Use of biochar to improve sewage sludge quality in Maluti-A-Phofung Municipality, South Africa

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Research on wastewater treatment processes in Maluti-a-Phofung Municipality (South Africa) has revealed that substandard wastewater management in this region contributes to terrestrial and aquatic pollution. Because this pollution poses a threat to the environment, there is a pressing need to reduce the environmental impact of poorly managed sewage sludge in the region. Biochar has been regarded as a cost-effective way of reducing chemical toxicity in terrestrial environments. In the present study, we aimed to investigate the effectiveness of biochar in the remediation of the toxicity of sewage sludge using the earthworm *Eisenia fetida*. Sewage sludge was collected from a local wastewater treatment plant and *E. fetida* were exposed to 0, 25, 50, and 100% non-amended and 10% biochar-amended sludge. After 28 days, survival, biomass and reproduction were assessed. Separately, in clean artificial soil, *E. fetida* was exposed to 5, 10 and 15% biochar amendment for 96 hours to determine if biochar amendment alone could be harmful to *E. fetida*. The results showed no significant differences in all parameters between the worms exposed to non-amended sludge and 10% biochar-amended sludge. Assessment of acetylcholinesterase and catalase activities in the earthworms that were exposed to biochar via clean soil revealed that 10% and 15% biochar amendment rates caused the worms to experience significant levels of neurotoxic and oxidative stress ($p < 0.05$). These findings reveal that biochar alone is likely to have adverse effects on soil organisms, and amendment rates higher or equal to 10% are not suitable to alleviate the toxic effects of sewage sludge.

Significance:

This study can be used as a reference in the usage of biochar as a toxicity remediator. Different biochar rates ($\geq$ 10%) can have different effects on soil-dwelling organisms. Policymakers can use this study when constructing laws regarding the disposal of sewage sludge by wastewater treatment plants.

Introduction

Sewage sludge is one of the by-products of the wastewater treatment process. It contains high levels of organic matter and nutrients and is valued for agricultural application.[1,2] Studies show that the addition of sludge to degraded soils leads to soil restoration and fertilisation which results in increased crop production.[1,3] Despite this benefit, sewage sludge is still regarded as waste with a potential for significant risk to the environment[4], especially if applied without proper chemical and ecotoxicological consideration[4,5].

Sewage sludge is usually disposed of via landfilling, incineration, and cropland application.[6] Other than its enhancing effect on soil productivity, biochar use in cropland application has gained attention as a means of waste disposal.[7] Some of the disadvantages of this method of disposal include odours, aesthetics, the high load of pathogenic microorganisms, and the high concentrations of both metals and organic pollutants.[8,9] Research has demonstrated the potential for sewage sludge to induce behavioural abnormalities, increase mortality and inhibit the growth of invertebrates.[4] Klee et al.[10] observed that sewage sludge can induce genotoxic effects in the earthworm *Eisenia andrei*. Malińska et al.[11] reported that the heavy metals from sewage sludge can bioaccumulate in earthworm tissues, which poses a risk to the food chain because of potential biomagnification.[12]

Specifications from the existing South African sludge management regulations indicate three categories of sewage sludge: a microbiological class, a stabilisation class, and a pollutant class.[13] The pollutant class depends on the concentration of eight potentially toxic metals: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn).[13] The concentration of these metals is used to further classify the sludge into three subclasses, which are: pollutant class a (best quality), pollutant class b (intermediate quality) and pollutant class c (worst quality).[13,14]

The known toxicity of sewage sludge has prompted the need to find affordable and effective means of decreasing its potential deleterious effects before it is discarded through landfilling or harnessed for agricultural application.[6] Biochar has been identified as a suitable candidate to play such a role in reducing the toxic effects of sewage sludge.[15] Biochar is a solid material obtained from the pyrolysis of a wide range of plant and animal biomasses.[16] Together with bio-oil and biofuel, biochar is one of the by-products of this process.[17-19] Several studies dealing with the application of select biochar types to contaminated soils have reported successful reduction in the toxicity, bioavailability and leachability of soil contaminants.[20-28]

In the present study, we aimed to investigate the effectiveness of biochar in the remediation of the toxicity of sewage sludge on the survival, reproduction, and biomass of the earthworm *Eisenia fetida*. A secondary aim was to assess, at the biomarker level, if biochar on its own could prove detrimental to earthworms. We hypothesised that the inclusion of biochar in the soil would improve the survival, reproduction and biomass of the earthworms. We also expected that the potential beneficial effects of biochar at the level of the whole organism would be supported by concurrent biomarker responses within the organism.

Materials and methods

Study area

Sewage sludge was collected from the wastewater treatment plant (WWTP) in Harrismith (28°16'46.5"S; 29°05'46.9"E). This town is located in the Maluti-A-Phofung municipality within the Drakensberg Afromontane region of the eastern Free State Province of South Africa. The area rises from 1500 m to 2400 m above sea level and experiences summer rainfall (www.floodmap.net). The chosen WWTP receives wastewater from neighbouring households and industries in the region, which is a densely populated peri-urban economic hub, with several industries such as textile, dairy, and aluminium industries.[29]

Experimental organism

Adult *E. fetida* earthworms bred in the Ecotoxicology Laboratory, housed in the Department of Zoology and Entomology of the University of the Free State, QwaQwa Campus, were used as the experimental organism. The earthworms were maintained on a diet consisting only of dried cow dung .

Preparation of exposure substrates

Preparation of soil

The artificial soil used during this study was prepared following the Organisation for Economic Co-operation and Development (OECD) guidelines.[30] The soil was composed of 20% kaolin clay, 70% air-dried quartz sand, and 10% sphagnum peat (pH of 5.5–6.5). The prepared OECD artificial soil was used both as a clean control substrate and in the preparation of varying concentrations (amendment rates) of sewage sludge.

Preparation of non-biochar amended sewage

The sewage sludge was dried at room temperature before use. After drying, it was blended and sieved through a 2 mm sieve. Non-biochar amended sewage was prepared by mixing OECD artificial soil with sewage sludge. The mixing was carried out to make the following concentrations of the sludge: 0 (OECD artificial soil control), 25, 50, and 100% sewage sludge. The total exposure substrate weight per treatment was 500 g. Distilled water was used to moisten each soil treatment to 40–60% of their respective water-holding capacity.[30]

Preparation of biochar amended sewage sludge

Biochar from pine wood obtained in pellet form was purchased from C FERT™ South Africa. According to the manufacturer, it was made at a pyrolysis temperature range of 400–500 °C. This produced a biochar with about 100 μm pore size on average and containing 30.35% carbon, 3.54% nitrogen, 0.13% potassium, 0.02% phosphorus, 20.31% calcium, 0.34% magnesium, and 0.14% sulfur. Biochar-amended substrates were made by replacing 50 g of the 500 g non-amended OECD/sewage substrates with 50 g of biochar for each concentration (including the control), thus making a 10% biochar amendment. Thus, a 10% biochar amendment rate was applied to the 0, 25, 50, and 100% sludge treatments. Distilled water was used to moisten each biochar-amended treatment to 40–60% of their respective water-holding capacity. All the treatments were prepared and left undisturbed for 7 days before exposure. This was necessary to allow for biochar activation by the microorganisms in the sewage sludge.

Metal contents of the sewage sludge

The total concentrations of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) in the sewage sludge were determined following the EPA 3052 digestion method[31], while the concentrations of metals readily available in the water phase of the soil were obtained through the water extraction method.[14] These analyses were performed at the Institute for Soil, Climate, and Water (an Institute of the South African Agricultural Research Council located in Pretoria, Gauteng Province). Inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Perkin-Elmer Optima 3000 DV) was used to determine the concentrations of the metals of interest.

Ecotoxicological assays

Prior to exposure, the earthworms were weighed, and their masses were recorded. Ten adult *E. fetida* were exposed to the biochar-amended and non-amended treatments and incubated at 20 ± 2 °C (in a Labcon low-temperature incubator) for 28 days. All exposures were carried out in triplicate. During the exposure period, the worms were fed 5 g of ground and moist cattle manure once a week. After 4 weeks, the earthworms were weighed once again, and their masses were recorded. The number of cocoons laid in each treatment was recorded. To assess whether biochar on its own could prove detrimental to earthworms at biomarker level, in another experiment, we exposed 10 adult *E. fetida* to 500 g of OECD artificial soil (no sewage sludge involved) amended with 0, 5, 10, and 15% biochar. The exposures conducted in triplicate were incubated at 20 ± 1 °C (in a Labcon low-temperature incubator) for a duration of 4 days. This exposure duration was chosen both to fill a gap in the literature because studies on biochar often last longer[26,32-34], and to increase the chances of observing biomarker responses, which can peak in the early days of the experiment and vanish after relatively longer exposure durations[35]. No feeding took place during the exposure period. After 96 h, the worms were stored at –80 °C until acetylcholinesterase (AChE) and catalase analyses.

Determination of acetylcholinesterase activity

Acetylcholinesterase (AChE) is an enzyme involved in the hydrolysis of acetylcholine (which is an essential neurotransmitter of the central nervous system) into choline.[36] The enzyme was measured to find out whether the sewage sludge had neurotoxic effects on the earthworms. To prepare tissue homogenates for AchE activity, two worms from each exposure treatment were defrosted. Three to five segments of the tail end were sectioned for the assessment of AChE activity and protein determination. Tris-buffer (pH 7.4) was used to homogenise the tissues, which were centrifuged at 9500 *g* for 10 min at 4 °C. Supernatants were then used for AChE and protein analyses. The protein concentrations of the homogenates were determined using the Bradford method.[37] Ellman's method[38] was used for the assessment of AChE activity. For each tissue homogenate, measurements were made in triplicate. AChE activity was determined by calculating the average absorbance of the readings at each time interval from time 0 to time 6 min. Readings were done at 412 nm in 1-min intervals over a 6-min period. The linear graph for each sample was drawn and expressed as the change in absorbance over time. Then, the gradient was calculated for each sample curve and divided by 6 (minutes). AChE activity was calculated as follows: (Absorbance/min/mg protein) = (Abs/min)/mg protein.

Determination of catalase activity

Catalase activity was determined following the method of Cohen et al.[39] The reaction mixture contained the sample homogenate (10 μL) in 10 μL of 0.09 M phosphate buffer (pH 7.0) and 93 μL of 6 mM (30%) hydrogen peroxide, 19 μL of 6 N sulfuric acid and 130 μL of 0.01 N potassium permanganate. The degradation of hydrogen peroxide by the catalase present in the samples was measured within 60 s at 490 nm and expressed in μmol H_2O_2/min/mg protein.

Statistical analysis

The data obtained from this study were subjected to statistical analysis using Microsoft Excel 2010 and GraphPad Prism version 13.2. Statistical analyses of survival, reproduction, biomass and acetylcholinesterase activity data were performed in GraphPad Prism using a one-way ANOVA followed by a Bonferroni post-test for pairwise comparisons. The level of significance was $p < 0.05$. Finally, ToxRat® version 2.10.05 was used to generate median lethal concentrations (LC_{50}) and half-maximal effective concentrations (EC_{50}) whenever possible.

Results and discussion

Concentration of heavy metals in sewage sludge

Using the EPA 3052, the concentration of metals observed in sewage sludge in this study were in the following order: Zn > Cu > Pb > Cr >

Ni > As > Hg > Cd, varying from 840 mg/kg for Zn to 0.66 mg/kg for Cd (Table 1). The water extraction method revealed the following order: Zn > Cu > Ni > Hg > As > Cd > Pb > Cr, varying from 4.76 mg/kg for Zn to 0.03 mg/kg for Cr. (Table 1). When comparing the EPA 3052 and the water extraction data, it was discovered that Zn was the chemical with the highest concentration in both methods, while Cd had the lowest concentration in the EPA 3052, and Cr had the lowest concentration in the water extraction method. This trend is consistent with the findings of Berrow and Webber[40], Zufiaurre et al.[41] and Mosolloane et al.[14], who reported concentrations of heavy metals in sewage sludge from treatment plants in England, Spain and South Africa, respectively. High total concentrations of metals indicate that the wastewater treatment plants receive raw sewage from anthropogenic sources like industries and domestic activities, while high concentrations of Zn and Cu can be attributed to the high organic content of the sewage sludge.[41,42] In South Africa, the works of Snyman and Herselman[43] and Herselman and Snyman[13] are used to classify sewage sludge based on the concentration thresholds of the following metals: As, Cd and Hg (40 mg/kg); Ni and Pb (420 mg/kg); Cr, Cu and Zn (2800 mg/kg). The total metal concentrations of the sewage sludge used in this study were evaluated using this classification system; consequently, the sewage was classified as 'best quality' pollutant class a sludge. It was found that, when combining the concentrations of As, Cd and Hg, they summed to 3.68 mg/kg, which is below the threshold of 40 mg/kg. The concentrations of Ni and Pb added up to 62.51 mg/kg, which did not exceed the threshold of 420 mg/kg. Finally, Cr, Cu and Zn added up to 1001.61 mg/kg, which was below the threshold of 2800 mg/kg (Table 1). Therefore, from the results of the total metal analysis, the concentrations of metals were below the specified limits.

Table 1: Total EPA 3052 and water extraction results for metals that were found in the sewage sludge

Metal (mg/kg)	EPA 3052 method	Water extraction
Cr	22.11	0.03
Ni	11.5	0.53
Cu	139.5	0.88
Zn	840	4.76
Cd	0.66	< 0.5
Pb	51.01	< 0.2
Hg	0.80	0.16
As	2.22	0.14

Nevertheless, the presence of these heavy metals in the sewage sludge and the water extract especially, implies that they could be a source of pollution and toxic stress in soils, especially in agricultural lands where they can accumulate to higher levels.[44,45] The relatively low concentrations of metals in the water extract nevertheless point to lower potential for significant toxic effects. These metals were similarly found in minute quantities in the sewage sludge sampled from the same WWTP by Mosolloane et al.[14]

Ecotoxicological assays

Survival of *Eisenia fetida* in biochar-amended and non-amended sewage sludge

The results show that there was no significant difference ($p > 0.05$) in the survival rates of *E. fetida* exposed to biochar-amended and non-amended sewage sludge for 28 days (Figure 1). This indicates that the presence of biochar did not statistically affect or improve the odds of survival of *E. fetida*. Nevertheless, survival rates in the 25, 50 and 100% sewage sludge treatments was 100% (Figure 1). In comparison, in the absence of biochar in the same treatments, survival rates were 60, 80 and 90% for 25, 50 and 100% treatments, respectively. Such beneficial attributes of biochar to the survival of the earthworm *E. fetida* have been reported by several authors: Elliston and Oliver[46], Malińska et al.[11], Malińska et al.[47], Kim et al.[48], Zhang et al.[49] and Nyoka et al.[26] Earthworms have been reported to thrive in sewage sludge amended with biochar by ingesting sewage and biochar particles, later dispersing them through casting.[50,51] Domínguez et al.[52] also reported that *E. fetida* utilises sewage sludge as a food source, thus its ability to do well in soil amended with sewage sludge. This report has been corroborated by the works of Contreras-Ramos et al.[53] and Malińska et al.[47] It is worth noting that a sewage sludge of 'best quality' class a should not inflict significant mortality on such terrestrial worms as previously reported by Mosolloane et al.[14] using the portworm *Enchytraeus albidus*.

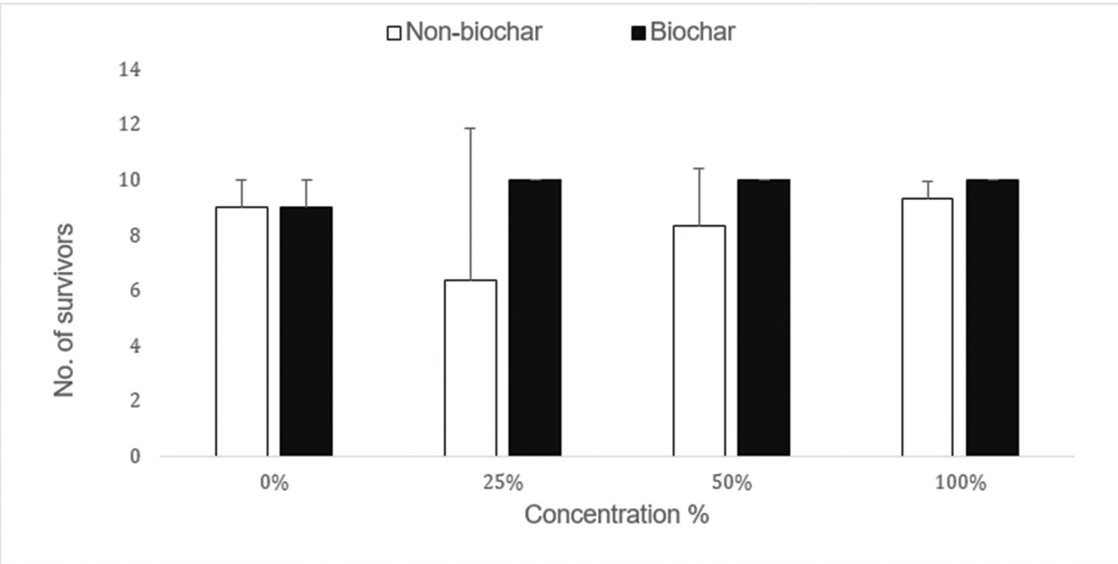

Figure 1: Survival rate of adult *Eisenia fetida* worms at 20 °C after 28 days of exposure to non-biochar and 10% biochar amended sewage sludge from the Harrismith Wastewater Treatment Plant. Error bars represent standard deviations.

Reproduction of *Eisenia fetida* in biochar-amended and non-amended sewage sludge

For both biochar-amended and non-amended sewage sludge, there was no significant reduction in cocoon production in the 100% (pure) sewage sludge when compared to the control ($p > 0.05$; Figure 2). Despite this observation, in pure sewage sludge, the reproduction of *E. fetida* was visibly hampered in both the biochar-amended and non-amended sewage sludge. This observation is similar to the findings of Mosolloane et al.[14] who reported a lack of reproductive output in the pot worm *Enchytraeus albidus* after exposure to the pure sewage sludge from the same WWTP.

In the present study, the lack of significant differences in cocoon production between all the treatments indicates that biochar amendment did not improve the odds of reproduction in *E. fetida*. Although some authors, such as Gong et al.[54] and Nyoka et al.[34], have reported improved reproduction rates in *E. fetida* in biochar-amended soil, others, such as Li et al.[33], have reported the contrary in the same species. The same dichotomy exists in enchytraeids, with some[26] finding improved reproduction rates in *Enchytraeus albidus* and others[55] reporting no effect of biochar on the reproduction of *Enchytraeus crypticus*. Dlamini and Voua Otomo[56] have argued that these seemingly heterogeneous findings could be explained by differences in both biochar amendment rates and the sources of the feed used to make the biochar. Indeed, in both studies by Nyoka et al.[26,34], an amendment rate of 10% biochar was used, and the biochar was made of pine wood. In the study by Gong et al.[54], the rates varied from 0% to 6% and bamboo was used to make the biochar. Li et al.[33] used apple wood chips as a source of their biochar and applied amendment rates ranging from 0% to 20%. Marks et al.[55] used even greater rates, ranging from 0% to 50% with biochar made from poplar and pine wood.

Biomass change in *Eisenia fetida* exposed to biochar-amended and non-amended sewage sludge

After 28 days of exposure, there were no significant changes in the biomass of *E. fetida* in all amended and non-amended treatments except for the 50% biochar amended treatment in which a significant weight gain was observed (Figure 3). The results from this study are similar to the results obtained by Liesch et al.[32] after they looked at the effect of pine chip biochar on the growth and survival of *E. fetida*. Although not always significant, Figure 3 reveals a slight increase in biomass in all biochar-amended treatments, indicating that the presence of biochar has a somewhat positive effect on the biomass of *E. fetida*. This could be attributed to the fact that there was an increase in nutrient content in the soils amended with biochar. It has been reported that biochar can increase the capacity of the soil to absorb and replenish plant nutrients, which in turn has a positive impact on soil invertebrates.[57,58] The observed increase in earthworm biomass is supported by the findings of Li et al.[33] who reported biomass gains after incubating *E. fetida* for 28 days in soil amended with different rates (0.1,10, and 20%) of biochar produced from apple wood chips. Atkinson et al.[59] reported that biochar can convert organic matter into useful and consumable nutrients or elements that soil invertebrates use as food, thus resulting in increases in biomass. However, other authors, such as Gomez-Eyles et al.[60], have reported weight loss in *E. fetida* exposed to soil treated with deciduous, hardwood-derived biochar.

Acetylcholinesterase activity of *Eisenia fetida*

After assessing the activity of AChE in different rates of biochar in the absence of sewage sludge, significantly high activity could only be observed in the 15% biochar amended soil (Figure. 4). These findings demonstrate that even in the absence of a pollutant, high biochar amendment levels, particularly above 15%, resulted in an increase in AChE activity. Khalid et al.[61] claimed that adding biochar from corncob biomass to soils alone can harm earthworms at a molecular level, in addition to affecting their behaviour, growth and reproduction. Furthermore, they reported a substantial increase in AChE inhibition (i.e. low AChE activity) in the earthworm *Pheretima posthuma* at 5% and 10% amendment rates after 30 days of exposure in soil amended with 0, 5, 10, and 25% biochar. Such inhibition in AChE activity was not observed in the present study. The pine tree biochar assessed herein favoured biochar activity, especially in the 15% amendment rate. The divergence in findings between our studies could be due to differences in both the biomass used to create the biochar and the duration of exposure, which was substantially shorter in our case (4 vs 30 days).

Catalase activity of *Eisenia fetida*

Catalase activity was similar in the earthworms exposed to all biochar amendment rates except for the 10% amendment in which it was significantly higher. This indicates an increase in oxidative stress associated with greater rates of biochar amendment (Figure 5), especially considering that catalase activity was statistically similar in both highest amendment rates.

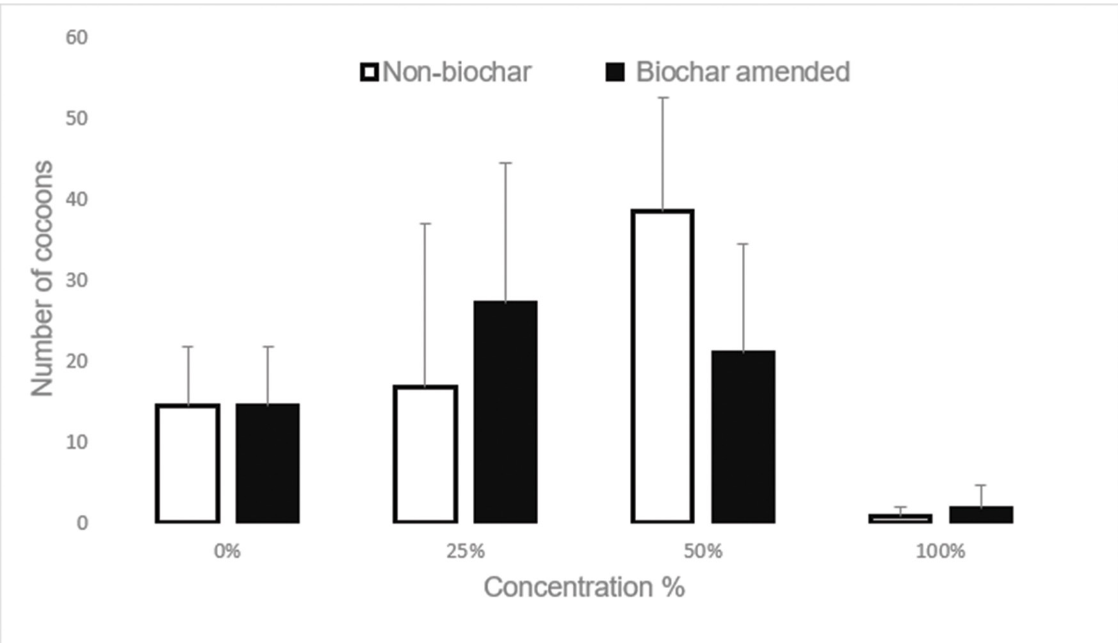

Figure 2: Number of *Eisenia fetida* cocoons produced at 20 °C after 28 days of exposure to non-biochar and 10% biochar amended sewage from the Harrismith Wastewater Treatment Plant. Error bars represent standard deviations.

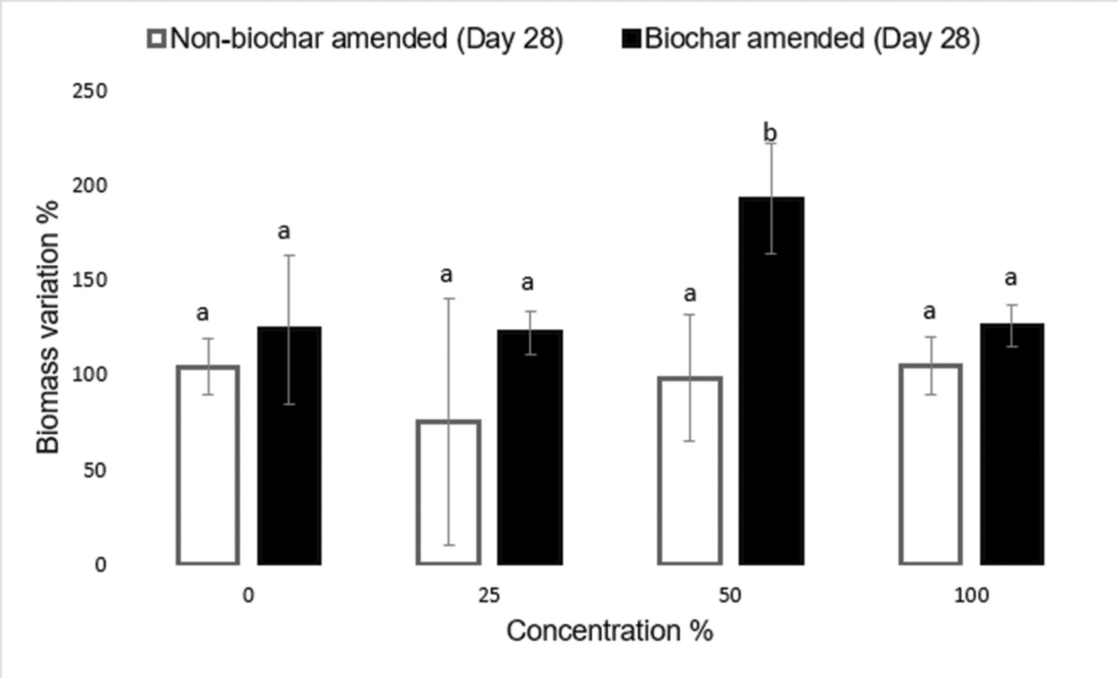

Figure 3: Comparison of the biomass of *Eisenia fetida* at 20 °C before and after 28 days of exposure to non-biochar and 10% biochar amended sewage sludge from the Harrismith Wastewater Treatment Plant. Error bars represent standard deviations.

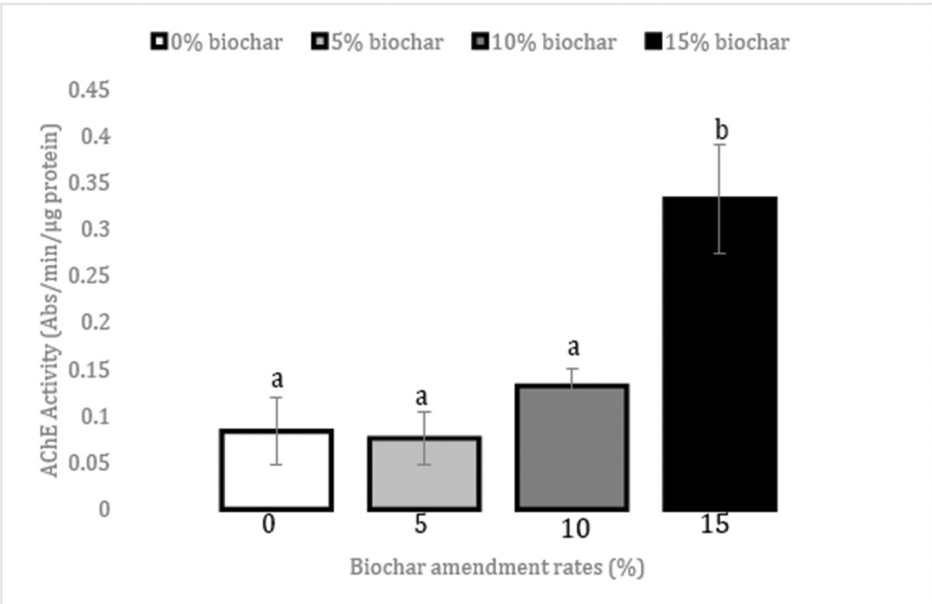

Figure 4: AChE activity in *Eisenia fetida* after a 96-h exposure to biochar-amended (5, 10 and 15%) and non-amended OECD artificial soil. Data represented are the means of three replicates. Error bars represent standard deviations and different letters represent significant differences.

Our findings are supported by those of Han et al.[62] who found no appreciable variations in the catalase activity of *E. fetida* exposed to 0–5% biochar made from rice straws, although other authors such as Shi et al.[63] have reported a significant increase in catalase activity in *E. fetida* exposed to soil amended with less than 5% biochar made from cow dung. In our study, such low biochar rates did not result in any significant alterations in catalase activity, indicating that the feed used to make the biochar might play a role in the observed variations. The pyrolysis temperature might also be a factor. Shi et al.[63] discovered that biochar made at a pyrolysis temperature of 550 °C resulted in more increased catalase activity than those at the lower temperature of 350 °C or the higher temperature of 750 °C. Similarly, Kim et al.[48] examined how biochar generated from biomasses of perilla, sesame, and pumpkin seeds affected the earthworm *E. fetida,* and discovered that at 5% amendment, the biochar produced at a pyrolysis temperature of 550 °C caused higher catalase activity than biochar produced at a temperature of 300 °C. The biochar utilised in this study was made at a temperature range that should cause relatively less catalase activity (400–450 °C), although our pine tree biochar did cause noticeable catalase increases at 10% amendment (Figure 5).

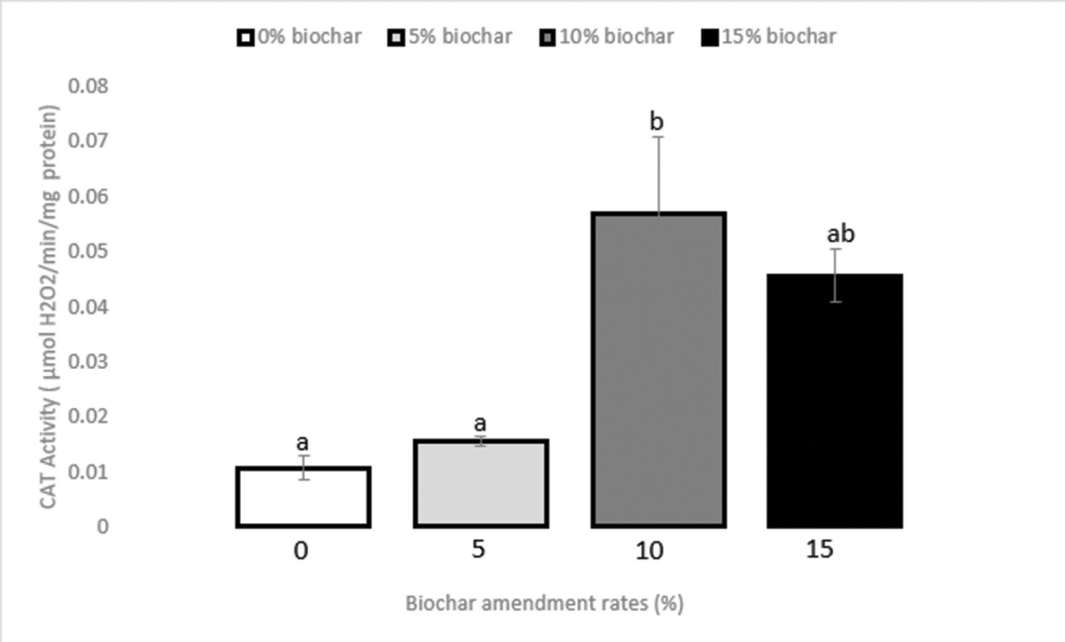

Figure 5: Catalase activity of *Eisenia fetida* after a 96-h exposure to biochar-amended (5, 10 and 15%) and 0% biochar amended OECD artificial soil. Data represented are the means of three replicates. Error bars represent standard deviations and different letters represent significant differences.

Conclusions

We set out to test whether biochar amendment could help improve the quality of sewage sludge by assessing multiple endpoints at biomarker and whole organism levels in the earthworm *Eisenia fetida*. Our results show little to no benefit to selected life-cycle parameters. A biochar rate experiment revealed that biochar rates of 10% and 15% could significantly increase catalase and AChE activities, respectively. Overall, the results indicate that biochar amendment could help organisms such as earthworms better withstand environmental stress brought about by sewage sludge application or be used as a prior step to environmental disposal of wastewater treatment waste.

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Competing interests

We have no competing interests to declare.

Authors' contributions

N.P.D.: Methodology; investigation; project administration; writing – first draft, reviewing and editing. P.V.O.: Supervision; writing – reviewing and editing; project management, project administration.

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