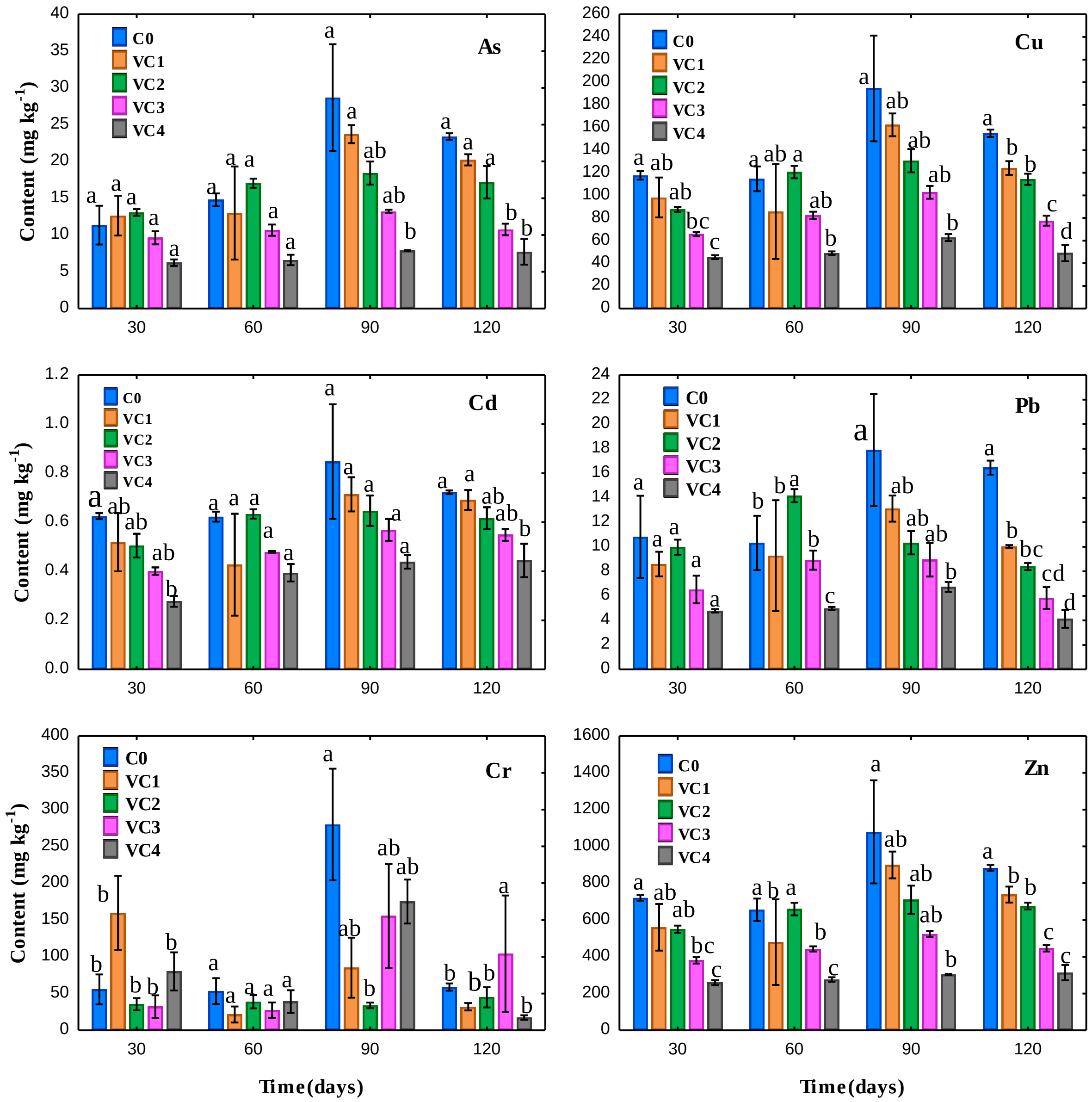


**Figure 1.** Variations of PTE content during vermicomposting. The bars indicate the standard deviation of the mean ($n$ = 3). Different letters indicate significant differences among the variants ($p < 0.05$).

### *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*).

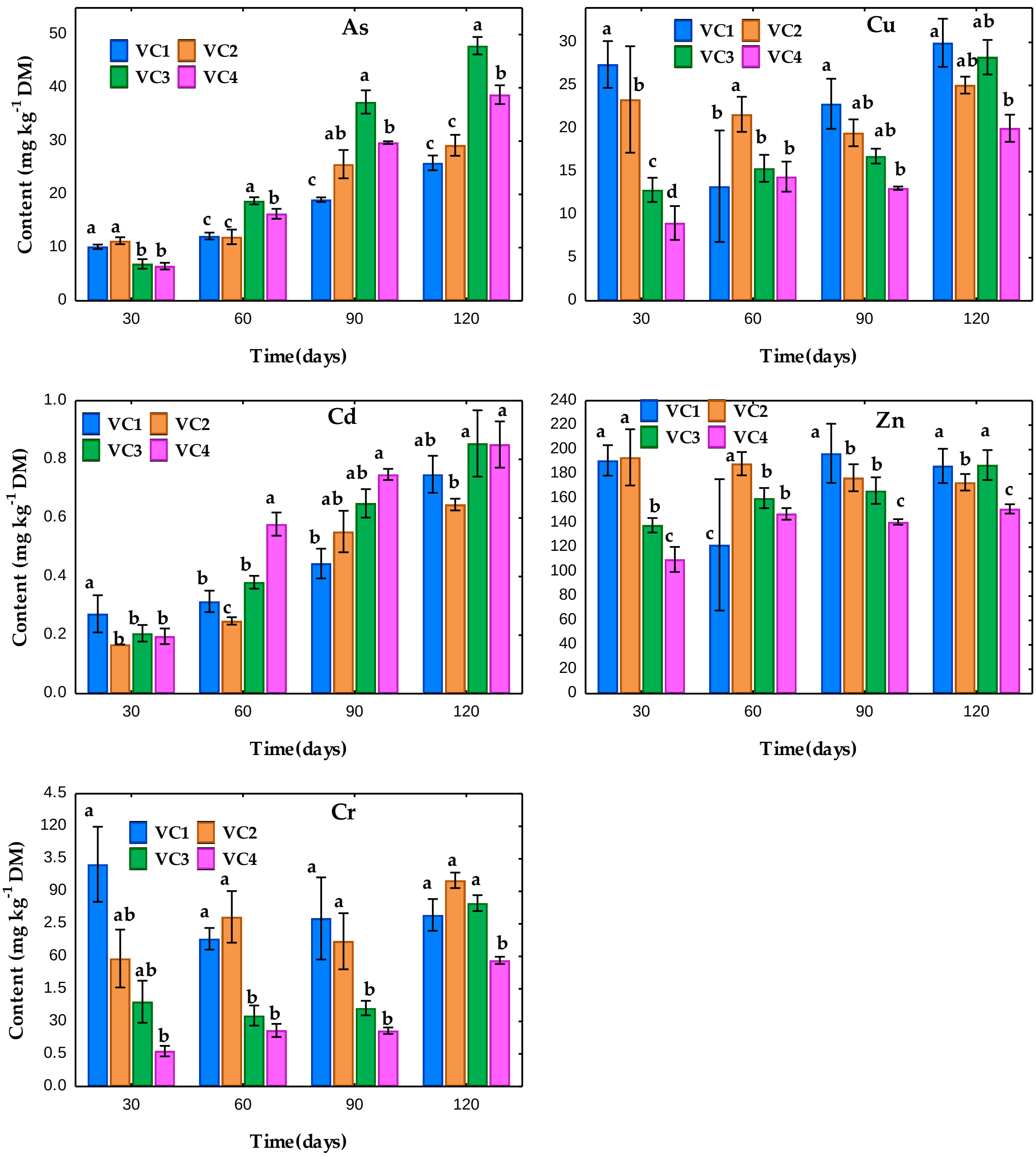


**Figure 2.** Variations of PTEs content in earthworm tissues. The bars indicate the standard deviation of the mean ($n$ = 3). Different letters indicate significant differences among the variants ($p < 0.05$).

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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