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Biochar amendment for integrated composting and vermicomposting of sewage sludge – The effect of biochar on the activity of *Eisenia fetida* and the obtained vermicompost

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HIGHLIGHTS

- Significantly increased number of cocoons and juveniles in the mixture amended with biochar before composting.
- Reduced bioavailability of Cd and Zn to *E. fetida* earthworms in the mixture amended with biochar before composting.
- Biochar is an efficient amendment in combined composting and vermicomposting.
- Acidification of the products – probably linked to nitrification – occurred during combined composting and vermicomposting.
- Biochar-added vermicompost as a growing medium or amendment for calcareous soils.

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ABSTRACT

Sewage sludge derived biochar (SSDB) was used as a supplementary material for municipal sewage sludge (SS) and wood chips mixtures (WC) treated by combined composting and vermicomposting. SSDB added to the mixture before composting resulted in significantly higher reproduction rate: on week 4 the number of cocoons increased by 213% when compared to the mixture with no biochar. On week 6 the average number of juveniles increased 11-fold in the mixture with biochar added before composting and 5-fold in the mixtures with biochar added after composting when compared to the mixture with no biochar. Biochar added before composting reduced bioavailability of Cd and Zn to *E. fetida*. The biochar-added vermicomposts showed good fertilizing properties except for elevated concentrations of Cr. The pH of all vermicomposts was in the range of 5.27–5.61. The obtained vermicomposts can be used as a growing medium for horticultural purposes or as an amendment in calcareous soils.

1. Introduction

Biochar and its potential applications have been extensively studied in recent years by many researchers worldwide (Qian et al., 2015). Biochar is a solid, carbon rich material that can be obtained from a wide range of plant and animal biomass through thermal conversion in the absence of air (Lehmann and Joseph, 2009). Undoubtedly, biochar shows interesting properties such as ability to bind contaminants, retain nutrients and water, and also foster microorganisms. Some of the potential applications can include the use of biochar as a soil improver or a fertilizer component (Ngo et al., 2013), a sorbent for organic and inorganic contaminants (Khan et al., 2013; Zhang et al., 2013; Ahmad et al., 2014) or a supplementary material in composting (Dias et al., 2010; Malińska et al., 2014; Czekała et al., 2016). Also, biochar can be considered a potential amendment in vermicomposting of various organic waste, including sewage sludge (Malińska et al., 2016).

The literature reports many studies on vermicomposting of municipal sewage sludge (Khwairakpam and Bhargava, 2009; Hait and Tare, 2012; Wang et al., 2013; Das et al., 2015; He et al., 2016), however managing sewage sludge through vermicomposting poses many challenges mostly due to the concentration of various contaminants present in sewage sludge such as heavy metals (Gogoi et al., 2015; He et al., 2016). During vermicomposting heavy metals present in sewage sludge can inhibit the activity

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of earthworms resulting in reduced growth and reproduction and also mortality, and thus the efficiency of converting sewage sludge into vermicomposts. Therefore, vermicomposting of sewage sludge should require the addition of supplementary materials to ensure optimal properties for reproduction and growth of earthworms, and thus improvement of the conversion rate and properties of the obtained vermicomposts. These supplementary materials can include bulking agents such as different kinds of crop straw, wood chips or saw dust as well as amendments such as soil, fly ash (Wang et al., 2013; He et al., 2016), tea factory coal ash (Goswami et al., 2014), phosphate rock (Wang et al., 2013) and also biochar (Malińska et al., 2016). These materials can be added to the mixtures before composting or after composting i.e. directly to precomposted mixtures, and then subjected to vermicomposting. The addition of supplementary materials, e.g. straw or sawdust can improve C/N ratio, and thus accelerate the stabilization of sewage sludge and eliminate the toxicity and sequester heavy metals (Nayak et al., 2013; He et al., 2016). Fly ash and phosphoric rock can reduce mobility and bioavailability of heavy metals in sewage sludge during vermicomposting (Wang et al., 2013). Biochar can reduce bioavailability of heavy metals in sewage sludge (Malińska et al., 2016). Moreover, it is hypothesized that biochar could provide some nutrients to earthworms during vermicomposting.

To our knowledge there are very few studies that have investigated the effect of biochar on vermicomposting dynamics, activity of earthworms and properties of vermicomposts (Malińska et al., 2016). Most studies reported in the literature focused on the effect of biochar on earthworms but mostly in soils (Liesch et al., 2010; Weyers and Spokas, 2011; Tammeorg et al., 2014). For example, Liesch et al. (2010) investigated the effect of pine chip and poultry litter biochars added to artificial soil at various rates. Poultry litter biochar due to heavy metals, and high Na and Mg contents that led to high salinity, had a negative effect on *Eisenia fetida*. This resulted in a significant change in weight and mortality of earthworms exposed to higher biochar rates. Tammeorg et al. (2014) studied the effect of biochar field application on the activity of earthworms and they found that under field conditions the highest earthworms densities and biomass were observed in soils that were amended with biochar. Other researchers investigated the effect of bamboo biochar added to vermicompost on its biological and chemical stability (Ngo et al., 2013).

Numerous studies reported that earthworms can bioaccumulate various contaminants including heavy metals present in waste or soils indicating the potential of earthworms for vermiremedation of contaminated soils (Beesley and Marmiroli, 2011; Goswami et al., 2014). It was reported that bioaccumulation of heavy metals by earthworms can depend on various factors such as physiological and morphological characteristics of these organisms, nutrition requirements and dietary intake of elements (Nannoni et al., 2011). Yadav and Garg (2011) indicated that the increase in the concentration of heavy metals in earthworms after vermicomposting can be mainly associated with two mechanisms, i.e. (1) chemical absorption of soluble elements through earthworms' skin and gut, and (2) digestion. In the present study we also made an attempt to test the hypothesis that biochar added to the mixture of sewage sludge and wood chips before composting could bind selected heavy metals in compost-biochar matrix, and thus make them less available to earthworms during vermicomposting.

Our previous study showed that the amendment of sewage sludge mixture with wood derived biochar before composting increased the reproduction rate of *Eisenia fetida* during laboratory vermicomposting (Malińska et al., 2016). Therefore, in this study the objectives included determination of the effects of biochar amendment: (1) prior and after composting of sewage sludge on the activity of earthworms and the obtained product and (2) on bioaccumulation of selected heavy metals in earthworm tissues. The paper presents the results from the 6 week laboratory vermicomposting of precomposted sewage sludge and woodchips mixtures amended with sewage sludge derived biochar. The scope of this study included: (1) physical and chemical analysis of composting substrates, sewage sludge derived biochar and the initial mixtures, (2) the 4-week laboratory composting of sewage sludge and woodchips mixtures in a set of 40-L insulated composting bioreactors, (3) the 6-week laboratory vermicomposting of sewage sludge and woodchips mixtures, (4) the analysis of *E. fetida* activity during vermicomposting based on survival rate, total biomass, number of cocoons and juvenile earthworms, (5) bioaccumulation of selected heavy metals in earthworm tissues, and (6) physical and chemical characteristics of vermicomposts.

2. Materials and methods

2.1. Composting substrates

Dewatered and anaerobically stabilized sewage sludge (SS) was sampled from a municipal wastewater treatment plant (Częstochowa, Poland) whereas woodchips (WC) were collected from a local green waste management facility. Moisture content was 80.37 ± 0.33% (SS) and 5.73 ± 0.09% (WC), organic matter content was 63.13 ± 1.49% (SS) and 96.26 ± 0.25% (WC). Total carbon was 408.15 ± 8.41 $mg \cdot g^{-1}$ (SS) and 464.75 ± 5.59 $mg \cdot g^{-1}$ (WC). Nitrogen was 4.52 ± 0.01% (SS) and 0.56 ± 0.02% (WC). pH was 7.69 (SS) and 6.07 (WC) and electrical conductivity was 2215 $\mu S \cdot cm^{-1}$ (SS) and 857 $\mu S \cdot cm^{-1}$ (WC).

2.2. Sewage sludge derived biochar

Sewage sludge derived biochar (SSDB) was produced in an experimental pyrolysis installation (at 550 °C) from municipal sewage sludge sampled from a local wastewater treatment plant (Opole, Poland). SSDB was crushed in a laboratory jaw crusher (LAB-01-65, EKOLAB) and sieved to size <2 mm in order to assure even distribution in a mixture. Biochar was subjected to chemical and physical analysis, which included moisture content (0.8 ± 0.2, % w/w), ash content at 550 °C (43.76, % w/w), total carbon (42.2 ± 2.8, % w/w), total organic carbon (39.1 ± 3.7, % w/w), total nitrogen (3.80 ± 0.59, % w/w), pH (6.3), electrical conductivity (147 $\mu S \cdot cm^{-1}$), total phosphorous (5.25%), magnesium (11.95, $g \cdot kg^{-1}$, dw), aluminum (9.82, $g \cdot kg^{-1}$, dw), iron (51.32, $g \cdot kg^{-1}$, dw), sulphur (0.399 ± 0.065, $g \cdot kg^{-1}$, dw), chloride (0.158, $g \cdot kg^{-1}$, dw), mercury (0.0049, $g \cdot kg^{-1}$, dw), zinc (1238 ± 492, $g \cdot kg^{-1}$, dw), cadmium (2.31 ± 0.68, $g \cdot kg^{-1}$, dw), manganese (692 ± 228, $g \cdot kg^{-1}$, dw), lead (46.2 ± 21.0, $g \cdot kg^{-1}$, dw).

Biochars from sewage sludge can differ in physicochemical properties due to the origin and composition of the initial feedstock (Méndez et al., 2013). SSDB showed lower organic matter content and total and organic carbon in comparison to biochars from e.g. woody biomass. The C/N ratio was 11, pH of 6.3 and electrical conductivity of 147 μS/cm. The pH of SSBD was somewhat lower than for biochars of this type reported in the literature (Méndez et al., 2013). Also, these biochars show high P content and also high concentrations of Al, Ca, Mg, Fe (Chen et al., 2014). Although SSDB contained heavy metals, their concentrations did not exceed the permissible values for sewage sludge itself applied to agricultural soils. Studies indicate that most biochars obtained from sewage sludge show higher concentration of Cr, Cu and Zn (Chen et al., 2014; Srinivasan et al., 2015). Similar characteristics of sewage sludge derived biochars were reported by other researchers (Zhang et al., 2013; Jin et al., 2014). Studies show that biochar obtained from pyrolysis of sewage sludge can be considered as an amendment for soil applications (Khan et al., 2013) but it can also be used for composting and vermicomposting. However, the biggest concern of introducing biochar into the environment is the content of heavy metals as the majority of heavy metals present in sewage sludge end up in pyrolysed material (except from volatile elements like Hg or Cd) (Lu et al., 2013). Some studies show that pyrolysis in fact can inhibit the release of heavy metals from biochar samples (Zhang et al., 2013). Moreover, biochar can show high potential for biding organic and inorganic contaminants and reducing bioavailability of various contaminants present in soils to plants (Beesley and Marmiroli, 2011; Tang et al., 2013; Srinivasan et al., 2015; Oleszczuk et al., 2014). For example, Lu et al. (2014) found that amendment of soils with bamboo and rice biochars resulted in significant reductions in Cd, Cu, Pb and Zn in the above-plant biomass and shoots of *S. plumbizincicola*. Jin et al. (2014) studied the effect of feedstock particle size on heavy metal leaching and they found that Cu, Zn and As in biochar derived from larger particles of sewage sludge could be easier leached to the environment. Some studies conclude that despite the presence of contaminants (such as PAHs and heavy metals), sewage sludge derived biochars could be an alternative to sewage sludge applied to soil (Ociepa, 2011; Niewiadomska et al., 2015; Pachura et al., 2016) as sewage sludge itself can pose significant risk due to phytotoxicity and pathogens when applied at high rates (Khan et al., 2013). Therefore, the potential risk of biochar associated with releasing toxic metals during vermicomposting can be acceptable. In addition, some studies point out that biochar added to soils resulted in lower leaching of Cu, Ni and Zn and lower availability of Ni, Zn, Cd and Pb to plants in comparison to sewage sludge (Tang et al., 2013).

2.3. Treatments

Sewage sludge (SS) was mixed with woodchips used as a bulking agent in the ratio of 1:0.3 wet weight (21 kg of sewage sludge and 6 kg of woodchips). The amendment of the mixtures was performed by adding 8% of sewage sludge derived biochar (SSDB) on wet weight. The amount of biochar addition was established based on our previous study (Malińska et al., 2016) and the results from other studies that investigated the effect of various supplementary materials in vermicomposting (He et al., 2016). The applied treatments are presented in Table 1.

2.4. Experimental set-up

2.4.1. Composting

The mentioned 4-week composting was performed in 40 L laboratory insulating composting reactors with forced aeration for 22 days. The process was monitored by daily measurements of temperature (data not presented here). Moisture and organic matter content, total nitrogen, pH and electrical conductivity were determined in the samples taken on day 0 and day 22. Composting of investigated mixtures prior to vermicomposting is essential as it stabilizes and reduces the mass of the waste and inactivates some of the pathogenic microorganisms due to thermophilic temperatures during composting (Nair et al., 2006). Also, composting prior to vermicomposting prevents from earthworm mortality as it allows releasing volatile gases that could be toxic to these organisms (Kaushik and Garg, 2003). Recent studies indicated that extended or chained processes are needed to obtain quality products (Cáceres et al., 2015, in press; Malińska et al., 2016).

2.4.2. Vermicomposting

Vermicomposting was performed in a laboratory system of vermireactors, i.e. the 2.5 L plastic boxes with tight-fitting lids, drainage holes in the bottom and air vents on the lids and sides. The top surface area of each vermireactor was $0.0324\ m^2$ (Malińska et al., 2016). *E. fetida* earthworms were purchased from a commercial vendor and kept in the laboratory culture till the experiments. The 500 g of the investigated mixtures was placed in each vermireactor. Then, 30 clitellated adult earthworms of the average total weight of 12.1 g were transferred to each vermireactor (average stocking density was $0.4\ kg\ m^{-2}$). The optimal population of *E. fetida* in vermicomposting of sewage sludge varies in the literature. Some researchers recommended the optimal stocking density as $0.5\ kg\ m^{-2}$ (Hait and Tare, 2011) whereas others suggested $1.6\ kg\ m^{-2}$ (He et al., 2016). In this study, we assumed the initial stocking density at $0.4\ kg\ m^{-2}$ in order to maintain the sufficient quantity of feed for the increasing number of earthworms throughout the 6 week experiment. Similar initial stocking density of $0.5\ kg\ m^{-2}$ was used by Wang et al. (2013). All treatments were run in 4 replications. The vermireactors were kept in dark at room temperature. *E. fetida* earthworm – an epigeic waste composting worm – was selected for this study due to high tolerance towards different toxic compounds and high temperature. Also, *E. fetida* shows high efficiency of converting diverse types of waste into vermicompost (Maboeta and Rensburg, 2003; Loh et al., 2005; Khwairakpam and Bhargava, 2009; Sinha et al., 2010). In addition, *E. fetida* has been applied for vermicomposting of various types of waste, including municipal sewage sludge, and the growth, mortality and reproduction of this species has been studied in detail (Nayak et al., 2013).

2.5. Process control

2.5.1. Chemical analysis

Composting substrates, composting mixtures and vermicomposts were tested using standard procedures for moisture (oven drying in 105 °C till constant weight) and organic matter content (loss on ignition in a muffle furnace in 550 °C for 6 h), pH and electric conductivity in water extract 1:10 w/w (pH and conductivity meter CPC 411), total carbon (Turin method) and nitrogen (Kjeldahl method) (Malińska et al., 2016). Sewage sludge derived biochar (SSDB) was analyzed for a number of parameters including macronutrients and trace elements (atomic absorption spectrometry) in order to ensure that it can be safely introduced to composting mixtures. Additionally, the accumulation of selected metals (Cd, Zn, Mn, Cr, Pb) in the earthworm tissues was determined upon the completion of vermicomposting. The earthworms were hand-picked from each vermireactor and separated from the

Table 1
Treatments applied in this study.

Treatment	Mixture	Composting	SSBD amendment	Vermicomposting
CONTROL	Mixture of sewage sludge and woodchips in the ratio of 1:0.3 (wet weight)	Yes. 4 weeks	No amendment.	Yes. 6 weeks
AM-PRECOM	Mixture of sewage sludge and woodchips in the ratio of 1:0.3 (wet weight)	Yes. 4 weeks	Amendment with 8% before composting (PRE)	Yes. 6 weeks
AM-POSTCOM	Mixture of sewage sludge and woodchips in the ratio of 1:0.3 (wet weight)	Yes. 4 weeks	Amendment with 8 % after composting (POST)	Yes. 6 weeks

vermicomposts, counted, washed in distilled water and weighed. Subsequently, a group of 12 earthworms was selected randomly from each treatment for metal content analysis. Each group was placed in a plastic box with perforated lid filled with 1% agar in order to remove the gut content. After 48 h of incubation in room temperature, earthworms were separated from agar, washed in distilled water and frozen. Then, samples were dried at 37 °C in a ventilated oven for 48 h, homogenized by grinding with an electric mill and stored at room temperature. Total metal contents were determined after acid digestion by atomic absorption spectrometry (ICP-MS) (Rorat et al., 2016; Malińska et al., 2016).

2.5.2. Activity of earthworms

During the 6-week laboratory vermicomposting the activity of the earthworms was monitored on week 1, 2, 3, 4, 5 and 6. The activity was determined based on survival rate, total weight and number of adult earthworms, cocoons and juveniles. Every week the earthworms were separated from the mixtures, hand sorted, counted, washed and weighed. Cocoons and juveniles were hand-picked and counted. After that all the earthworms, cocoons and juveniles were placed back in the vermireactors. Moisture content in the vermireactors was maintained at 60–70% throughout the entire experiment. After completion of vermicomposting the adult and juvenile earthworms and cocoons were separated from the vermicompost (Malińska et al., 2016).

2.5.3. Bioaccumulation factors

Bioaccumulation factors (BAFs) were calculated for selected metals, i.e. Cd, Zn, Mn, Cr, Pb according to the following equation (Eq. (1)):

$$BAF_{Me} = \frac{C_{\text{Me earthworm}}}{C_{\text{Me vermicompost}}} \tag{1}$$

where $C_{\text{Me earthworm}}$ is the total concentration of a selected metal in earthworm body ($mg \cdot g^{-1}$) and $C_{\text{Me vermicompost}}$ is the total concentration of this metal in vermicompost ($mg \cdot g^{-1}$).

2.5.4. Statistical analysis

All statistical analyses were performed using SAS® software (v9.1, SAS Institute, Cary, NC, USA). All sample data were analyzed using one-way or repeated time analysis of variance. Mean separation among treatments was obtained by Tukey's test.

3. Results and discussion

3.1. Characteristics of the mixtures prior to vermicomposting

The addition of biochar before composting to the mixture of sewage sludge and woodchips (AM-PRECOM) resulted in slightly higher contents of organic matter and higher nitrogen than in the CONTROL (Table 2). Similar results were observed by Vandecasteele et al. (2016) who investigated the effect of biochar added to the feedstock mixture before and after composting. They found that adding biochar before composting to the feedstock mixture resulted in the enhanced rate of organic matter decomposition and reduction of nitrogen loss. Also, Czekała et al. (2016) observed the increase in organic matter in poultry mixtures amended with wood derived biochar. In this study organic matter decreased which was due to the fact that sewage sludge derived biochar shows lower organic matter content (56%) in comparison to wood derived biochars (e.g. 94%). The pH of all the mixtures was suitable for vermicomposting.

In the AM-PRECOM treatment the increase in the concentration of P, K, Ca and Na was observed (Table 3). The increase in phosphorous in feedstock with the addition of biochar prior to composting was also observed by Vandecasteele et al. (2016) who studied the effect of blending wood derived biochar with feedstock mixture and mature composts. The total content of heavy metals i.e. Cd, Zn, Mn, Cr and Pb in the AM-PRECOM was slightly lower than in the AM-POSTCOM. This could result from leaching of these metals during composting. Lower contents of heavy metals in the CONTROL than in both AM-PRECOM and AM-POSTCOM could be due to the fact that the CONTROL did not contain biochar. SSDB showed higher concentrations of Cd (2.31 ± 0.68 mg kg^{-1}), Zn (1238 ± 492 mg kg^{-1}), Mn (692 ± 228 mg kg^{-1}), Cr (88.5 ± 3.92 mg kg^{-1}), Pb (46.2 ± 21.0 mg kg^{-1}).

3.2. Effects of biochar amendment on the activity of E. fetida during vermicomposting

3.2.1. Earthworm survival and growth rate

Throughout this study no mortality of earthworms in the investigated mixtures was observed. The earthworm growth during the first 3 weeks was somewhat at a similar rate except for earthworms in the AM-PRECOM treatment where the increase in the total biomass was observed after week 1 (Fig. 1).

Similar tendency was observed by other researchers (Gupta and Garg, 2008; Nayak et al., 2013). After week 4 the total biomass started to decrease in all mixtures. In our previous study the highest earthworm biomass was observed on week 8 as more organic matter was available to earthworms (i.e. 500 g of feed for 20 adult earthworms) and the population density was lower. Assuming that one earthworm consumes the feed up to half of its body weight per day, the amount of the substrate, i.e. 500 g consumed by 30 earthworms was sufficient for maximum of 10–11 weeks. Therefore, the decline in the total earthworm biomass after week 4 could be attributed to the significant increase in the population density due to increasing number of juveniles. Similar results were observed by Yadav and Garg (2016) who observed that average worm biomass – but also growth and cocoon production – were lesser at higher population densities. The highest increase in the earthworm biomass was observed for the AM-PRECOM throughout the entire process. The least significant change in the earthworm weight during 6 weeks of vermicomposting was reported for the AM-POSTCOM. After 3 weeks of vermicomposting the earthworm biomass was higher about 4.5% for the AM-PRECOM and lower about 3.8% for the AM-POSTCOM in comparison to the CONTROL.

Table 2
Characteristics of the materials in each treatment.

Parameters	Units	CONTROL	AM-PRECOM	AM-POSTCOM
Moisture content	%	69.08 ± 0.24	63.72 ± 0.23	65.27 ± 1.03
Organic matter content	%	77.51 ± 0.33	72.18 ± 0.43	69.95 ± 0.95
C_{org}	%	43.06 ± 0.18	40.10 ± 0.24	38.86 ± 0.53
N	%	2.69 ± 0.18	3.03 ± 0.07	3.39 ± 0.06
C/N	–	16:1	13:1	11:1
pH	–	7.14	7.11	6.88
Electrical conductivity	$\mu S \cdot cm^{-1}$	617	886	693

Table 3
Macro and microelements in the mixtures in each treatment.

Elements	Units	CONTROL	AM-PRECOM	AM-POSTCOM	*p*
Macroelements					
P	$g\ g^{-1}\cdot100$, dw	1.45 b	2.51 a	1.48 b	*0.0020*
K	$g\ g^{-1}\cdot100$, dw	0.35	0.56	0.39	*0.2999*
Ca	$g\ g^{-1}\cdot100$, dw	1.99	2.36	1.62	*0.6127*
Mg	$g\ g^{-1}\cdot100$, dw	0.31	0.42	0.26	*0.5093*
Na	$mg\cdot kg^{-1}$, dw	553.3 b	1076.7 a	926.7 a	*0.0008*
Microelements/heavy metals					
Cd	$mg\cdot kg^{-1}$, dw	1.40	2.06	2.13	
Zn	$mg\cdot kg^{-1}$, dw	833.3	923.3	960.0	*0.1004*
Mn	$mg\cdot kg^{-1}$, dw	196.7 b	283.3 a	286.7 a	*0.0042*
Cr	$mg\cdot kg^{-1}$, dw	186.7	186.7	196.7	*0.7703*
Pb	$mg\cdot kg^{-1}$, dw	29.7	34.3	35.0	*0.1929*

Means followed by the same letter in a column are not significantly different at $p \leqslant 0.05$.
dw: dry weight basis; *p:* probability.

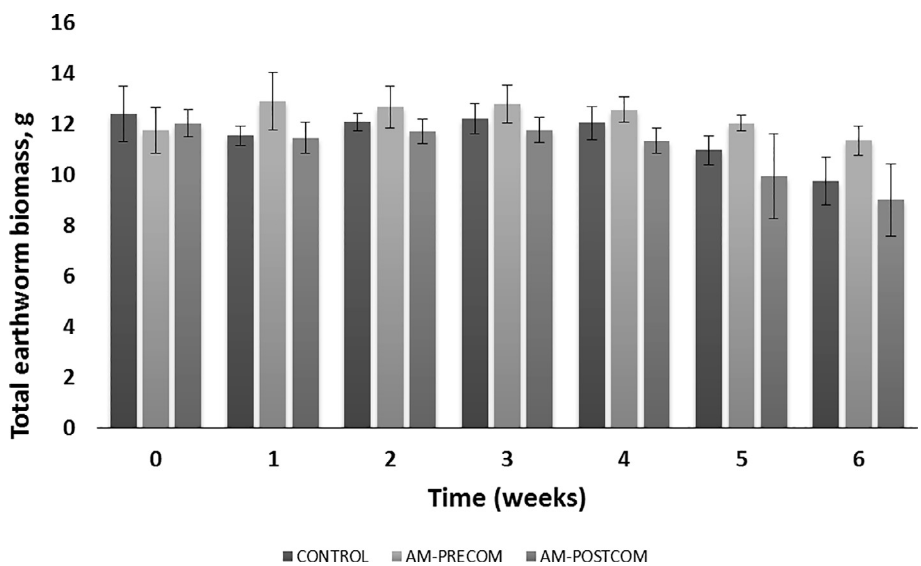

Fig. 1. The total earthworm biomass during 6-week vermicomposting with different treatments (the average from 4 replications).

This implies that the AM-PRECOM treatment was the most suitable habitat for the earthworms.

3.2.2. Reproduction rate

First cocoons appeared in all mixtures on the second week of vermicomposting and the highest number was observed on week 4 (Fig. 2).

On week 4 the average number of cocoons was 15 ± 8, 47 ± 11 and 4 ± 3 for the CONTROL, AM-PRECOM and AM-POSTCOM, respectively. This means that amendment of sewage sludge mixture with biochar before composting resulted in the increase in the cocoon number by 213% in the comparison to the mixture with no biochar. Similar results were obtained in our previous study where the amendment of 8% biochar prior to composting resulted in the increase of cocoons by 66% when compared to the mixture without biochar. The addition of biochar to sewage sludge mixture after composting led to impaired reproduction for *E. fetida* – the number of cocoons on week 4 was lower by 73% in the comparison to the mixture with no biochar. A significant increase in the number of cocoons throughout of the experiment was reported for the AM-PRECOM treatment. Since week 5 the number of cocoons started to decrease which could be due to the depletion of organic matter and increased stocking density (Yadav and Garg, 2016).

The number of juveniles during 6 weeks of vermicomposting was the highest for the AM-PRECOM treatment (Fig. 3). On week 6 the average number of juveniles in the CONTROL, AM-PRECOM and AM-POSTCOM was 3 ± 1, 33 ± 7, 7 ± 2, respectively. This indicates that the AM-PRECOM treatment provided the most favorable environment for development of juvenile earthworms resulting in almost 11-fold increase in the number of juveniles in comparison to the CONTROL and the AM-POSTCOM (5-fold increase in the number of juveniles). Amendment of sewage sludge mixture with biochar before and after composting led to enhanced development of juveniles in comparison to the mixture with no addition of biochar.

The obtained data on the reproduction of *E. fetida* during vermicomposting of sewage sludge mixtures amended with biochar compares well with the previous study where we found that the addition of biochar before composting resulted in the increased number of cocoons and juveniles in the comparison to the mixture with no biochar (Malińska et al., 2016). This could be due to a number of factors such as immobilization of heavy metals by biochar, improvement of total porosity and water holding capacity, and also provision of macro and microelements (Tammeorg et al., 2014). Other factors could include nutrient retention and enhancement of microbial activity. A significant difference in the reproduction of earthworms was reported for the AM PRECOM which was amended with biochar before composting. It is hypothesized that biochar-compost matrix could bind heavy metals during composting and make them less bioavailable to earthworms, and also to

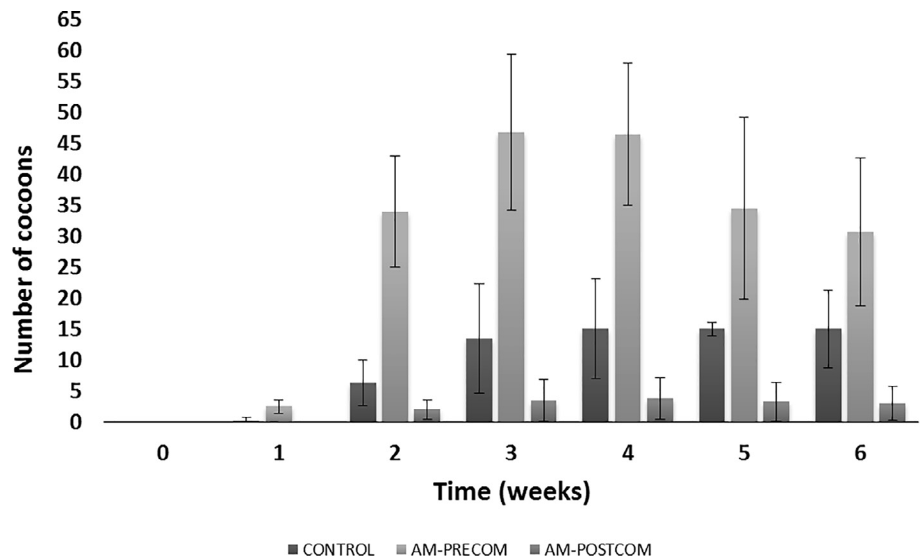

Fig. 2. Number of cocoons produced during the 6 week vermicomposting with different treatments (the average from 4 replications).

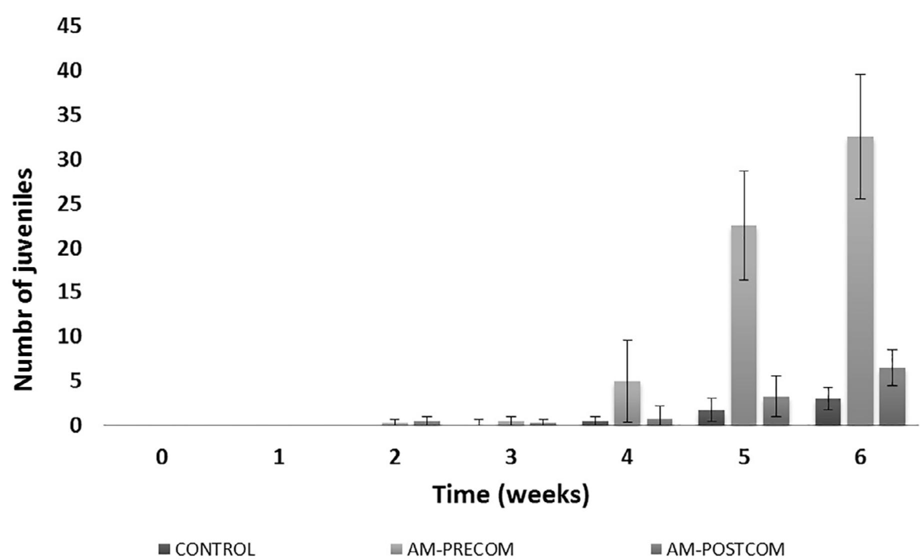

Fig. 3. Number of juveniles during the 6 week vermicomposting with different treatments (the average from 4 replications).

juveniles, allowing for reproduction and growth. Maboeta and Rensburg (2003) pointed out that the growth and reproduction can be considered as a sensitive parameter in evaluating the effects of heavy metals.

3.2.3. Bioaccumulation of heavy metals in the earthworm tissues

The total concentration of selected heavy metals, i.e. Cd, Zn, Mn, Cr and Pb was determined in the earthworm tissues after the completion of vermicomposting (Table 4).

Bioaccumulation of Cd and Zn in the tissues of *E. fetida* grown in the mixture with biochar added before composting was lower than in other two mixtures. The bioaccumulation of Mn, Cr and Pb in the earthworm tissues was similar for all investigated mixtures. For all earthworms grown in the mixtures with the CONTROL, AM-PRECOM AND AM-POSTCOM treatments the bioaccumulation factors (BAFs) showed the same trend: Cd > Zn > Mn > Pb > Cr, i.e. decreasing with the increase of the concentrations of these metals in the investigated vermicomposts. For Cd the BEFs values were

Table 4
Bioaccumulation of heavy metals in the earthworm tissues.

Treatment	Cd mg·kg^{-1}, dw	Zn mg·kg^{-1}, dw	Mn mg·kg^{-1}, dw	Cr mg·kg^{-1}, dw	Pb mg·kg^{-1}, dw
CONTROL	5.63 a	70.33 a	9.73 a	0.53	0.56
AM-PRECOM	3.20 b	57.00 a	10.8 a	0.50	0.50
AM-POSTCOM	5.90 a	60.67 a	9.73 a	0.50	0.73
p	*0.0068*	*0.0863*	*0.9197*	*0.4219*	*0.5178*

Means followed by the same letter in a column are not significantly different at $p \leqslant 0.05$.
dw: dry weight basis; *p:* probability.

4.000 (CONTROL), 1.333 (AM PRECOM) and 3.105 (AM POSTCOM). For Zn the BEFs values were 0.081 (CONTROL), 0.058 (AM PRECOM) and 0.067 (AM POSTCOM). For Mn the BEFs values were 0.046 (CONTROL), 0.036 (AM PRECOM) and 0.035 (AM POSTCOM). For Cr the BEFs values were 0.003 (CONTROL, AM PRECOM, AM POSTCOM). For Pb the BEFs values were 0.017 (CONTROL), 0.016 (AM PRECOM) and 0.017 (AM POSTCOM).

The bioaccumulation of Cd from the AM-PRECOM mixture was lower by 43.2% and 45.8% in comparison to the CONTROL and th AM-POSTCOM mixtures, respectively, whereas the bioaccumulation of Zn from the AM-PRECOM mixture decreased by 19% and from the AM-POSTCOM mixture by 13.7% when compared to the CONTROL. No significant changes in the bioaccumulation of Mn, Cr and Pb in the earthworm tissues between the treatments were observed. This indicates that addition of biochar before composting had a significant effect on the bioaccumulation of Cd by the earthworms. As for Zn bioaccumulation no significant difference was observed between the mixtures. Some studies showed that biochar can be effective in reducing high concentrations of soluble Cd and Zn from a contaminated soil mostly through sorption (Beesley and Marmiroli, 2011). Tang et al. (2013) indicated that removal of heavy metals with biochar can be related to electrostatic interactions, precipitation and other more complex interactions. They also point out that due to the presence of many functional groups (e.g. carboxylic, alcohol and hydroxyl groups) on the surface of biochar, heavy metals can form complexes with these groups.

During composting of sewage sludge amended with biochar heavy metals could be bound by the compost-biochar matrix, and thus limiting the solubility and bioavailability to earthworms and later to plants when applied to soil (Amir et al., 2005; Kang et al., 2011; Wong and Selvam, 2006). Practical implication of this hypothesis is that amendment of sewage sludge (or other waste materials that are rich in nutrients but also contain contaminants such as heavy metals) with biochar could allow efficient conversion of sewage sludge into vermicomposts that despite the content of heavy metals can be safely applied to soils. At the same time could allow production of earthworm biomass for various applications such as producing feed for fish and animals or obtaining bioactive compounds for pharmaceutical industry (Kostecka and Pączka, 2006; Sinha et al., 2010; Fiołka et al., 2013; Cock et al., 2013). However, more studies is needed to better understand the mechanisms of heavy metal binding by biochar and the fate of heavy metals during composting and vermicomposting. Also, bioavailability of heavy metals in biochar-added vermicomposts to plants requires further research.

3.3. Effects of biochar amendment on vermicomposts

Vermicomposting of the sewage sludge mixtures resulted in significant changes in the obtained vermicomposts. The main characteristics of the investigated mixtures are presented in Table 5.

The pH in all treatments decreased from 6.88–7.14 to slightly acidic 5.27–5.61 – similar results were obtained by other researchers (Hait and Tare, 2012; Sahariah et al., 2015; He et al., 2016). This drop in pH can be due to the formation of carbon dioxide and organic acids that were produced by microorganisms in the process of degradation, and also can be related to substrate specificity (Goswami et al., 2014). Results show that pH values of the obtained products were moderately low. This fact would be favorable for using the product as a horticultural substrate or as an organic amendment in calcareous soils. The process of natural acidification is attributed to the nitrification process that occurs during the composting process at the end of the thermophilic period or during the curing phase (Cáceres et al., 2006, in press), a part of the acidification/nitrification held in vermicomposting (Malinska et al., 2016). However, other researchers observed an increase in pH during vermicomposting of municipal sewage sludge (Khwairakpam and Bhargava, 2009; Nayak et al., 2013).

Electrical conductivity increased significantly in all treatments. This was also reported in other studies (Nayak et al., 2013; He et al., 2016). This increase is attributed to the loss of organic matter and the presence of mineral salts in available forms (Nayak et al., 2013). The increase in moisture content at the end of vermicomposting was a result from weekly moisture adjustment. Organic matter loss was the highest in the AM-PRECOM mixture (9.6%) in the comparison to the CONTROL (6.1%) and the AM-POSTCOM (4.2%) mixtures. Significant losses in organic matter indicate that degradation and mineralization of organic waste was enhanced in the presence of earthworms (Contreras-Ramos et al., 2005; Hait and Tare, 2012). Total nitrogen was somewhat similar or slightly higher after vermicomposting for all mixtures. This could be due to the earthworm nitrogenous excreta (Nayak et al., 2013). The C/N ratio decreased in the CONTROL whereas in the AM-PRECOM and the AM-POSTCOM was slightly lower or similar to the values before vermicomposting. The concentration of macro and trace elements in the obtained vermicomposts is presented in Table 6.

Total phosphorous increased in all treatments. Similar trend was reported by other researchers (Khwairakpam and Bhargava, 2009; Hait and Tare, 2012; Yadav et al., 2012; Nayak et al., 2013). The average increase in total phosphorous was by 15.9%, 62.9% and 53.4% for the CONTROL, the AM-PRECOM and the AM-POSTCOM, respectively. In the mixtures amended with biochar the concentration of phosphorous was significantly higher than in the mixture without biochar. This could be due to the fact that biochar contained significant amount of phosphorous. Also, Nayak et al. (2013) indicated that the increase in total phosphorus could result from mineralization and mobilization of phosphorous by worm gut enzymes and micro flora. During vermicomposting earthworms can convert the insoluble phosphorous forms into soluble forms, and thus increasing their availability to plants (Porkodi and Amruththa, 2014). Other macronutrients (K, Ca and Mg) increased in the vermicomposts except from sodium. Trace elements in small quantities are essential for plant growth. However, elevated concentration of such elements like Cd, Pb, Cr, Zn can be detrimental to plants, and thus limit the application of vermicomposts for fertilization. Generally, the concentration of the investigated trace elements increased after vermicomposting. This is a

Table 5
Characteristics of the obtained vermicomposts.

Parameter	Units	CONTROL	AM-PRECOM	AM-POSTCOM
Moisture content	%	74.43 ± 0.61	70.40 ± 0.37	68.67 ± 0.66
Organic matter content	%	72.82 ± 0.70	65.24 ± 1.29	67.14 ± 1.48
C_{org}	%	40.45 ± 0.39	36.95 ± 0.72	37.30 ± 0.22
N	%	2.96 ± 0.05	3.21 ± 0.50	3.04 ± 0.01
C/N	–	14:1	12:1	12:1
pH	–	5.27	5.61	5.37
Electrical conductivity	μS/cm	1365	1293	1435

Table 6
Macro elements and selected trace elements in the vermicomposts.

Elements	Units	CONTROL	AM-PRECOM	AM-POSTCOM	*p*
Macroelements					
P	$g\,g^{-1}\cdot 100$, dw	1.68	4.09	2.27	*0.6264*
K	$g\,g^{-1}\cdot 100$, dw	0.47	0.46	0.66	*0.2402*
Ca	$g\,g^{-1}\cdot 100$, dw	3.19	1.88	3.04	*0.1802*
Mg	$g\,g^{-1}\cdot 100$, dw	0.64	0.32	0.49	*0.1071*
Na	$mg\cdot kg^{-1}$, dw	480.0 b	900.0 a	890 a	*0.0003*
Trace elements					
Cd	$mg\cdot kg^{-1}$, dw	2.4 a	1.43 b	1.90 ab	*0.03*
Zn	$mg\cdot kg^{-1}$, dw	863.3 b	986.7 a	900.0 ab	*0.0127*
Mn	$mg\cdot kg^{-1}$, dw	210.0 b	296.7 a	276.7 a	*0.0006*
Cr	$mg\cdot kg^{-1}$, dw	190.0	193.3	173.3	*0.2406*
Pb	$mg\cdot kg^{-1}$, dw	30.0	32.0	30.7	*0.7958*

Means followed by the same letter in a column are not significantly different at $p \leqslant 0.05$ dw: dry weight basis; *p*: probability.

typical trend observed in vermicomposting of sewage sludge (Nayak et al., 2013). The concentration of Cd, Zn, Mn and Pb did not exceed the permissible values for organic fertilizers and soil improvers, however in case of Cr the concentration was almost twofold (permissible value is 100 mg/kg) in all mixtures.

4. Conclusions

Amendment of sewage sludge mixtures with biochar before composting had a significant effect on the activity of *E. fetida* during 6 weeks of vermicomposting. The amendment of the initial mixtures before composting can reduce the toxicity of heavy metals to earthworms during vermicomposting. However, more information on the distribution and bioavailability of heavy metals in vermicomposts amended with biochar is needed to better understand the mechanisms behind the reduction of bioavailability of heavy metals to earthworms during vermicomposting.

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