ARTICLE IN PRESS

Journal of Hazardous Materials Advances xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Hazardous Materials Advances

journal homepage: www.elsevier.com/locate/hazadv

Ecotoxicity of two contrasting soils with biochar derived from cattle manure and rice husk

Guillermina Cantou [a], Jim J. Wang [b,*], Baoyue Zhou [b], Jeong-Min Lee [c], Jong-Hwan Park [c,**]

[a] Departamento de Sistemas Agrarios y Paisajes Culturales, Centro Universitario Regional Este (CURE), Universidad de la República (UdelaR), Treinta y Tres, Uruguay
[b] School of Plant, Environment & Soil Sciences, Louisiana State University AgCenter, Baton Rouge, LA 70803, USA
[c] Department of Smart Green Resources, Dong-A University, 37, Nakdong-daero 550 beon-gil, Saha-gu, Busan, 49315, South Korea

ARTICLE INFO

Keywords:
Biochar
Cattle manure
Rice husk
Ecotoxicity
Soil

ABSTRACT

This study investigates the ecotoxicological effects of two contrasting biochars, produced from cattle manure (CMB) and rice husk (RHB), applied to two agricultural soils with divergent properties: a calcareous silt loam (Norwood) and an acidic clay (Alligator). The biochars differed markedly in their physicochemical profiles: CMB exhibited high pH (11), electrical conductivity (12 dS m^{-1}), and K content (89 g kg^{-1}), while RHB showed much greater surface area (174 vs. 1.7 m^2 g^{-1}). Ecotoxicity was assessed through four bioassays (*Vibrio fischeri, Pseudokirchneriella subcapitata, Sorghum saccharatum, and Lepidium sativum*). CMB caused strong toxicity in all aqueous bioassays, while RHB showed no adverse effects. When applied to soils, CMB reduced the toxicity of acidic Alligator soil to *P. subcapitata* (from 54 % to 16 %) and reclassified its hazard level from class III to I, likely due to aluminum immobilization (Al^{3+}). Conversely, CMB slightly increased toxicity in Norwood soil while RHB improved ecotoxicological profiles in both soils. These results highlight the critical role of soil-biochar interactions in modulating environmental risk. This study links biochar composition and soil properties to bioassay outcomes, offering a practical approach to assess bioavailability-driven toxicity across realistic combinations of biochar and agricultural soils under controlled conditions. These findings underscore the need to align biochar–soil combinations with specific management goals, whether agronomic, environmental, or both.

1. Introduction

Biochar has been considered an eco-friendly management tool that can improve agricultural production systems and contribute to environmental sustainability. Various research studies have shown that biochar application enhances soil productivity by improving soil quality and fertility (Lehmann and Joseph, 2015). Specifically, biochar amendment affects soil physicochemical characteristics such as cation exchange capacity (CEC), water-holding capacity, soil porosity, bulk density, carbon content and stability, and pH (Xu et al., 2012; Tao et al., 2016). Moreover, it has been demonstrated that adding biochar to the soil modifies soil biological properties, which may influence nutrient and organic matter cycles, soil processes, and plant growth (Luo et al., 2013; Liang et al., 2010; Yang et al., 2016). These changes in microbial community activity and diversity have a direct impact on soil functionality (Lehmann and Joseph, 2015).

The characterization of biochar's physicochemical properties and contaminant content has provided essential information for risk assessment strategies (IBI, 2015). However, several studies have emphasized the need for a more holistic and precautionary approach to biochar application, highlighting the importance of incorporating ecotoxicological criteria even when chemical safety thresholds are met (Oleszczuk, 2013; Domene, 2015; Dong et al., 2025). This is particularly relevant because biochar, despite complying with chemical standards, can still negatively affect soil biota, such as by immobilizing microbes, disrupting microbial community structure, and altering nutrient cycling, ultimately compromising soil health (Brtnicky et al., 2021; Dai et al., 2021). Bioassay methods offer a rapid, reliable assessment of bioavailable toxicants, complementing traditional chemical and physical characterization (Tsui and Chu, 2003). Ecotoxicity testing provides direct insight into the biological availability of undetected compounds and the integrated impact of a mixture of chemical substances present in a complex and heterogeneous matrix such as biochar. Although bioassay tests have been commonly used in water/soil systems contaminated

* Corresponding author at: School of Plant, Environmental, and Soil Sciences, Louisiana State University AgCenter, Baton Rouge, LA 70803, USA.
** Corresponding author at: Department of Smart Green Resources, Dong-A University, Busan, 49315, South Korea.
E-mail addresses: JJWang@agcenter.lsu.edu (J.J. Wang), zoqlsqkr@dau.ac.kr (J.-H. Park).

https://doi.org/10.1016/j.hazadv.2025.100832
Received 1 April 2025; Received in revised form 27 June 2025; Accepted 17 July 2025

Please cite this article as: Guillermina Cantou et al., *Journal of Hazardous Materials Advances,* https://doi.org/10.1016/j.hazadv.2025.100832

with various chemicals, it has been seldom performed with biochar products. Oleszczuk et al. (2013) conducted the first study on the toxicity of biochar and introduced the use of some innovative biological assays for assessing the ecotoxicity of aqueous solutions.

Some studies have reported potential negative effects of biochar on biota (Oleszczuk et al., 2013; Spokas et al., 2010). The most likely cause of toxicity following biochar addition to soil has been attributed to the presence of polycyclic aromatic hydrocarbons (Koltowski and Oleszczuk, 2015), high levels of volatile organic compounds (Smith et al., 2013), ammonia (Liesch et al., 2010; Domene et al., 2015), as well as high pH, base cation content, or salinity (Buss et al., 2015, 2016; Lehmann et al., 2012).

While biochar amendment can alter the soil environment, benefits or deleterious effects likely depend not only on its intrinsic properties, but also on the type of soil, the applied dose, and the response of the organisms that inhabit that ecosystem (Spokas and Reicosky, 2009). Researchers have reported that certain biochars improved the productivity of some soil types, while in others, the same biochar did not affect or even harm their productivity (Shaaban et al., 2018). Soil, biochar, and organisms interact with each other immediately after biochar addition to soil (Lehman and Joseph, 2015), and biochar's function highly depends on these complex relationships. Soil organic matter, nutrient and toxic content, mineralogy and texture, pH, electrical conductivity (EC), moisture, and biota and plant composition can all influence biochar's behavior in soil (Joseph et al., 2010). Agronomic benefits of biochar have to be checked for different types of soil and crops (Van Zwieten et al., 2010), in order to minimize potential detrimental environmental effects. The Organisation for Economic Co-operation and Development (OECD) artificial soil is commonly used in soil ecotoxicity tests as a standardized soil-like medium (Hofman et al., 2009). However, it is important to conduct toxicity tests on different soil types to develop an understanding of the role of their properties on the bioavailability and toxicity of biochar, which has not been adequately evaluated.

The bioavailability of toxic compounds added to soil is known to be strongly influenced by various soil physical and chemical properties. For example, soil CEC is important for the sorption of metals, while organic matter adsorbs organic hydrophobic contaminants very strongly (Ahmed et al., 2015). Soil texture and clay mineralogy also affect pollutant behavior through their influence on CEC and water-holding capacity (van Gestel and van Dis, 1988). Furthermore, microbiological activity and community composition may vary with different soils, and affect organic compounds degradation. Once biochar is applied to soil, it can undergo biogeochemical interactions with soil constituents that may affect ionic strength and pH of soil solution (Shackley et al., 2013). This can, in turn, influence the bioavailability and toxicity of some elements, resulting in both direct and indirect toxic effects on the exposed organisms (Hilber et al., 2017).

Given that biochar application can exert either positive or negative effects on soil biota, depending on both biochar physicochemical properties and soil characteristics, this study specifically examines biochar-soil interactions across distinct soil types. Our hypothesis posits that soil attributes critically modulate the ecotoxic impact of biochar, leading to divergent biological responses in different soil matrices. Based on the literature, we anticipated that: (i) biochars that exacerbate abiotic stress (e.g., through changes in pH, salinity or other soil properties) may tend to induce greater ecotoxicity depending on the soil context (Nkoh et al., 2021; Hilber et al., 2017); (ii) contrasting soils would exhibit differential sensitivity to the same biochar (Singh et al., 2022; Shang et al., 2024); and (iii) certain biochar-soil combinations might mitigate toxic effects (Nkoh et al., 2021). Consequently, this work evaluates the ecotoxic characteristics of two distinct agricultural soils amended with two contrasting biochars to elucidate how soil attributes interact with biochar properties to drive ecotoxic outcomes.

2. Materials and methods

2.1. Production and characteristics of biochar

The CM and RH used in this experiment for biochar production were collected from LSU AgCenters Rice Research Station, Crowley, LA and LSU AgCenters Iberia Research Station, LA, respectively. The CMB and RHB were produced at a pyrolysis temperature of 550 °C. Briefly, raw materials were first oven-dried (40 °C), and then pyrolyzed at 550 °C for 2 h in a muffle furnace (Thermolyne FA1730). The rest of the production conditions were the same for all samples. The heating rate was ~4 °C min^{-1}. N_2 was continuously flushed throughout the pyrolysis process and during the cooling period at a flow rate ranging from 0.5 to 1.0 L min^{-1}, to provide oxygen-limited conditions. Biochars were cooled down to room temperature overnight and then ground and sieved to yield a uniform 2 mm size fraction.

The following biochar parameters were determined: pH and EC (biochar:water = 1:10) with an Orion model 150 (Thermo Fisher Scientific, MA); ash and moisture content were determined by ASTM D1762–84; elemental composition (C and N) was measured by dry combustion method using a CN Elemental Analyzer (Elementar Americas Inc., NJ); specific surface area was determined by physisorption at 77 K (Micromeritics ASAP 2020 Plus); and chemical analysis was conducted through wet acid digestion (nitric acid and 30 % H_2O_2) followed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, SPECTRO Plasma 3200, Germany).

2.2. Soils amended with biochar

Two agricultural soils, one calcareous and the other acidic, from Louisiana, U.S., were selected for this study. The calcareous soil was a Red River Alluvial Norwood silt loam (fine-silty, mixed, superactive, hyperthermic Fluventic Eutrudepts) collected from a soybean field at the Dean Lee Research and Extension Station, LSU. The acidic soil was a Mississippi River Alluvial Alligator clay (very-fine, smectitic, thermic Chromic Dystraquerts), from a sugarcane field in Iberia Parish. Composite soil samples were collected at 15 cm depth, air-dried, ground, and passed through a 2-mm sieve before use. The physical and chemical

Table 1
Physical and chemical properties of the selected soils.

Parameter	Norwood	Alligator
OM, g kg^{-1}	6.9	23
pH	8.0	4.6
CEC, cmol kg^{-1}	8.5	26
Ca[Ŧ], mg kg^{-1}	2848	3748
Mg[Ŧ], mg kg^{-1}	244	707
P[ƥ], mg kg^{-1}	291	86
K[Ŧ], mg kg^{-1}	106	243
Na [Ŧ], mg kg^{-1}	34	45
B[¥], mg kg^{-1}	0.74	0.69
Cu[ƕ], mg kg^{-1}	0.72	1.91
Fe[ƕ], mg kg^{-1}	15	96
Mn[ƕ], mg kg^{-1}	9.6	27
Zn[ƕ], mg kg^{-1}	0.25	0.96
Al[€], mg kg^{-1}	0.34	119
S[§], mg kg^{-1}	45	28
Texture	Silt loam	Clay
Particle size distribution, g kg^{-1}		
Sand	20	130
Silt	860	240
Clay	120	630

OM: Organic matter; CEC: Cation exchange capacity.
Ŧ NH_4OAc extraction.
¥ DTPA extraction.
ƥ NH_4F - HCl extraction.
ƕ DTPA-TEA extraction.
€ Titrimetric method by 1 N KCl.
§ KCl extraction.

properties of these soils are presented in Table 1.

The CMB and RHB were applied at 20 g kg^{-1} of the agricultural soils, and the samples were mixed thoroughly on a rotary shaker for 24 h. The biochar-amended soils and a control of each soil (no amendment) were wetted with deionized water to reach 70 % of the water-holding capacity, and then incubated in the dark for 7 days at room temperature (24 ± 0.5 °C). After incubation, the treatments were air-dried at 40 °C for 6 days, ground, and stored in airtight plastic bags before ecotoxicological analysis.

2.3. Ecotoxicity assay

The ecotoxicological assessment in this study was based on a battery of four standardized bioassays using different test organisms: *Vibrio fischeri* (bacteria), *Pseudokirchneriella subcapitata* (algae), *Lepidium sativum* and *Sorghum saccharatum* (plants), aiming to capture a range of biological responses across different trophic levels. The assays were conducted separately for biochar samples (via water extracts) and for soils and soil-biochar mixtures (via liquid-phase elutriates or solid-phase exposure, depending on the assay).

For biochar samples, water extracts were prepared following the EN 12,457–2 protocol, using a 10:1 liquid-to-solid (L/S) ratio. Samples were shaken at 10 rpm for 24 h at 23 ± 0.5 °C using a roller device and then filtered through 0.45 µm membrane filters. The bacterial luminescence inhibition test was performed using the standard Microtox® protocol with *V. fischeri*. Freeze-dried bacteria were reconstituted with the manufacturer's reconstitution solution (JJS Technical Services, Schaumburg, IL, USA) and exposed to 45 % (v/v) extract concentration. Luminescence was measured after 5 and 15 min using a Microtox M500 analyzer (Strategic Diagnostics Inc., Newark, DE, USA), and compared with a deionized water control. All tests were performed in triplicate at 15 °C.

Algal growth inhibition was evaluated using *P. subcapitata* (CPCC 37, obtained from the Canadian Phycological Culture Centre), following the 72-h microplate toxicity protocol developed by Environment Canada (2007). Algae were grown axenically under continuous vertical fluorescent illumination (75 µmol $m^{-2}s^{-1}$), with orbital shaking at 70 rpm and temperature maintained at 25 ± 1 °C. The test consisted of 0.22 mL bioassay volumes in sterile 96-well flat-bottom microplates, where 100 % biochar extract, nutrient medium, and algal inoculum were combined to yield an initial cell density of 1×10^4 cells mL^{-1}. After 72 h of static incubation, algal cell density was quantified using a particle counter (Beckman Coulter Z Series, Indianapolis, USA). Growth inhibition was calculated relative to controls exposed to ultrapure water.

Phytotoxicity of biochar extracts was assessed using the liquid-phase Phytotoxkit F™ (MicroBioTests Inc., Belgium), with L. *sativum* and *S. saccharatum* as test plants. Transparent biocompartment plates were prepared by placing a foam pad and filter paper at the bottom, which were soaked with 20 mL of either biochar extract or deionized water (control). A second filter paper was placed above, and ten seeds were positioned equidistantly along the central ridge. Plates were incubated vertically in the dark at 24.5 ± 1 °C for 3 days. Root length and germination percentage were measured using Image Tool 3.0 software (UTHSCSA, San Antonio, TX, USA).

Ecotoxicity tests for soils and soil-biochar mixtures followed similar principles but with some adaptations. For bacteria and algae assays, liquid-phase elutriates were prepared by mixing soil samples with deionized water at a liquid-to-solid (L/S) ratio of 1:4, as described by Pereira et al. (2011). These suspensions were shaken at 10 rpm in darkness for 24 h at 23 ± 0.5 °C, then allowed to settle and filtered through 0.45 µm membranes. The resulting elutriates were used in the Microtox® 81.9 % Basic test and the standardized algal microplate toxicity test, following the same protocols described above for biochar extracts. In both cases, test responses were compared to those from corresponding water-only controls.

Phytotoxicity in soils and amended soils was assessed using the solid-phase Phytotoxkit F™, with the same plant species. Test units were filled with homogenized test soil or soil-biochar mixtures, and compared against a standardized artificial reference soil provided by MicroBioTests Inc., consisting of 85 % sand, 10 % kaolin, and 5 % peat. Plates were incubated at 24.5 ± 1 °C in darkness for 3 days, and root length and germination were recorded as described previously.

As both bacteria and algae are highly sensitive to matrix pH, extract samples were adjusted with NaOH or HCl (≤ 1 N) to remain within the neutral range of pH 6–9 when needed, to isolate potential toxic effects from pH-related interference. Potassium dichromate ($K_2Cr_2O_7$) was used as a positive control to monitor assay precision and reproducibility over time.

All bioassays were conducted in triplicate, and ecotoxicity results were expressed as percent effect (PE) relative to the control. To classify the potential hazard of the samples, we adopted the acute toxicity ranking system originally proposed by Persoone et al. (2003), which uses PE values derived from a battery of bioassays. This system assigns each sample to one of five hazard categories based on the highest PE observed across tests: I: no acute hazard, II: slight acute hazard (20 % ≤ PE < 50 %), III: acute hazard (50 % ≤ PE < 100 %), IV: high acute hazard (PE = 100 % in at least one test), and V: very high acute hazard (PE = 100 % in all tests). Although this classification was originally developed for aquatic systems, it has been successfully extended to eluates of waste materials, sludge, and soils (Ozcan et al., 2013; Rodríguez-Liébana et al., 2013; Malara and Oleszczuk, 2013; Martínez-Gallardo et al., 2025; Grzegórska et al., 2023), supporting its relevance for our study.

2.4. Statistical analysis

Ecotoxicological properties were also evaluated using Bray–Curtis distance-based redundancy analysis as an ordination method, carried out in RStudio software (RStudio, Inc., MA, U.S.). The combination of two soils and three biochar variables (no addition, 20 g kg^{-1} of CMB, and 20 g kg^{-1} of RHB) resulted in a factorial design of six treatments. Analysis of variance was performed using the SAS package (version 9.7, SAS Institute Inc., N.C., U.S.), and differences were evaluated with Tukey HSD's post-hoc test at a 0.05 level. Three replications for each combination of the two factors were considered, unless otherwise stated.

3. Results and discussions

3.1. Biochar characteristics

The pH of CMB and RHB was strongly alkaline and weakly alkaline at 11 and 8.9, respectively (Table 2). In general, it was reported that the pH of biochar is affected by oxides and hydroxides originating from alkali metals (Ahmad et al., 2014). Zornoza et al. (2016) reported that the magnitude of pH increase varies depending on the type of feedstock that contains alkaline metal components. The EC content in CMB was 12 dS

Table 2
Physical and chemical properties of biochars derived from cattle manure and rice husk.

Parameters	CMB	RHB
pH	11 ± 0.15	8.9 ± 0.11
EC, dS m^{-1}	12 ± 0.07	0.38 ± 0.01
Ash, %	41 ± 1.3	40 ± 1.5
C [Ŧ], g kg^{-1}	490 ± 2.3	510 ± 1.4
N [Ŧ], g kg^{-1}	33 ± 0.57	3.4 ± 0.10
K [Ŧ], g kg^{-1}	89 ± 2.7	2.9 ± 0.17
Ca, g kg^{-1}	21 ± 1.3	0.90 ± 0.04
Mg [Ŧ], g kg^{-1}	8 ± 0.25	0.54 ± 0.07
Na [Ŧ], g kg^{-1}	10 ± 0.24	0.04 ± 0.001
P [Ŧ], g kg^{-1}	16 ± 0.85	0.69 ± 0.09
SA_{BET}, m^2 g^{-1}	1.70	174

[Ŧ] Total content.

m^{-1}, which was much higher than in RHB (0.4 dS m^{-1}), and this was closely related to the content of inorganic components present in CMB. The carbon contents in CMB and RHB were 490 and 510 g kg^{-1}, respectively, showing no significant difference between the two biochars. The nitrogen and phosphorus contents in CMB were higher than those in RHB, and this is thought to be mainly influenced by the raw materials. In general, cattle manure contains a large amount of protein, which is partially vaporized during the thermal decomposition process, and most of the remainder is converted into pyrrole-N or pyrimidine-N structures (He et al., 2024). The content of inorganic components in CMB was significantly higher than that in RHB, which explains why CMB exhibits high pH and EC. RHB (174 m^2 g^{-1}) has a very well-developed specific surface area, whereas that of CMB (1.70 m^2 g^{-1}) is extremely limited. This result suggests that the specific surface area of CMB is lower than that of RHB because most of the pores are blocked by inorganic crystals formed during the thermal decomposition of cattle manure. According to Cao and Harris (2010), the development of specific surface area in biochar is critically affected by the mineral components of raw materials. For example, livestock manure containing calcium crystals may form more compact crystal structures during the pyrolysis process, which reduces the specific surface area. On the other hand, the pores in agricultural residues and plant residues with relatively low inorganic compound content tend to expand during the thermal decomposition process, thereby improving the specific surface area within biochar (Martins et al., 2007).

3.2. Biochar ecotoxicity

A large variation in the responses of organisms to the biochars was observed, although CMB elicited similar responses across the four biological tests performed (Fig. 1). According to the toxicity classification system Persoone et al. (2003), all values above 50 % were considered toxic, while negative values indicated stimulation of luminescence, growth, or root length stimulation for bacteria, algae or plants, respectively. CMB showed the highest toxicity to all test organisms, suggesting that the causal factor of this toxicity probably originated from the feedstock material. The International Biochar Initiative (IBI, 2015) has established a range of maximum threshold values for toxicants in biochar, and also prescribed the use of the germination test to assay biochar toxicity. This test uses “fail” or “pass” to indicate if there is a difference in germination compared to the control. The results of germination inhibition by CMB and RHB are shown in Table 3. Based on IBI criteria, CMB failed the germination of *S. saccharatum* and L. sativum. Both germination (Table 3.1) and root elongation tests (Fig. 1C and D) proved to be effective endpoints to detect phytotoxicity parameters. In a comparison of the integrated index of bioassay results for the produced biochars (Persoone et al., 2003), CMB was classified as highly toxic (class IV). In contrast, RHB did not show any negative effects in any of the toxicity tests performed and was particularly effective in stimulating bacteria, algae, or plant growth/root length.

According to these results, the biological toxicity effect of biochar is considered to be closely related to the biochar properties. Although biochar with high EC value can be considered a nutrient-rich amendment, it can also pose a threat to plant and microbial health (Domene et al., 2015). In this research, the EC was a good indicator to predict biochar ecotoxicity related to total salt concentrations. Owojori et al. (2009) reported that juvenile production and reproduction in *F. candida* (the least sensitive of the four soil organisms studied) was inhibited at values above 1.03 and 1.62 dS m^{-1}, respectively. Domene et al. (2015)

Table 3
Germination inhibition test of biochars derived from cattle manure and rice husk.

Biochar	*Sorghum saccharatum*	Lepidium sativum
Control[T]	9.6	10.0
CMB	8.3	7.2
RHB	9.4	9.8

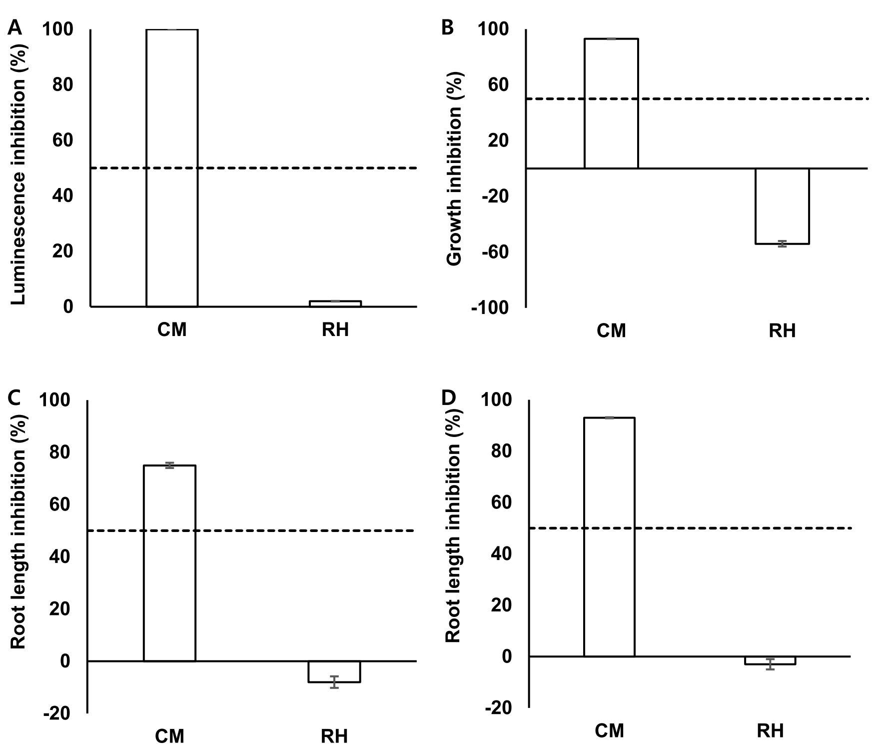

Fig. 1. Toxicity (% inhibition with respect to the control) of biochars. (A) Vibrio fischeri, (B) *Pseudokirchneriella subcapitata*, (C) *Sorghum saccharatum*, and (D) Lepidium sativum. The dashed line indicated 50 % of inhibition, which corresponds to the limit from which the sample was considered toxic, according to the hazard classification system employed.

also reported biochar salinity varied according to the original feedstock. The alkali metal potassium (K) is not only an essential macronutrient but also an important osmotic agent. As K is involved in the salinization process, it could produce toxicity even at very low concentrations. It has been shown that K can increase the toxicity of other chemicals, especially soluble species (e.g. nitrate) in the environment (Romano and Zeng, 2007). Negative effects of biochar on L. *sativum* growth were reported due to the high pH (>10.8) of biochar (Godlewska et al., 2021). Gascó et al. (2016) reported that the toxicity caused by biochar was dependent on the plant species and feedstock used in the experiment. In particular, biochars with high pH were harmful to the growth of most plants.

3.3. Ecotoxicity in soil amended biochar

It has been well established that biochar application to soil can impact microbial diversity and activity due to the changes it generates in soil physico-chemical properties (Masiello et al., 2011; Lehmann et al., 2011). However, there was no clear trend related to the potential ecotoxicological risk of biochar addition, since responses were influenced by multiple factors such as feedstock type, production conditions, tested organism, soil characteristics, application rate or extract concentration (Smith et al., 2013; Oleszczuk et al., 2013; Bastos et al., 2014). Oleszczuk et al. (2013) investigated the toxicity of four commercially manufactured biochars and observed that their inhibitory effect depended both on biochar characteristics and the test applied. The authors concluded that biochar may constitute a threat to some organisms. Smith et al. (2013) also found that water extract of pinewood-derived biochar (L/S 4) was toxic to two photosynthetic algae, while water extracts of chicken litter- and peanut shell-based biochars did not produce any growth inhibition effects on these organisms. Furthermore, Bastos et al. (2014) detected a species-specific biological response and dose-response pattern while investigating the ecotoxicity of a mixture of soil and pine-wood biochar (4 % w^{-1}) on various species. The authors found that *V. fischeri* was the most sensitive organism to the aqueous soil extracts (L/S 2), while *P. subcapitata* was not affected in its growth, and *Daphnia magna* showed 25 % mobility impairment. Likewise, other researchers also observed a variable response in earthworms, including a short-term avoidance behavior toward biochar (Weyers and Spokas, 2011). However, there was no attempt to link these effects to the presence of inherently potentially toxic elements in the biochar.

The effect of biochar-soil interactions on the response of the test organisms was presented in Fig. 2. There was a clearly different influence of soil type on the response of the tested biological targets at different levels. While Norwood soil significantly and negatively affected the luminescence of V. fischeri ($p < 0.10$) and the growth of *S. saccharatum* seedling roots ($p < 0.05$), the Alligator soil showed an inhibitory effect on algae growth ($p < 0.05$). Although the inhibitory effect of Norwood on V. fischeri and *S. saccharatum* tended to increase by the incorporation of CMB, the biochar impact was not significantly different from the unamended soil. On the other hand, RSB did show a significant effect in reducing the adverse impact of calcareous Norwood soil on V. fischeri, suggesting its beneficial role. A significant effect of the type of biochar was only found in the *P. subcapitata* bioassay, where the mixture of CMB with Alligator soil showed a positive effect by significantly decreasing the growth inhibition of *P. subcapitata* exerted by acidic Alligator clay from 54 to 16 % ($p < 0.05$). No phytotoxicity was observed in L. sativum following the addition of both biochars. In fact, biochar addition, especially CMB, promoted root growth of L. sativum in acidic Alligator clay soil but slightly retarded the growth in calcareous Norwood silt loam soils as compared to RHB. However, these latter effects were not statistically significant.

Previously, Oleszczuk et al. (2013) evaluated the extracts (1:10 liquid/solid ratio) of four commercial biochars mixed with OECD soil at 1 %, 5 %, and 10 %, and established that the alga *S. capricornutum* and the protozoa *T. thermophile* were the least sensitive bioassays studied, while the crustaceans *D. magna* was the most susceptible organism. Besides, the biochar-soil extracts were more toxic to *V. fischeri* than to L. *sativum*. Bastos et al. (2014) also observed that while *V. fischeri* got an inhibition of 45 %, there was no effect on the growth of *P. subcapitata* when these organisms were exposed to water-extractable fractions (1:2 soil/water ratio) of pine-wood-based biochar mixed with Lufa 2.2 soil (4 % w^{-1}).

According to the hazard classification system developed by Persoone et al. (2003), the toxicity of soil-biochar interactions observed in the current study is shown in Table 4. This result suggested that the ecotoxicity was more influenced by the effect of the soil type than by the

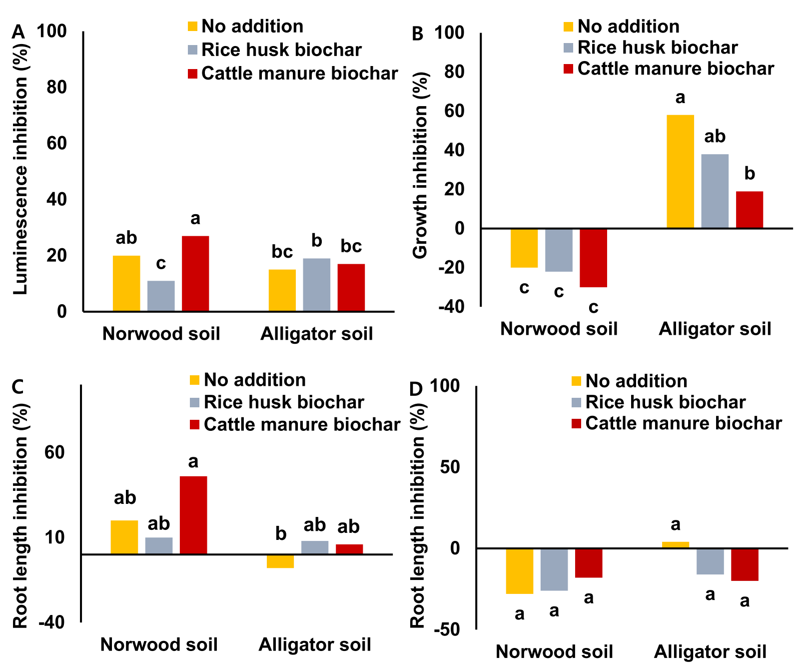

Fig. 2. Inhibition effect (%, with respect to the control) of biochar amended soils. (A) Vibrio fischeri, (B) *Pseudokirchneriella subcapitata*, (C) *Sorghum saccharate*, and (D) Lepidium sativum. Mean values followed by different letters indicate significant differences at Tukey's test $p < 0.05$.

Table 4
Hazard classification for soils amended with biochars.

Soil	Biochar	Hazard classification[Ŧ]
Norwood	—	II (25 %)
	RHB	I
	CMB	II (50 %)
Alligator	—	III (25 %)
	RHB	II (50 %)
	CMB	I

RHB: rice husk biochar; CMB: cattle manure biochar.
[Ŧ] Hazadous class in Roman numerals, and class weight score (%) in parenthesis (Persoone et al., 2003); I: no acute hazard, II: slight acute hazard, III: acute hazard, IV: high acute hazard, and V: very high acute hazard.

biochar studied. However, biochar type modified the biota response to different soil conditions. The slight toxicity found in Norwood soil (toxicity class II) could be explained by the *V. fischeri* behavior (23 % of inhibition), whereas the classification of Alligator soil as a toxic sample (class III) was mainly due to its water extract inhibitory effect on *P. subcapitata* (54 %). On the other hand, the effect of biochar varied depending on the type of soil. While the incorporation of RHB improved the ecotoxicological characteristics of both soils (Table 4), CMB showed both a beneficial and a slight detrimental effect depending on the type of soil to which it was added. This aligns with the findings of Smider and Singh (2014), who also noted a positive impact of the use of a highly alkaline and salty biochar in an acid soil with high clay content and organic C, but a phytotoxic effect when the same biochar was applied to a poorly buffered soil at 5 and 15 g kg^{-1} soil. Domene et al. (2015), on the other hand, reported low toxicity risk of biochars at application rates in the normal range used in the field (< 20 t ha^{-1}).

In this research, the toxicity found in Alligator acidic soil could be attributed to its high content of exchangeable Al (376 mg kg^{-1} by Mehlich 3 extraction). We hypothesized that the concentration of this element was decreased with the addition of CMB, and consequently alleviated its toxicity, which could be explained by the high ash content associated with the alkaline chemical composition of CMB (Table 2). Previously, Van Zwieten et al. (2010) observed the positive response of biochar amendment on plant biomass production in the ferrosol soil and attributed it to the decrease of available Al. Several other studies have also shown that biochar improved acidic soil properties by increasing pH and fertility (Lin et al., 2018; Biederman et al., 2013). Further, in a meta-analysis of published data, Jeffery et al. (2011) found that the benefit of biochar addition to soil on crop productivity was greater in acidic and neutral soils with coarse to medium textures than with alkaline soils.

Past studies showed that Al caused pH-dependent toxicity and under acidic conditions (pH < 5), Al could be solubilized into monomeric and ionic species (e.g. Al^{3+}, $Al(OH)^{2+}$) that were reported to be toxic to plants and aquatic organisms (Kopittke et al., 2015; Blaser et al., 2008). Al toxicity to aquatic organisms was the result of iono-regulatory disturbance due to exposure to these dissolved species (Gensemer et al., 2018). Thus, CMB altered aluminum speciation by promoting the conversion of biologically available aluminum monomeric forms into insoluble non-toxic compounds that precipitate out of the soil solution due to the liming effect. Qian et al. (2013) also showed that $Al(OH)^{2+}$ and $Al(OH)^{2+}$ monomers were strongly adsorbed in manure-based biochar pyrolyzed at 400 °C, through surface complexation of the carboxyl groups rather than through electrostatic attraction. Besides, base cations associated with high ash content in CMB could protect against Al toxicity in algae by cation competition for surface binding sites (Gensemer and Playle, 1999). Likewise, RHB also showed a lower toxicity concerning the unamended Alligator soil, but at a smaller magnitude as CMB. This difference in response was probably attributed to its lower liming value and pH, as evidenced by low Ca, K, Mg, and Na levels. Particularly, the CM addition changed the Alligator soil pH from 4.6 to 5.2, whereas RHB increased pH only by 0.1 unit.

On the other hand, the use of CMB in Norwood soil was not as beneficial as was observed for RHB, with a slight negative effect on the bioluminescence of *V. fischeri* and the growth length of *S. saccharatum* seedlings. Animal waste-based biochar was frequently characterized by high ash content, elevated pH, EC and high concentrations of alkaline chemical species (Chan et al., 2008; Lima and Marshall, 2005). Although the adverse effects of CMB were associated with its high pH and salinity, it is important to note that such characteristics have also been observed in biochars derived from other feedstocks, including tomato green waste (Smider and Singh, 2014), rice straw (Meng et al., 2018), food waste (Domene et al., 2015), and coffee husks (Domingues et al., 2017), among others. These findings suggest that potentially ecotoxic properties are not restricted to animal-waste biochars, and similar caution should be applied when evaluating other high-ash or high-salt biochars for soil application.

The adverse effects due to the use of alkaline biochars in alkaline soils were also observed by others (Luo et al., 2013; Liesch et al., 2010; Domene et al., 2015). According to Luo et al. (2013), the addition of *M. giganteus* straw-based biochar in a high pH soil (clay loam soils of pH 7.6, mixed at 50 mg C g^{-1} soil) was toxic to microbial biomass-C, resulting in less biomass C and ATP. Liesch et al. (2010) also found that the impact of biochar amendment to a near-neutral artificial soil (pH 7.2) on earthworm growth and survival was influenced by the type of biochar and the rate of application used. While slightly basic pine chip biochar did not affect both end-points even at high doses, the addition of highly basic poultry litter-based biochar impaired earthworms at doses greater than 4.7 % w^{-1}. The authors attributed these results to the presence of ammonia, high pH, and excessive soluble salt (Na) in the poultry litter biochar. Domene et al. (2015) evaluated 14 different biochars and reported a strong inhibition effect on collembolan reproduction with only food waste-derived biochar at application rates between 20 and 540 Mg ha^{-1} (0.5–14.0 % w^{-1}). Again, soluble Na was pointed out as the main factor for this inhibition.

These results suggest that identifying a biochar as "toxic" based on bioassays alone does not necessarily imply it is unsuitable for use. The final effect of its application depends strongly on soil properties and interactions. In our study, CMB exhibited high toxicity when tested in isolation, likely due to its high pH, salinity, and available K content. However, when applied to the acidic Alligator soil, CMB reduced overall ecotoxicity, particularly by decreasing the inhibition of *P. subcapitata* growth from 54 % to 16 %, suggesting a beneficial effect through pH buffering and Al immobilization. Conversely, in the calcareous Norwood soil, where the baseline pH was already high, CMB caused a slight increase in toxicity, especially affecting *V. fischeri* luminescence and *S. saccharatum* root growth.

This highlights the context-dependent nature of biochar toxicity and reinforces the importance of assessing soil–biochar compatibility. According to the Paracelsus principle, toxicity is dose-dependent: while low doses may have neutral or beneficial effects, higher application rates can exacerbate risks. For instance, Lehmann et al. (2012) recommend keeping biochar doses below 2 % to avoid potential negative impacts from salinity or Na, particularly in animal-waste-derived biochars. Similarly, Buss et al. (2016) reported that biochar added at 5 % (>100 t ha^{-1}) to sterile sand inhibited L. *sativum* root growth due to high pH and K levels, but the same biochar at 1–10 t ha^{-1} showed positive effects on plant development.

Likewise, Bielská et al. (2018) observed that the addition of wood- and rice-husk-derived biochars at 1 % and 5 % to contaminated soils reduced the reproductive toxicity effects on *Folsomia candida*, whereas at higher doses (>10 %), the biochar itself induced toxicity. In our study, the excessive salt content and high levels of available K in CMB were particularly concerning. Although K toxicity was not necessarily expected in the K-deficient Norwood soil, the high salinity and alkaline nature of the biochar could still represent a risk at elevated application rates (>2–5 %). Overall, our results highlight that biochars can produce both beneficial and adverse effects, depending on their interaction with specific soil conditions. These findings underscore the need for site-specific evaluations to ensure safe and effective biochar use.

Conclusion

This study shows that the ecotoxicological impact of biochar depends largely on the interaction between its physicochemical properties and those of the receiving soil. CMB characterized by high pH, salinity, and K levels, exhibited high toxicity in aqueous extracts (hazard class IV), whereas RHB showed no toxicity and even stimulated organism responses. When applied to soil, the effects varied by soil type: in acidic Alligator soil, CMB reduced toxicity from class III to I, likely due to Al immobilization via liming. In contrast, in calcareous Norwood soil, CMB maintained or slightly increased toxicity levels, while RHB consistently improved the ecotoxicological response in both soils. These results support the need for site-specific assessments prior to field application. Thresholds based solely on total contaminant concentrations are insufficient to predict ecotoxicological outcomes, as they fail to capture soil-mediated changes in bioavailability or indirect stress effects. Bioassays provided sensitive endpoints for detecting organism-level effects and should be incorporated into environmental risk assessment protocols. Moreover, the optimal combination of biochar properties and soil characteristics may vary depending on the intended objective, whether it is to enhance soil fertility, mitigate Al toxicity, or remediate contamination. Therefore, incorporating ecotoxicological evaluation alongside agronomic and environmental criteria is essential for guiding the safe and effective use of biochar under diverse conditions.

CRediT authorship contribution statement

Guillermina Cantou: Writing – original draft, Data curation, Conceptualization. **Jim J. Wang:** Writing – review & editing, Conceptualization. **Baoyue Zhou:** Investigation, Formal analysis. **Jeong-Min Lee:** Investigation. **Jong-Hwan Park:** Writing – review & editing, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported, in part, by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (RS-2020-NR049596), by the Biomaterials Specialized Graduate Program through the Korea Environmental Industry & Technology Institute (KEITI) funded by the Ministry of Environment (MOE)

Data availability

No data was used for the research described in the article.

References

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S., 2014. Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere 99, 19–33.

Ahmed, A.A., Thiele-Bruhn, S., Aziz, S.G., Hilal, R.H., Elroby, S.A., Al-Youbi, A.O., Leinweber, P., Kühn, O., 2015. Interaction of polar and nonpolar organic pollutants with soil organic matter: sorption experiments and molecular dynamics simulation. Sci. Total Environ. 508, 276–287.

Bastos, A., Prodana, M., Abrantes, N., Keizer, J., Soares, A., Loureiro, S., 2014. Potential risk of biochar-amended soil to aquatic systems: an evaluation based on aquatic bioassays. Ecotoxicology 23 (9), 1784–1793.

Biederman, L., Harpole, W., 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. Glob. Change Biol. Bioenergy 5 (2), 202–214.

Bielská, L., Škulcová, L., Neuwirthová, N., Cornelissen, G., Hale, S.E., 2018. Sorption, bioavailability and ecotoxic effects of hydrophobic organic compounds in biochar amended soils. Sci. Total Environ. 624, 78–86.

Blaser, P., Walthert, L., Pannatier, E.G., 2008. The sensitivity of swiss forest soils to acidification and the risk of aluminum toxicity. J. Plant Nutr. Soil Sci. 171 (4), 605–612.

Brtnicky, M., Datta, R., Holatko, J., Bielska, L., Gusiatin, Z.M., Kucerik, J., Hammerschmiedt, T., Danish, S., Radziemska, M., Mravcova, L., Fahad, S., Kintl, A., Sudoma, M., Ahmed, N., Pecina, V., 2021. A critical review of the possible adverse effects of biochar in the soil environment. Sci. Total Environ. 796, 148756.

Buss, W., Graham, M., Shepherd, J., Mašek, O., 2016. Suitability of marginal biomass-derived biochars for soil amendment. Sci. Total Environ. 547, 314–322.

Buss, W., Graham, M.C., Shepherd, J.G., Mašek, O., 2015. Risks and benefits of marginal biomass-derived biochars for plant growth. Sci. Total Environ. 569–570, 496–506.

Cao, X., Harris, W., 2010. Properties of dairy-manure-derived biochar pertinent to its potential use in remediation. Bioresour. Technol. 101 (14), 5222–5228.

Chan, K., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Using poultry litter biochars as soil amendments. Aust. J. Soil Res. 4, 437–444.

Dai, Z., Xiong, X., Zhu, H., Xu, H., Leng, P., Li, J., Tang, C., Xu, J., 2021. Association of biochar properties with changes in soil bacterial, fungal and fauna communities and nutrient cycling processes. Biochar 3, 239–254.

Domene, X., Enders, A., Hanley, K., Lehmann, J., 2015. Ecotoxicological characterization of biochars: role of feedstock and pyrolysis temperature. Sci. Total Environ. 512–513, 552–561.

Domingues, R., Trugilho, P., Silva, C., Melo, I., Melo, L., Magriotis, Z., Sánchez-Monedero, M., 2017. Properties of biochar derived from wood and high-nutrient biomasses with the aim of agronomic and environmental benefits. Plos ONE 12 (5), 1–19.

Dong, M., Jiang, M., He, L., Zhang, Z., Gustave, W., Vithanage, M., Niazi, N.K., Chen, B., Zhang, X., Wang, H., He, F., 2025. Challenges in safe environmental applications of biochar: identifying risks and unintended consequence. Biochar 7, 12.

Environment Canada, 2007. Biological Test Method: Growth Inhibition Test Using a Freshwater Alga, Report EPS 1/RM/25.

Gascó, G., Cely, P., Paz-Ferreiro, J., Plaza, C., Mendez, A., 2016. Relation between biochar properties and effects on seed germination and plant development. Biol. Agric. Hortic. 32, 237–247.

Gensemer, R., Gondek, J., Rodriquez, P., Arbildua, J., Stubblefield, W., Cardwell, A., Santore, R., Ryan, A., Adams, W., Nordheim, E., 2018. Evaluating the effects of pH, hardness, and dissolved organic carbon on the toxicity of aluminum to freshwater aquatic organisms under circumneutral conditions. Environ. Toxicol. Chem. 37 (1), 49–60.

Gensemer, R., Playle, R., 1999. The bioavailability and toxicity of aluminum in aquatic environments. Crit. Rev. Environ. Sci. Technol. 29 (4), 315–450.

Godlewska, P., Ok, Y.S., Oleszczuk, P., 2021. THE DARK SIDE OF BLACK GOLD: ecotoxicological aspects of biochar and biochar-amended soil. J. Hazard. Mater. 403, 123833.

Grzegórska, A., Czaplicka, N., Antonkiewicz, J., Rybarczyk, P., Baran, A., Dobrzyński, K., Zabrocki, D., Rogala, A., 2023. Remediation of soils on municipal rendering plant territories using Miscanthus × giganteus. Environ. Sci. Pollut. Res. 30 (9), 22305–22318.

He, Z., Zhao, A., Liu, S., Chen, Y., Liu, J., Zhao, W., Yin, M., Dong, Q., Zhang, J., Zhang, G., Bi, D., 2024. Preparation of nitrogen-containing chemicals from lignocellulosic biomass and nitrogen-rich organic solid waste by pyrolysis: characteristics, reaction mechanisms, and feedstock interaction. Chem. Eng. J. 496, 153793.

Hilber, I., Bastos, A.C., Loureiro, S., Soja, G., Marsz, A., Cornelissen, G., Bucheli, T.D., 2017. The different faces of Biochar: contamination risk versus remediation tool. J. Environ. Eng. Landsc. Manag. 25 (2), 86–104.

Hofman, J., Hovorková, I., Machát, J., 2009. Comparison and characterization of OECD artificial soils. Ecotoxicological Characterization of Waste 223–229.

IBI, 2015. Standardized Product Definition and Product Testing Guidelines For Biochar That is Used in Soil. International Biochar Initiative (IBI), version 2.1.

Jeffery, S., Verheijen, F., van der Velde, M., Bastos, A., 2011. Review: a quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agric. Ecosyst. Environ. 144, 175–187.

Joseph, S., Camps-Arbestain, M., Lin, Y., Munroe, P., Chia, C., Hook, J., van Zwieten, L., Kimber, S., Cowie, A., Singh, B., Lehmann, J., Foidl, N., Smernik, R., Amonette, J., 2010. An investigation into the reactions of biochar in soil. Aust. J. Soil Res. 48 (6–7), 501–515.

Koltowski, M., Oleszczuk, P., 2015. Toxicity of biochars after polycyclic aromatic hydrocarbons removal by thermal treatment. Eco. Eng. 75, 79–85.

Kopittke, P., Moore, K., Lombi, E., Gianoncelli, A., Ferguson, B., Blamey, F., Menzies, N., Nicholson, T., McKenna, B., Wang, P., Gresshoff, P., Kourousias, G., Webb, R., Green, K., Tollenaere, A., 2015. Identification of the primary lesion of toxic aluminum in plant roots. Plant Physiol 167 (4), 1402–1411.

Lehmann, J., Joseph, S., 2015. Biochar For Environmental Management: Science and Technology and Implementation, 2nd ed. RoutledgeEarthscan Ltd., LondonLondon.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – a review. Soil Biol. Biochem. 43, 1812–1836.

Lehmann, J., Zimmerman, A., Rajkovich, S., Enders, A., Hanley, K., Hyland, C., Lehmann, J., Zimmerman, A., 2012. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. Biol. Fertil. Soils 48, 271–284.

Liang, B., Lehmann, J., Sohi, S.P., Thies, J.E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E.G., Luizao, F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Org. Geochem. 41 (2), 206–213.

Liesch, A.M., Weyers, S.L., Gaskin, J.W., Das, K.C., 2010. Impact of two different biochars on earthworm growth and survival. Annals of Environmental Science 4, 1–9.

Lima, I.M., Marshall, W.E., 2005. Granular activated carbons from broiler manure: physical, chemical and adsorptive properties. Bioresour. Technol. 96, 699–706.

Lin, Q., Zhang, L., Riaz, M., Zhang, M., Xia, H., Lv, B., Jiang, C., 2018. Assessing the potential of biochar and aged biochar to alleviate aluminum toxicity in an acid soil for achieving cabbage productivity. Ecotoxicol. Environ. Saf. 161, 290–295.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Devonshire, B.J., Brookes, P.C., 2013. Microbial biomass growth, following incorporation of biochars produced at 350 °C or 700 °C, in a silty-clay loam soil of high and low pH. Soil Biol. Biochem. 57, 513–523.

Malara, A., Oleszczuk, P., 2013. Application of a battery of biotests for the determination of leachate toxicity to bacteria and invertebrates from sewage sludge-amended soil. Environ. Sci. Pollut. Res. 20, 3435–3446.

Martínez-Gallardo, M.R., Jiménez, R., Suárez-Estrella, F., Toribio, A., Estrella-González, M.J., Mira-Urios, M.A., Jurado, M.M., López-González, J.A., Martínez-Culebras, P.V., López, M.J., 2025. Cataloging olive oil mill wastewater sludge based on toxicological profiles and functional microbial diversity. Sci. Total Environ. 976, 179348.

Martins, A.F., Cardoso, A.L., Stahl, J.A., Diniz, J., 2007. Low temperature conversion of rice husks, eucalyptus sawdust and peach stones for the production of carbon-like adsorbent. Bioresour. Technol. 98, 1095–1100.

Masiello, C., Thies, J., Crowley, D., Rillig, M., Lehmann, J., Hockaday, W., Lehmann, J., Thies, J., Rillig, M., Masiello, C., Hockaday, W., Crowley, D., 2011. Biochar effects on soil biota - A review. Soil Biol. Biochem. 43 (9), 1812–1836.

Meng, J., Tao, M., Wang, L., Liu, X., Xu, J., 2018. Changes in heavy metal bioavailability and speciation from a Pb-Zn mining soil amended with biochars from co-pyrolysis of rice straw and swine manure. Sci. Total Environ. 633, 300–307.

Nkoh, J.N., Baquy, M.A.Al, Mia, S., Shi, R., Kamran, M.A., Mehmood, K., Xu, R., 2021. A critical-systematic review of the interactions of biochar with soils and the observable outcomes. Sustainability 13 (24), 13726.

Oleszczuk, P., Jośko, I., Kuśmierz, M., 2013. Biochar properties regarding to contaminants content and ecotoxicological assessment. J. Hazard. Mater. 260, 375–382.

Owojori, O., Reinecke, A., Voua-Otomo, P., Reinecke, S., 2009. Comparative study of the effects of salinity on life-cycle parameters of four soil-dwelling species (Folsomia candida, Enchytraeus doerjesi, Eisenia fetida and Aporrectodea caliginosa). Pedobiologia (Jena) 52 (6), 351–360.

Ozcan, A., Mehmet, A.T., Aydin, M.E., 2013. Investigation on the levels of heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in sewage sludge samples and ecotoxicological testing. CLEAN-Soil Air Water 41 (4), 411–418.

Pereira, H., Barreira, L., Mozes, A., Florindo, C., Polo, C., Duarte, C.V., Custodio, L., 2011. Microplate-based high throughput screening procedure for the isolation of lipid-rich marine microalgae. Biotechnol. Biofuels 4, 1.

Persoone, G., Marsalek, B., Blinova, I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L., Kolar, B., 2003. A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters. Environ. Toxicol. 18 (6), 395–402.

Qian, T., Zhang, X., Hu, J., Jiang, H., 2013. Effects of environmental conditions on the release of phosphorus from biochar. Chemosphere 93, 2069–2075.

Rodríguez-Liébana, J.A., Mingorance, M.D., Peña, A., 2013. Pesticide sorption on two contrasting mining soils by addition of organic wastes: effect of organic matter composition and soil solution properties. Colloid. Surf. A 435, 71–77.

Romano, N., Zeng, C., 2007. Acute toxicity of sodium nitrate, potassium nitrate, and potassium chloride and their effects on the hemolymph composition and gill structure of early juvenile blue swimmer crabs (Portunus pelagicus Linnaeus, 1758) (Decapoda, Brachyura, Portunidae). Environ. Toxicol. Chem. 26 (9), 1955–1962.

Shaaban, M., Van Zwieten, L., Bashir, S., Younas, A., Núñez-Delgado, A., Chhajro, M.A., Kubar, K.A., Ali, U., Rana, M.S., Mehmood, M.A., Hu, R., 2018. A concise review of biochar application to agricultural soils to improve soil conditions and fight pollution. J. Environ. Manag. 228, 429–440.

Shackley, S., Sohi, S., Ibarrola, R., Hammond, J., Mašek, O., Brownsort, P., Cross, A., Prendergast-Miller, M., Haszeldine, S., 2013. Biochar, tool for climate change mitigation and soil management. Geoengineering Responses to Climate Change 73–140.

Shang, Q., Chi, J., 2024. Mechanistic insight into the effects of interaction between biochar and soil with different properties on phenanthrene sorption. J. Environ. Manag. 367, 121961.

Singh, H., Northup, B.K., Rice, C.W., Prasad, P.V.V., 2022. Biochar applications influence soil physical and chemical properties, microbial diversity, and crop productivity: a meta-analysis. Biochar 4, 8.

Smider, B., Singh, B., 2014. Agronomic performance of a high ash biochar in two contrasting soils. Agriculture Ecosystems & Environment 191, 99–107.

Smith, C., Buzan, E., Lee, J., 2013. Potential Impact of Biochar Water-Extractable Substances on Environmental Sustainability. ACS Sustain. Chem. Eng. 1 (1), 118–126.

Spokas, K.A., Reicosky, D., 2009. Impacts of sixteen different biochars on soil greenhouse gas production. Annals of Environmental Science 3, 179–193.

Spokas, K.A., Baker, J.M., Reicosky, D.C., 2010. Ethylene: potential key for biochar amendment impacts. Plant Soil 333, 443–452.

Tao, X., Sadasivam, B.Y., Reddy, K.R., Chengwen, W., Spokas, K., 2016. Review of the effects of biochar amendment on soil properties and carbon sequestration. J. Hazard. Toxic Radioact. Waste 20 (1), 1–14.

Tsui, M.T.K., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52, 1189–1197.

Van Gestel, C.A.M., Van Dis, W.A., 1988. The influence of soil characteristics on the toxicity of four chemicals to the earthworm Eisenia fetida andrei (Oligochaeta). Biol. Fertil. Soils 6 (3), 262–265.

Van Zwieten, L., Kimber, S., Morris, S., Chan, K.Y., Downie, A., Rust, J., Joseph, S., Cowie, A., 2010. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil 327, 235–246.

Weyers, S.L., Spokas, K.A., 2011. Impact of Biochar on Earthworm Populations: a Review. Appl. Environ. Soil Sci. 2011, 541592.

Xu, G., Lv, Y., Sun, J., Shao, H., Wei, L., 2012. Recent advances in biochar applications in agricultural soils: benefits and environmental implications. Clean-Soil Air Water 40 (10), 1093–1098.

Yang, D., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., Zheng, B., 2016. Biochar to improve soil fertility. A review. Agron. Sustain. Dev. 36, 1–18.

Zornoza, R., Moreno-Barriga, F., Acosta, J.A., Munoz, M.A., Faz, A., 2016. Stability, nutrient availability and hydrophobicity of biochars derived from manure, crop, residue, and municipal solid waste for their use as soil amendments. Chemosphere 144, 122–130.