Liesch et al., Annals of Environmental Science / 2010, Vol 4, 1-9

IMPACT OF TWO DIFFERENT BIOCHARS ON EARTHWORM GROWTH AND SURVIVAL

Amanda M. Liesch[1], Sharon L. Weyers[2*], Julia W. Gaskin[3], and K.C. Das[3]

[1] Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA
[2] USDA - Agricultural Research Service, North Central Soil Conservation Research Laboratory, 803 Iowa Ave., Morris, MN 56267, USA
[3] Agricultural and Biological Engineering, University of Georgia, Athens, GA 30602, USA

Received December 1, 2009; in final form February 22, 2010; accepted April 20, 2010.

ABSTRACT

Interest in the use of biochar as a soil amendment to increase soil fertility and sequester carbon is increasing. However, the effects of biochar on the survival and reproduction of earthworms are unknown. The toxicity of two different biochars (pine chip and poultry litter char) on *Eisenia fetida* applied to an artificial soil (70% sand, 20% kaolin, and 10% sphagnum) was investigated at five different application rates of 0, 22.5, 45, 67.5, and 90 Mg ha^{-1}. Earthworm mortality and weight loss reached 100% at the two highest application rates of poultry litter biochar, whereas mortality and weight loss with pine chip biochar did not differ from control treatments. Soil pH, which also increased in controls and pine chip biochar treatments over the course of the incubation, was the most likely cause of earthworm mortality in all treatments. However, it was apparent that poultry litter biochar provided a more stressful environment to earthworms since many worms died in the first five days of incubation. This stressful environment was most likely due to the presence of ammonia gas in addition to high pH, which increased from 7.2 to 8.9 with increasing application rates of poultry litter biochar (pH 10.3). Potentially toxic micronutrients, including As, Zn, Cu, Fe, and Al, present at sub-toxic levels in poultry litter biochar treatments on an individual basis were not likely to have contributed to earthworm mortality; additive effects, however, were not established. Poultry litter biochar also had high Na and Mg content, which could have led to high salinity. As biochar characteristics depend on the feedstock and conditions of pyrolysis, toxicity screening of biochars, particularly those likely to increase soil pH, prior to land application is recommended.

Keywords: Pyrolysis, biochar, *Eisenia fetida*, toxicity, artificial soil test

1. INTRODUCTION

Pyrolysis is thermoconversion of biomass with the exclusion of oxygen. This pyrolysis produces syngas, bio-oil and biochar [1]. Interest has been increasing in using biochar as a soil amendment to sequester carbon C, improve soil quality and also reduce the potential negative impacts of bioenergy production [2,3]. Biochar is thought to be recalcitrant [4,5] and thus is a stable source of C. Biochar has effects on other soil characteristics, including CEC [6], pH [7] and fertility [8-11]. However, these effects depend on the biochar feedstock and pyrolysis conditions [12,13] as well as on the soil itself [14]. Proposed rates of biochar addition for C sequestration and soil quality enhancement can be quite high. Reported application rates in studies of the fertility and crop growth effects of biochar range from 10 to 100 Mg ha^{-1} for low or moderate nutrient feedstocks in tropical and subtropical soils [10,11,15,16]. Biochar from high nutrient poultry litter feedstock was applied at rates of 10 to 50 Mg ha^{-1} in an Alfisol [8]. Biochar addition at these rates is likely to have significant effects on soil properties [6-14].

Although there has been some research on the effect of biochar addition on the soil microbiological community [4,17-19], there is relatively little information on the effects of biochar application on macrofauna such as earthworms. Application of charcoal from a traditional charcoal kiln mixed with sawdust decreased cocoon density of the earthworm *Pontoscolex corethurus* Muller compared to a fallow control in French Guiana, but no effects were seen in the adult/sub-adult populations [20]. *P. corethrurus* did not avoid naturally produced charcoal-soil mixtures and was able to burrow through mixtures and even ingest charcoal-laden soil [21,22]. Topolaintz and Ponge [21,22] suggested that *P. corethrurus* likely benefits from actively incorporating wood-based charcoal (pH 7.1) into soil because of the associated increase in soil pH from acidic (pH 4.63) to

*Corresponding author: Phone: (320)589-3411 ext. 146, Fax: (320)589-3787, Email: <Sharon.Weyers@ars.usda.gov>

more neutral values (pH 6.9). Similarly, Chan et al. [8] reported that earthworms preferred Alfisol soils (pH 4.5) mixed with poultry litter biochar produced at 450°C (pH 9.9) to unamended soil or soil mixed with poultry litter biochar produced at 550°C (pH 13). At amendment rates of 50 Mg ha^{-1}, addition of the 450°C poultry litter biochar increased soil pH from 5.0 to 7.1 and addition of the 550°C poultry litter biochar increased soil pH from 4.83 to 7.78. The biochar amendment rates of soil mixtures and earthworm species used in the avoidance tests were unclear.

The earthworm reports cited above using biochar or charcoal substances indicate that the resulting soil pH after application may be the main factor driving earthworm behavior. Cited research also indicates that feedstock material and processing influence the pH of the resulting biochar. Of concern also is the concentration of potentially toxic micronutrients (e.g., Zn and Cu) in biochar made by feedstock materials such as poultry litter [13]. In order to develop appropriate recommendations for the application of biochar, the potential impacts on soil biota, specifically earthworms, need to be identified. Our first step in determining these impacts was to conduct a toxicity test modeled after standard short-term artificial soil tests using the earthworm *Eisenia fetida* [23,24]. This type of testing is often used by regulators to approve substances for land application. Although *E. fetida* does not occur naturally in agricultural soils, it has become the standard species for toxicity studies and numerous toxicology data using this species are available in the literature. The aim of this study was to determine if two different types of industrially-created biochar, one low nutrient of near neutral pH, and one high nutrient of more basic pH, were detrimental to *E. fetida* growth and survival in an artificial test soil.

2. METHODS

We studied the effect of pine chip biochar and poultry litter biochar on earthworm growth and survival in incubated, controlled cylindrical containers. The containers had a surface area of 32.2 cm^2, and contained a 12-13 cm depth of artificial soil (approx 300 g soil per container). Artificial soil was prepared by mixing 10.5 kg of sand, 3 kg of kaolin clay, and 1.5 kg of sphagnum peat moss, 100g of $CaCO_3$ and moistened to 35% by weight (modified from US-EPA guidelines [24]). $CaCO_3$ is added to moderate the acidity of the sphagnum. A side test showed that *E. fetidia* was tolerant of this prepared soil substrate which had an initial pH of 7.2. The soil mixture was weighed out into 36 replicate chambers. Soil pH was tested at the beginning on one replicate and the end of the experiment on all replicates using a 2:1 water to soil ratio.

The pine chip and poultry litter biochars were obtained by pyrolysis with a maximum temperature of 400°C, a holding time of 0.5 hr, and N_2 as a carrier gas. Approximately 95% of the material was between 1 to 2 mm in size. Selected chemical properties of these two chars are listed in Table 1. Two biochar treatments each at 0, 22.5, 45, 68, and 90 Mg ha^{-1} application rates, corresponding to 0, 7.1, 14.2, 21.3, and 28.3 g of biochar added per replicate chamber were tested. Biochar was homogenously mixed into the soil-containing chambers with four replicates per biochar application rate, except the zero (0) application, which was a single four-replicate set of controls. Moisture contents were not adjusted to the amount of biochar, which was immiscible in water, but starting weights were maintained throughout the experiment. Mesocosms were completely randomized in the incubation chamber, covered in parafilm to maintain moisture and prevent earthworm escape, and incubated at 20°C for 28 days.

Ten sub-adult *E. fetida* earthworms obtained from a commercial vendor were randomly chosen and added to each mesocosm with the total earthworm weight and number per replicate recorded at the beginning and end of the experiment. Earthworms were rinsed to remove surface soil and blotted dry for total fresh weights. Observations of survival were made at five days and again at 28 days at the end of the experiment. Dead earthworms found at the surface of mesocosms were noted and removed. An earthworm was judged to be dead if it did not respond to stimulus with a blunt probe. As dead tissue decomposes rapidly in soil, earthworms not found after sampling after 28 days were assumed to have died sometime during the incubation period. Total earthworm weight per replicate chamber was measured to the nearest ± 0.01 g. Statistical comparisons were made on the total initial weight for all ten earthworms added, and on average final weight, which was calculated on the basis of total number of surviving worms per replicate. This average weight approach avoided bias due to the different sizes of earthworms added or any potential impact of that individual weight on survivorship. Percent change in weight was calculated as:

$$\frac{(\text{final fresh weight biomass g worm}^{-1} - \text{initial fresh weight biomass g worm}^{-1})}{\text{initial fresh weight biomass g worm}^{-1}} \times 100$$

Table 1 Readings for pH, electrical conductivity (EC) and total nutrient concentrations of poultry litter (PL) and pine chip (PC) - derived biochars

Feedstock	pH	C (%)	N (%)	S (%)	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Na ($g\ kg^{-1}$)
PL	10.27	42.3	4.24	0.97	42.9	70.6	54.4	15.2	19.5
PC	7.32	73.3	0.15	0.03	1.21	2.83	3.13	8.90	5.60
	EC mS	Al ($mg\ kg^{-1}$)	As ($mg\ kg^{-1}$)	Cd ($mg\ kg^{-1}$)	Cr ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Pb ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
PL	15.5	2530	51.7	1.84	10.4	177	2650	<4.60	1080
PC	0.112	285	<0.86	<0.86	1.55	164	415	<4.31	71

Statistical analyses were performed to determine significant differences in pH and earthworm growth and survival across application rates at the termination of the incubation. The pH value was converted into antilog [pH] for statistical tests, and converted back to pH for discussion of results. One-way ANOVA (PROC ANOVA in SAS 9.1) was used to determine significant differences across application rates within a biochar treatment. A two-factorial ANOVA (Proc GLM in SAS 9.1) was used to make inferences between biochars. However, because of the single set of control treatments, the statistical design for the two-factor ANOVA was balanced by replicating the data for the four control replicates in the second biochar treatment. We confirmed the validity of the two-factorial analysis by running six successive tests of randomly assigned pairs of control replicates to each of the biochar treatment factors; these results were not different, and only data from the complete design are reported. For ANOVA tests that showed significant differences among treatments, multiple comparisons were made using Fisher's LSD (T) test; for insignificant ANOVA findings, planned multiple comparisons were made using the Bonferroni test. Linear regressions (Proc Reg in SAS 9.1) were performed to determine linear trends in the pH (converted to antilog) with increasing application rates as discussed below. Pearson's and Spearman's correlations were conducted within biochar treatments between pH, initial total weight, and percent survival. Data are presented as means ± one standard error (s.e.).

3. RESULTS

The addition of slightly basic or highly basic biochars to a near neutral artificial soil (pH 7.2) altered initial and final soil pH (Table 2). At the outset of the incubation, initial pH levels of the treatment soils became slightly more acidic with the addition of pine chip biochar, but became more basic with addition of poultry litter biochar. At the end of the 28-day incubation, pH values in all treatments were higher than initial pH and were significantly different between the two biochar treatments at the higher application rates (Table 2). Linear regressions on the antilog of pH versus application rate within each biochar treatment indicated that pH had a significant and strong linear response to increasing poultry litter biochar ($r^2 = 0.75$, $p > 0.0001$), but a weak response to increasing pine chip biochar ($r^2 = 0.45$, $p = 0.0011$).

Earthworm additions to controls and pine chip biochar treatments were successful as the earthworms eventually burrowed into the substrate. Addition of earthworms into poultry litter biochar treatments, however, was marked by trauma, and within many of the amended treatments several earthworms were found dead on the surface within the first five days. At increasing application rates from 22 to 90 Mg ha^{-1} of poultry litter biochar, the average number of dead earthworms, respectively, were 2, 5, 7, and 8 individuals (n=4). At the same time, only one earthworm out of all four replicates had died on the surface in each of the 45 Mg ha^{-1}, and 90 Mg ha^{-1} pine chip biochar treatments. Since we did not destructively sample, we did not determine total mortality at five days. Initial total weight (10 earthworms) averaged 1.54 g (±0.03 s.e.) across all treatments and replicates. Although initial weights were not significantly different among the nine replicate sets nor within the replicate sets used for the poultry litter biochar treatments, they were significantly different between the 45 Mg ha^{-1} and 90 Mg ha^{-1} treatments within the pine chip biochar set after randomization (Table 3). Although these differences occurred, there were no significant correlations between initial total weight and percent survival.

At the termination of the incubation the mean survival was 27% across all mesocosm chambers, and survival was significantly better across pine chip biochar treatments than across poultry litter biochar treatments, respectively 40.5 % and 13.5%, with Fisher's LSD = 10.1% (Table 3). Percent survival was also significantly different among application rates, differing by 15.9%; the significant differences among application rates were primarily due to the significant differences among rates just within the poultry litter biochar treatments (Table 2). Although the test on the interaction term was not significant, Bonferoni comparisons indicated that treatment differences greater than 42.5% were significant among the biochar application rate combinations, which were essentially between the 22.5 Mg ha^{-1} pine chip biochar treatment and the 67.5 and 90 Mg ha^{-1} poultry litter biochar treatments (Table 3). Percent survival was significantly correlated to pH but only within the poultry litter biochar treatments (Pearson's = -0.642, p = 0.0023; Spearman's = -0.6513, p = 0.0019).

Total biomass within the controls dropped from 1.54 g to 0.35 g from the loss on average of seven earthworms. For the surviving earthworms, however, this amounted to a loss of only 0.04 g of fresh weight per worm (26% of initial body weight). Although surviving earthworms in two of the pine chip biochar treatments actually maintained or slightly increased in weight, over all treatments surviving earthworms averaged a loss of 0.06 g fresh weight (Table 3). There were no significant differences among application rates within or across biochar treatments; however, there were significant differences between the two biochars over all application rates (Table 3). Although the interaction term between biochar and application rates were not significant, Bonferoni multiple comparison indicated that there was a significant difference for treatments differing by more than 0.12 g of fresh weight, which essentially was limited to the 67.5 Mg ha^{-1} pine chip biochar treatment and the 67.5 and 90 Mg ha^{-1} poultry litter treatments, where no earthworms survived. Put on a percentage basis of initial total biomass per earthworm, weight loss among the biochar application rate comparisons were also significantly different among treatments (p = 0.0383; Figure 1). Differences in percent change in fresh weight per earthworm were also significant for the biochar factor (poultry litter biochar treatment mean (including control) = -63.2 (±10.7) %, pine chip biochar treatment mean (including control) = -13.7 (± 8.9) %, p < 0.0004) but there was no significant difference due only to application rate (p = 0.2028).

Table 2 Artificial soil pH readings (2:1 water:soil) in mesocosms treated with increasing application rates of pine chip (PC) and poultry litter (PL) - derived biochars at start and finish of a 28-day incubation; and results of statistical tests for final pH readings. Final values given are means (standard error) where n=4

Application Rate (Mg ha^{-1})	Initial PC	Initial PL	Final PC	Final PL	Final Mean
0	----------------- 7.19 --------------		------------- 7.70 (0.04) ----------		7.70 (0.04)
22.5	7.12	7.71	7.64 (0.17)	7.67 (0.04)	7.65 (0.03)
45	7.08	7.95	7.47 (0.04)	8.09 (0.18)	7.77 (0.15)
67.5	6.98	8.07	7.42 (0.09)	8.62 (0.12)	8.02 (0.23)
90	6.94	7.96	7.44 (0.39)	8.87 (0.02)	8.15 (0.27)
Mean	6.22	7.78	7.53 (0.03)	8.19 (0.29)	7.85 (0.08)
Statistics					
Within biochar	PC: p = 0.0115, LSD = 0.17		PL: p < 0.0001, LSD = 0.35		
Biochar	p < 0.0001, LSD = 0.12				
Application rate	p < 0.0001, LSD = 0.19				
Biochar * Application rate	p < 0.0001, LSD = 0.62				

 www.aes.northeastern.edu, ISSN 1939-2621

Table 3 Means (s.e.) and statistical comparisons for total initial weight, percent survival and weight loss by pine chip (PC) and poultry litter (PL) biochar and application rate

	Initial Total Weight (g fresh weight)			Survival (%)			Average weight loss per surviving earthworm (g fresh weight)		
Application Rate (Mg ha^{-1})	PC	PL	Mean	PC	PL	Mean	PC	PL	Mean
0		1.54 (0.09)			30 (5.8)			0.04 (0.02)	
22.5	1.45 (0.06)	1.54 (0.14)	1.50 (0.07)	57.5 (6.3)	30 (12.2)	43.8 (8.2)	0.00 (0.01)	-0.07 (0.04)	-0.03 (0.02)
45	1.82 (0.13)	1.61 (0.08)	1.71 (0.08)	50 (4.1)	7.5 (4.8)	43.8 (8.2)	-0.08 (0.03)	-0.09 (0.06)	-0.08 (0.03)
67.5	1.63 (0.12)	1.58 (0.12)	1.60 (0.08)	32.5 (11.1)	0 (0)	16.3 (8.0)	0.02 (0.04)	-0.16 (0.01)	-0.07 (0.04)
90	1.31 (0.05)	1.40 (0.07)	1.35 (0.04)	32.5 (13.8)	0 (0)	16.3 (8.9)	-0.03 (0.04)	-0.14 (0.01)	-0.08 (0.03)
Mean	1.55 (0.05)	1.53 (0.04)	1.54 (0.03)	40.5 (4.4)	13.5 (4.1)	27 (3.7)	-0.02 (0.01)	-0.10 (0.02)	-0.06 (0.01)
Statistical comparisons [a]									
Within char	0.28*	0.31^{NS}					0.14^{NS}	0.16^{NS}	
Biochar	0.12^{NS}			10.1****			0.04**		
Application rate	0.20*			15.9**			NS[b]		
Biochar Application rate	0.51^{NS}			42.5^{NS}			0.12^{NS}		

[a] Values shown are least significant difference for multiple comparisons by LSD/T test where ANOVA result is significant or Bonferoni minimum significant difference for multiple comparison by Bonferoni method where ANOVA result was not-significant; p – values provided are those for the ANOVA test, $p < 0.05$ for multiple comparisons. See explanation of statistical methods in text for further clarification. NS – non significant, *p <0.05, ** p <0.01, ***p < 0.001, ****p < 0.0001.
[b] Neither ANOVA nor Bonferoni test were significant.

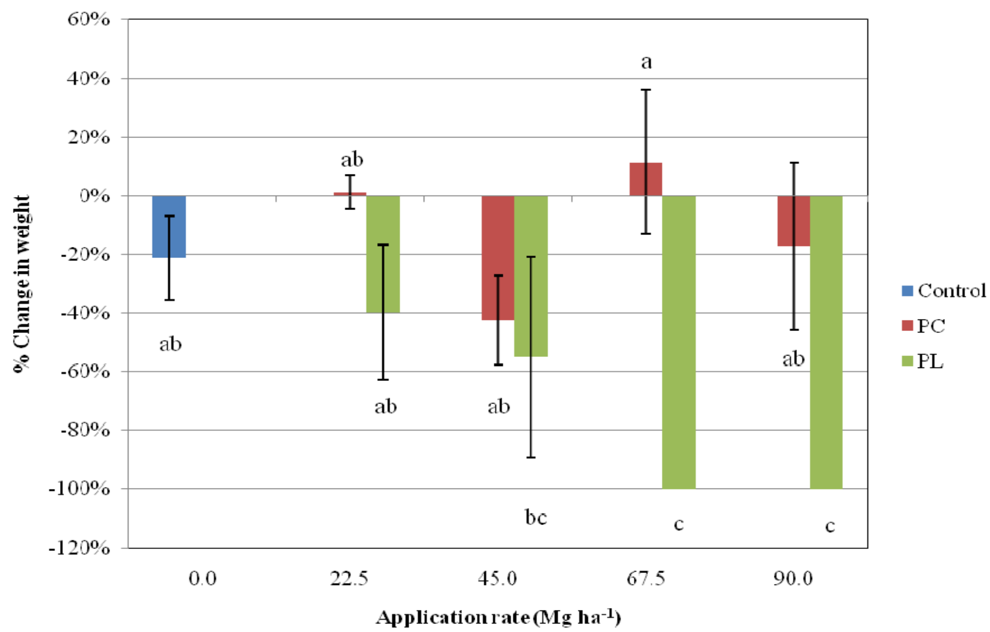

Figure 1 Percent change in average fresh biomass of *E. fetida* in the controls, pine chip (PC) and poultry litter (PL)-derived biochar treatments at increasing application rates. For each application rate mean n = 4, error bars show ± 1 s.e.

4. DISCUSSION

Our findings contrast the results of experiments with *P. corethrurus* showing 100% survival and selective ingestion of powdered charcoal/soil mixtures in mesocosms using natural char and native soil [21,22]. The mesocosms in those experiments with *P. corethrurus* had a non-char amended refuge in one half, but the earthworms were active in both halves. Bioturbation due to earthworm activity was minimal in pine chip biochar and control treatments and nonexistent in the poultry litter biochar treatments. In our side-test we determined that *E. fetida* was tolerant of the prepared test soil and burrowing activity was unaffected, so such low activity in the control treatment under experimental conditions was not expected. The change in burrowing behavior indicates that some limitation on biological incorpo-ration through the action of earthworms might occur in the field. The potential nutritive value or ingestion of biochar by *E. fetida* was not a consideration with our toxicity tests but it was clear that activity, i.e. burrowing behavior, was affected by the presence of poultry litter biochar.

Although sphagnum moss serves as a food source and appeared to be actively ingested, lack of sufficient resources or microbial activity is a possible contributing factor to weight loss. Less mature earthworms, as indicated by lower initial total biomass, may have been more susceptible to detrimental conditions imposed by the higher biochar treatments; however, initial total weight did not correlate significantly with percent survival. Other than increases in pH, which occurred in both biochar treatments and controls by the conclusion of the experiment, we are uncertain of any other cause of death or weight loss.

Many species of earthworms show intolerance to acidic soils with preference for soils typically at neutral pH [25]. However, reports of preferences or tolerances of earthworms to soils above pH 8 are scarce. As discussed previously, earthworm preference of poultry litter biochar-amended soils at neutral pH was shown over more acidic unamended soil or more basic amended soil [8]. More directly, biomass of juvenile *E. fetida* cultured on a mixed vermicompost bedding declined as pH was increased up to 9.5 [26]. The death of earthworms in the high application rate poultry litter biochar treatments at the beginning of the experiment was likely due to the toxicity of ammonia, although the higher pH in poultry litter biochar treatments likely contributed to continued death of earthworms as the incubation proceeded.

Poultry litter biochar contains ammonium (measured in water leachate, see [13]). Some portion of this may be ammonium salts that decompose to ammonia with sufficient moisture [27-29]. Although earthworms excrete nitrogenous wastes in the form of ammonia or urea, other nitrogenous compounds and ammonium salts, particularly ammonium chloride, ammonium citrate or glutamic acid, can be toxic [30]. The toxicity of animal manures to earthworms has been attributed to ammonia or ammonia salt contents [31-33], although negative effects of some ammonium salts such as ammonium sulfate were probably linked to soil acidification [31], which obviously was not a problem in the present study.

Biochars have a range of pH depending on feedstock and pyrolysis conditions, but most biochars are slightly acidic to basic (range 6.2 to 9.9 [12]). Although increasing temperature of pyrolysis can increase the pH of the resulting biochar [3], the pine chip and poultry litter biochars were produced at the same temperature and under the same pyrolysis conditions. Thus, the pH difference between the biochars is likely due to the higher ash content, as indicated by greater concentrations of non-volatile minerals (Table 1) of the poultry litter, and attributed to hydrolysis of Ca, K, and Mg salts [7].

Some poultry litters contain appreciable levels of potentially toxic elements, such as As, that can be preserved in the production of low-temperature biochars [34]. Poultry litter biochar used in this study contained high concentrations of metals and other micronutrients, including Na, Mg, Al, Cu, Fe, Zn and As (Table 1). High concentrations of these micronutrients may affect earthworm survivorship, growth and reproductive capacity, particularly for *E. fetida*, and especially at concentrations above 200 mg kg^{-1} [35-37]. There is also evidence of As and Al toxicity to other species of earthworms [38-40]. However, the calculated concen-tration of these elements in the present study ranged between 4 to 17 mg Cu kg^{-1} soil, 26 to 102 mg Zn kg^{-1} soil, 1 to 5 mg As kg^{-1} soil, and 60 to 239 mg Al kg^{-1} soil, far below reported toxic concentrations and unlikely to have contributed to earthworm death.

Although metal toxicity was unlikely in this case, the high concentration of metals, the presence of ammonia, high pH and salinity imparted by the high concentrations of Na, as supported by high EC measurements, in poultry litter biochars are still cause for concern. In many artificial and field soil toxicity tests, the form and/or availability of metals present as well as soil pH, texture, salinity, and organic matter content have an impact on toxicity [41-47]. Further research on water-soluble concentrations, bioavailability and additive effects from the whole complex of toxic substances would be necessary to ascertain metal toxicity as a potential concern for mortality of soil biota with land application. Additional analyses would be necessary to elucidate a salt effect and determine the bioavailability of other components.

5. CONCLUSIONS

The effects of biochar on earthworm growth and survival depend on the chemical composition of the feedstock, biochar produced and the application rate of material used. There was no difference in survivorship between pine chip biochar and the control treatments even at high rates of application (above 45 Mg ha^{-1}), and earthworm survivorship was apparently improved with the pine chip biochar compared to the poultry litter biochar. Poultry litter biochar, however, was harmful to earthworms at rates above 45 Mg ha^{-1}. Poor survival and growth of *E. fetida* in poultry litter biochar treatments is likely due to presence of ammonia and a rapid increase in pH, but not due to the presence of toxic metals. Although *E. fetida* does not occur naturally in agricultural soils, impacts on this standard test species give positive indication of the potential sensitivity of naturally occurring earthworms to the material being tested. We determined that the increase in soil pH was the main factor causing earthworm fatality in all treatments, including the controls. The negative impact of poultry litter biochar in this artificial soil test indicates that caution should be used when considering this type of biochar for the high rates of application needed for C sequestration. More specific tests evaluating bioavailability of components and impact on soil biota in field soil should be used to screen biochars, particularly those that are likely to increase soil pH, and to develop recommendations for use as a soil amend-ment.

6. ACKNOWLEDGMENTS

We greatly appreciate the assistance of Alan Wilts, Nancy Barbour, and Tyson Mastin at the USDA ARS in Morris, MN, and Keith Harris and Brian Bibens at the University of Georgia for their contributions in completing this experiment. We thank three anonymous reviewers for their comments, which greatly helped to improve this manuscript.

7. REFERENCES

[1] Antal MJ Jr., Gronli M. The art, science, and technology of charcoal production. *Ind. Eng. Chem Res.*, 2003, 42: 1619-1640.

[2] Laird DA. The charcoal vision: A win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agron. J.*, 2008, 100: 178-181.

[3] Lehmann J A. Handful of carbon. *Nature*, 2007, 447: 143-144.

[4] Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, Xu X. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. *Soil Biol. Biochem*, 2009, 41: 210-219.

[5] Schmidt MWI, Noack AG. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. *Global Biogeochem. Cycl.*, 2000, 14: 777-793.

[6] Liang B, Lehmann J, Solomon D, Kinyangi J, Grossman J, O'Neill B, Skjemstad JO, Thies J, Luizao FJ, Petersen J, Neves EG. Black carbon increases cation exchange capacity in soils. *Soil Sci. Soc. Am. J.*, 2006, 70: 1719-1730.

[7] Tryon EH. Effect of charcoal on certain physical, chemical, and biological properties of forest soils. *Ecol. Monograph.*, 1948, 18: 81-115.

[8] Chan KY, Zwieten LV, Meszaros I, Downie A, Joseph S. Using poultry litter biochars as soil amendments. *Aust. J. Soil Res.*, 2008, 46: 437-444.

[9] Lehmann J, Pereira da Silva Jr J, Steiner C, Nehls T, Zech W, Glaser B. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant Soil*, 2003, 249: 343-357.

[10] Novak JM, Busscher WJ, Laird DL, Ahmedna M, Watts DW, Niandou MAS. Impact of biochar amendment on fertility of a southeastern coastal plain soil. *Soil Sci.*, 2009, 174: 105-112.

[11] Steiner C, Teixeira WG, Lehmann J, Nehls T, de Macêdo JLV, Blum WEH, Zech W. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant Soil*, 2007, 291: 275-290.

[12] Chan KY, Xu Z. Biochar: Nutrient properties and their enhancement. In: Lehmann J, Joseph S, eds. *Biochar Environmental Management*. London, Sterling, VA: Earthscan, 2009, 67-84.

[13] Gaskin JW, Steiner C, Harris K, Das KC, Bibens

B. Effect of low-temperature pyrolysis conditions on biochar for agricultural use. *Trans. ASABE*, 2008, 51: 2061-2069.

[14] Speir RA. Use of pyrolysis char as an amendment in soil of the southeastern United States, M.S. Thesis. University of Georgia, Athens, 2008.

[15] Chan K.Y, Zwieten .V, Meszaros I, Downie A, Joseph S. Agronomic values of greenwaste biochar as a soil amendment. *Aust. J. Soil Res.*, 2007, 45: 629-634.

[16] Gaskin JW, Speir RA, Lee RD, Harris K, Morris L, Das KC, Fisher D. Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status and yield. *Agron. J.*, 2010, 102:623-633.

[17] Pietikainen J, Kiikkila O, Fritze H. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos*, 2000, 89: 231-242.

[18] Steiner C, Teixeira W, Lehmann J, Zech W. Microbial response to charcoal amendments of highly weathered soil and Amazonian dark earths in Central Amazonia - Preliminary results. In: Glaser B, Woods W, eds. *Amazonian Dark Earths: Explorations in Space and Time.* New York: Springer-Verlag, 2004, p. 195-212.

[19] Warnock DD, Lehmann J, Kuyper TW, Rillig MC. Mycorrhizal responses to biochar in soil - concepts and mechanisms. *Plant Soil*, 2007, 300: 9-20.

[20] Topoliantz S, Ponge J-F, Ballof S. Manioc peel and charcoal: a potential organic amendment for sustainable soil fertility in the tropics. *Biol. Fert. Soils*, 2005, 41: 15-21.

[21] Topoliantz S, Ponge JF. Burrowing activity of geophagus earthworm *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) in the presence of charcoal. *App. Soil Ecol.*, 2003, 23: 267-271.

[22] Topoliantz S, Ponge JF. Charcoal consumption and casting activity by *Pontoscolex corethrurus* (Glossoscolecidae). *App. Soil Ecol.*, 2005, 28: 217-224.

[23] OECD. Earthworm, acute toxicity tests. OECD Guidelines for testing of chemicals, Section 2, Effects on biotic systems. Paris: Organization for Economic and Cooperative Development 1984.

[24] US EPA. Ecological Effects Test Guidelines OPPTS 850.6200 Earthworm Subchronic Toxicity Test U.S. Environmental Protection Agency. 1996. http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-6200.pdf

[25] Edwards CA, Bohlen PJ. *Biology and Ecology of Earthworms.* London: Chapman & Hall, 1996, 426p.

[26] Tripathi G, Bhardwaj P. Comparative studies on biomass production, life cycles and composting efficiency of *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg). *Bioresc. Tech.* 2004, 92:275-283.

[27] Cantrell K, Ro K, Mahajan D, Anjom M, Hunt PG. Role of thermochemical conversion in livestock waste-to-energy treatments: obstacles and opportunities. *Ind. Eng. Chem. Res.*, 2007, 46: 8918-8927.

[28] Sheth AC, Turner AD. Kinetics and economics of catalytic steam gasification of broiler litter. *Tran. ASAE*. 2002. 45:1111-1121.

[29] Whitely N, Ozao R, Cao Y, Pan WP. Multi-utilization of chicken litter as a biomass source. Part II. Pyrolysis. *Energy Fuels*, 2006, 20: 2666-2671.

[30] Cohen S, Lewis HB. The nitrogenous metabolism of the earthworm (*Lumbricus terrestris*). *J. Biol. Chem.*, 1949, 180: 79-91.

[31] Curry JP. Factors affecting the abundance of earthworms in soils. In: Edwards CA, ed. *Earthworm Ecology*. Boca Raton: CRC Press, 2004, pp 91-114.

[32] Edwards CA, Bohlen PJ, Linden DR, Subler S. Earthworms in agroecosystems. In: Hendrix PF, ed. *Earthworm Ecology and Biogeography in North America*. Boca Raton: Lewis Publishers-CRC Press, 1995, p 185-213.

[33] Hansen S, Engelstad F. Earthworm populations in a cool and wet district as affected by tractor traffic and fertilisation. *App. Soil Ecol.*, 1999, 13: 237-250.

[34] Arai Y, Lanzirottii A, Sutton S, Davis JA, Sparks DL. Arsenic speciation and reactivity in poultry litter. *Environ. Sci. Technol.*, 2003, 37: 4083-4090.

[35] Lukkari T, Aatsinki M, Väisänen A, Haimi J. Toxicity of copper and zinc assessed with three different earthworm tests. *App. Soil Ecol.*, 2005, 30: 133-146

[36] Neuhauser EF, Loehr RC, Milligan, HL, Malecki MR. Toxicity of metals to the earthworm *Eisenia fetida*. *Biol. Fert. Soils,* 1985, 1: 149-152.

[37] Reinecke AJ, Reinecke SA. The influence of heavy metals on the growth and reproduction of the compost worm Eisenia fetida (Oligochaeta).

Pedobiologia, 1996, 40: 439-448.
[38] Langdon C J, Piearce TG, Meharg AA, Semple KT. Interactions between earthworms and arsenic in the soil environment: a review. *Environ. Pollut.*, 2003, 124: 361-373
[39] Lock K, Janssen CR. Toxicity of Arsenate to the Compostworm *Eisenia fetida*, the Potworm *Enchytraeus albidus* and the Springtail *Folsomia candida* Bull. *Environ. Contam. Toxicol.*, 2002, 68: 760-765.
[40] van Gestal CAM, Hoogerwerf G. Influence of soil pH on the toxicity of aluminium for *Eisenia andrei* (Oligochaeta: Lumbricidae) in an artificial soil substrate. *Pedobiologia*, 2001, 45: 385-395.
[41] Conder JM, Lanno RP. Evaluation of surrogate measure of cadmium, lead, and zinc bioavailability to *Eisenia fetida*. *Chemosphere*, 2000, 41: 1659-1668.
[42] Ma W. Sublethal toxic effects of copper on growth, reproduction and litter breakdown activity in the earthworm *lumbricus rubellus*, with observations on the influence of temperature and soil pH. *Environ. Pollut. Series A*, 1984, 33: 207-219.
[43] Nahmani J, Hodson ME, Black S. A review of studies performed to assess metal uptake by earthworms. *Environ. Pollut.*, 2007, 145: 402-424.
[44] Nahmani J, Hodson ME, Black S. Effects of metals on life cycle parameters of the earthworm *Eisenia fetida* exposed to field-contaminated, metal-polluted soils. *Environ. Pollut*, 2007, 149: 44-58.
[45] Owojori OJ, Reinecke AJ, Rozanov AB. Effects of salinity on partitioning, uptake and toxicity of zinc in the earthworm *Eisenia fetida*. *Soil Biol. Biochem.*, 2008, 40: 2385-2393.
[46] Spurgeon DJ, Lofts S, Hankard PK, Toal M, McLellan D, Fishwick S, Svendsen C. Effect of pH on metal speciation and resulting metal uptake and toxicity for earthworms. *Env. Toxicol. Chem*, 2006, 25: 788-796.
[47] Spurgeon DJ, Hopkin SP. Effects of variations of the organic matter content and pH of soils on the availability and toxicity of zinc to the earthworm *Eisenia fetida*. *Pedobiologia*, 1996, 40: 80-96.

AES 91201

.