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Characterization and Determination of the Toxicological Risk of Biochar Using Invertebrate Toxicity Tests in the State of Aguascalientes, México

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Abstract: Following a quantitative analysis of adequate feedstock, comprising 11 woody biomass species, four biochars were generated using a Kon-Tiki flame curtain kiln in the state of Aguascalientes, Mexico. Despite the high quality (certified by European Biochar Certificate), the biochars contain substantial quantities of hazardous substances, such as polycyclic aromatic hydrocarbons, polychlorinated dibenzo-*p*-dioxins and dibenzofurans, polychlorinated biphenyls, and heavy metals, which can induce adverse effects if wrongly applied to the environment. To assess the toxicity of biochars to non-target organisms, toxicity tests with four benthic and zooplanktonic invertebrate species, the ciliate *Paramecium caudatum*, the rotifer *Lecane quadridentata*, and the cladocerans *Daphnia magna* and *Moina macrocopa* were performed using biochar elutriates. In acute and chronic toxicity tests, no acute toxic effect to ciliates, but significant lethality to rotifers and cladocerans was detected. This lethal toxicity might be due to ingestion/digestion by enzymatic/mechanic processes of biochar by cladocerans and rotifers of toxic substances present in the biochar. No chronic toxicity was found where biochar elutriates were mixed with soil. These data indicate that it is instrumental to use toxicity tests to assess biochars' toxicity to the environment, especially when applied close to sensitive habitats, and to stick closely to the quantitative set-point values.

Keywords: acute toxicity test; LC_{50}; elutriate; interstitial water; hazardous substances; enzymatic/mechanic digestion

1. Introduction

Within the last decade, research on the dark, fertile, charcoal-rich anthropogenic Terra preta soils of Amazonia [1,2] has stimulated the idea to sequester charcoal-like pyrogenic carbon obtained by thermochemical conversion of various biogenic materials, under the absence of oxygen, into soils. The so-called biochar (BC), adds several advantageous features if applied to soil, such as: (a) Greenhouse gas emissions reduction; (b) improvement of the physicochemical and microbial properties, as well as generation of agronomic win–win situations like generic soil fertility increases; and (c) absorption of pernicious substances and reduction of ecological threats, such as N leaching and soil-water remediation [3,4]. BC research sharply increased within the last ten years; however, BCs can

be tremendously different produced from a broad range of different types of biomass and thus serve different purposes and trigger unwanted effects [5–7].

Within the last six years, considerable progress has been made with regard to standardization, pyrolysis techniques, quality, and sustainability control of BC. In 2012 the European Biochar Foundation established an accredited control system (ACS) for BC named European Biochar Certificate (EBC) that certifies the compliance with quality parameters and thresholds for contaminants, the use of accredited analytical methods and the sustainability of production [8]. Together with the United States (US) induced International Biochar Initiative (IBI) [9], EBC led to considerable improvements in BC quality and the sustainability of its production and hazard-free application for environmental safety, both in agriculture and livestock feed.

The certification under the EBC (as the US counterpart IBI) solicits, in addition to technology-related requirements (e.g., heat recovery, complete combustion of the pyrolysis gases, etc.), compliance with certain limit values of predefined physical and chemical parameters of the products. The physical parameters serve primarily to classify the coals affiliation or category (e.g., pyrolytic coal, hydrochar, BC, etc.) and quality (e.g., degree of inertization and recalcitrance (molar elemental ratios), surface area, density, storage capacity, etc.). The chemical parameters are used to determine the hazard potential of char-bounded contaminants (e.g., polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), fluorine, polychlorinated biphenyls (PCBs), and heavy metals). Heavy metals are either already contained in the feedstock or not and become concentrated by the thermochemical process. Aromatic and polychlorinated toxicants instead emerge during carbonation, reduction, and aromatization phases [8,10–12]. Potential contamination of soils when using BC in agriculture, as well as possible intoxications when used as animal feed, should be avoided as far as possible by adhering to the limit values specified by the current standard.

The hazard potential of BC is generally limited, as the hazardous substances contained in BC are strongly bound, due to the high adsorption potential caused by the porous structure and increased redox potential [7,13]. In addition aforementioned hazardous substances are very hydrophobic, exhibit low biological availability and are hardly metabolizable, as they are incorporated into the aromatic benzene ring skeleton [14,15]. The concentration of PAHs is strongly dependent on feedstock, temperature, and design of the pyrolysis unit [13]. However, even if the strong adsorption capacity of BCs limits the noxiousness, mineralization and metabolization processes propelled by microfauna could potentially trigger a bioavailable release of pollutants.

Oleszczuk et al. [16] state, that pollutants in BC can result in toxicity to organisms during environmental application of BC. When toxic potencies of BC were assessed by different methods, a significant correlation between concentrations of PAHs and toxicity was observed. Re-condensation of volatile organic compounds (VOCs) during pyrolysis can result in BC containing compounds that are bioavailable and phytotoxic [17]. Thus, even if the limit values defined according to an ACS, such as the EBC, are adhered to, residual risk remains that adverse effects result from the use of BC, especially when used in the vicinity of sensitive habitats, such as water bodies or nature reserves.

Concerning these arguments, the harmlessness (respectively the quality) of BC cannot be assessed solely by analyzing physicochemical parameters. Toxicity tests permit an evaluation of the degree to which a chemical substance has an adverse effect in live organisms, either acute or chronic [18]. We hypothesize that the aforementioned toxicological risk of BC used as a soil amendment can be proved with the help of aquatic invertebrate toxicity tests.

Toxicity testing in which live organisms, such as aquatic invertebrates, are exposed to contaminants under laboratory conditions, has become a powerful tool in the past years for organisms in marine, estuarine, and freshwater environments [19]. The purpose of toxicity testing is to obtain appropriate insight, which will acquaint decision makers and practitioners about the levels of toxicity and the associated risk created by anthropogenic activities in ecosystems [20]. Eco-toxicological tests provide a substantial tool to assess the toxicity potential of a complex mixture of toxicants typically found in BCs.

The toxicity assessment of chemical cocktails and organic contaminants is a growing necessity since single dangerous substances are rarely existent in the environment of anthropogenic activities.

Aquatic invertebrates, especially rotifers, are mainly found in freshwater environments but also in moist soil, where they inhabit the thin films of water that are formed around soil particles, which is known as interstitial water [21]. Some aquatic invertebrates, instead of living in particle-free water, prefer to dwell in planktonic, periphytic, and benthic ecosystems, where food availability such as bacteria, eukaryotic cells, algae, and detritus are abundant. Pore or interstitial water in soils is the most bioavailable material for aquatic organisms, whereas fertilized eggs in parched environments become dormant, are able to overwinter, and survive drought unscathed for years [22–24]. This is why several aquatic invertebrate species are most likely adequate as a suitable bioassay approach to examine the toxicity potential of BC application in the soil, as invertebrates are ubiquitous and the soil is a continuously interacting and cross-biocoenotic ecotope.

Most common toxic substances tested with aquatic invertebrates, especially rotifers and cladocerans, comprise natural toxins, pesticides, and heavy metals [25]. The biota contamination by such elements deserves attention, because of cumulative effects within trophic networks [26]. Transportation of heavy metals through trophic networks often commences with the assimilation of these by bacteria and protists [27]. Various heavy metals and organic pollutants, such as antifouling agents, pesticides, PAHs, and PCBs, which are traceable in BCs, have shown reproducible biological responses when tested in rotifers [28]. However, apart from this work, no data obtained by standard acute toxicity tests is available on the susceptibility of aquatic invertebrate species to different toxicants detected in BCs.

Most of the acute toxicity tests with aquatic invertebrates measure mortality after an exposure period of 24 or 48 h. These tests have standardized protocols, approved, for example, by the American Society for Testing and Materials (ASTM). Perhaps the most accepted test worldwide is the 48 h acute test using *Daphnia magna* Strauss [29,30]. Although Mexico has embraced the *D. magna* test, this European cladoceran species has never been found in Mexican reservoirs nor terrestrial water bodies [31]. As the contamination of reservoirs involves deposition of pollutants in sediments [18,32], elutriates are an approximation to soil pore water, and as the idea of this study is to expose benthic species suited for living in sediments to BCs, it is expedient to work with elutriates.

The purpose of this contribution was to use acute and chronic toxicity tests [33] with the ciliate *Paramecium caudatum*, the rotifer *Lecane quadridentata*, and the cladocerans *Daphnia magna* and *Moina macrocopa* to assess toxicity of hazardous substances identified in four different BCs that have been generated using local feedstock in Aguascalientes, Mexico. First, the BCs were analyzed with regard to heavy metal, PAHs, PCDD/Fs, and PCBs levels. Afterwards, the toxic responsiveness of the invertebrates to the BC elutriates in a controlled laboratory test environment has been checked, to estimate lethal concentration where 50% and 10% of test animals die (LC_{50}, LC_{10},) and define no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values. Subsequently, the expected toxicity range due to the concentration of the four BCs in the environmental samples, using the equations obtained from the toxicity tests, was calculated. The present work shows that the application of a toxicological test using aquatic invertebrates could substantially increase BC application safety in and close to sensitive habitats.

2. Materials and Methods

2.1. Study Site, Feedstock, and Biochar Generation

The state of Aguascalientes, Mexico ranges from 22°27′ to 21°38′ N and from 101°53′ to 102°52′ W (Figure 1) [34]. The state holds substantial untapped biomass potentials for BC generation, which could be of higher interest for the semi-desert state as BC addition to soil can increase water absorbance and water holding capacity (WHC) [35–38]. Data concerning quantity and woody species composition was acquired and analyzed from the municipal composting site and the sanitary landfill. Further samples were collected from forest residues in the northern municipality of Rincón de Romos. The total

identified woody biomass potential totaled up to 233,000 tons of dry matter, encompassing 11 different wood species. The collected feedstocks were used to generate four different BCs. The pyrolysis process was conducted by the use of a locally assembled Kon-Tiki flame curtain kiln at Universidad Panamericana (UP). Pyrolysis temperature ranged from 600 to 680 °C at the surface of the blaze. Duration of pyrolysis ranged from 3.5 to 4.5 h. Figure 1 illustrates the study related locations within the state of Aguascalientes.

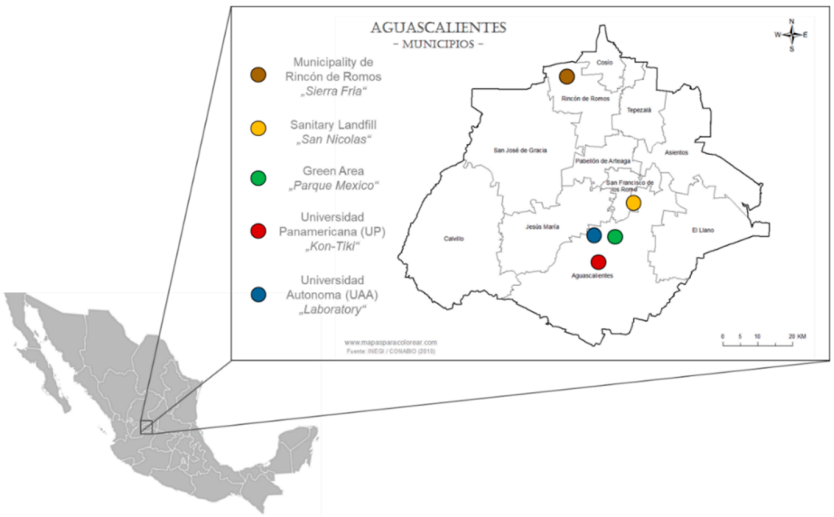

Figure 1. Location of three feedstock sampling sites, pyrolysis, and the laboratory site in the state of Aguascalientes, Mexico. Brown dot: Municipality of Rincón de Romos—"Sierra Fria"; yellow dot: Sanitary Landfill—"San Nicolas"; green dot: Green Area—"Parque Mexico"; Red dot: Universidad Panamericana—"Kon-Tiki flame curtain kiln"; blue dot: Universidad Autónoma de Aguascalientes—"Laboratory".

This study attempts to assess ways of biomass use for BC production under real conditions. Therefore, the collected feedstocks were not separated and distinguished into pure types, fractions, and species. This approach is substantiated because the feedstocks are found mixed-up in the forest and the collection centers and separation would be neither economically reasonable nor could a high-tech-based separation guarantee pure fractions.

Sample 1: Consisted of a mixture of 65% manchineel tree and 35% pine with average moisture of 8% for manchineel tree and 7% for pine. The branch diameter ranged from 0.5 to 8 cm for both types of wood. The collection took place in the forests of Rincón de Romos.

Sample 2: Was collected from the municipal composting site. It was a mixture of different species of wood, which were mainly branches. The following Table 1 illustrates the composition as the biomass was piled at the composting site. The branch diameter ranged from 0.5 to 10 cm for all types of branches. The average moisture was 18%.

Sample 3: Was collected from the municipal composting site and was a mixture of different species of wood, but was composed of much thicker trunks. The trunks were split into logs, with a diameter range from 4.5 to 12 cm in final size. Table 2 illustrates the composition of the biomass species and the percentage share applied in sample 3. The average moisture was 27%.

Sample 4: Was also collected from the municipal composting site. Its woody composition was similar to sample 2, but the feedstock of sample 4 was chopped to grain size 0.5 to 8 cm long and 0.5 to 2 cm thick. The average moisture measured 45%.

Table 1. Woody composition of sample 2, municipal composting.

Timber Species		
Common Name	**Scientific Name**	**Content (%)**
Velvet Mesquite	*Prosopis velutina*	18
Ash Tree	*Fraxinus excelsiur*	16
Australian Pine	*Casuarina equisetifolia*	15
Eucalyptus	*Eucalyptus spp.*	15
Ficus Tree	*Ficus benjamina*	12
Peruvian Mastic Tree	*Schinus molle*	11
Pynion Pine	*Pinus cembroides*	8
Bugambilia	*Bougainvillea spp.*	4
Palm	*Family Arecaceae*	1

Table 2. Woody composition of sample 3, municipal composting.

Tree Species		
Colloquial Name	**Scientific Name**	**Content (%)**
Velvet Mesquite	*Prosopis velutina*	38
Ash Tree	*Fraxinus excelsiur*	24
Manchineel Tree	*Hippomane mancinella*	21
Australian Pine	*Casuarina equisetifolia*	17

2.2. Biochar Analysis and Elutriate Preparation

The four BCs were sent to the Eurofins Umwelt Ost GmbH laboratory in Germany to assess the elementary composition, H/C and O/C molar ratios, specific inner surface, bulk density, ash and salt content, water content, and water holding capacity (WHC), as well as the content of ten different heavy metals, 16 PAHs (EPA 16 PAHs), PCDD/Fs, PCBs, and dl-PCBs according to the EBC guideline [39].

The BC elutriates were prepared for acute toxicity testing based on procedures described in the American Society for Testing and Materials Guide E 1391 [40] and US EPA-U.S. Army Corps of Engineers [41] with slight modifications (see also US EPA 823-B-01-002 [42]). After grinding BC to powder (grain size <1 mm), the BCs were mixed in a 1:4 (v/v) ratio of BC to EPA water in a beaker and placed on a rotary shaker table for 1 h, at a speed of 100 rpm. After shaking, the samples were centrifuged at 6000 rpm for 20 min. The aqueous fraction (elutriate sample) was pipetted and stored in jars at 20 ± 1 °C.

2.3. Toxicity Tests

We performed acute toxicity tests with each of the four BC elutriates using the ciliate *Paramecium caudatum*, the rotifer *Lecane quadridentate*, and two cladocerans: *Daphnia magna* and *Moina macrocopa*. In tests where acute toxicity was low or undetected, sublethal tests were conducted choosing parameters like growth inhibition in *Lecane quadridentata*, *Moina macrocopa*, and *Paramecium caudatum*. Finally, *L. quadridentata* and *M. macrocopa* were exposed to an elutriate generated from a soil–biochar mixture, to assess the acute toxicity if BC is mixed with soil.

During this additional test, only a 100% concentration of elutriate was applied. A common leptosol soil from Aguascalientes was used, mixed in volumetric ratio 8:1 with BC (V_{soil}/V_{BC}). This ratio was chosen based on the assumption of having a top soil with 10 cm thickness and the application of 30 t_{DM} ha^{-1} (120 m^3 ha^{-1}) of BC to the soil with a specific bulk density of 250 kg m^{-3}. This value refers to the maximum amount of BC that is allowed to be applied to acreage, for example, in Germany, predetermined by the German Fertilizer Application Ordinance (DüV) [43] and the German Federal Soil Protection Act (BBodSchG) [44]. As far as the authors know, México does not specify the application of BC in agriculture, which is the reason to refer to German thresholds. Based hereupon, 880 mL leptosol was mixed with 110 mL of BC in order to gain a representative mixture. Thereof 10 mL were used to prepare the elutriate, following the same protocol applied to prepare elutriates with the pure BC.

2.3.1. *Paramecium caudatum* 24 h Acute Toxicity Test

The ciliates were obtained from samples collected at the reservoir at the campus of the Autonomous University of Aguascalientes and identified through in vivo observation following the protocol by Dieckmann [45]. Selected *P. caudatum* specimens were cultivated in Petri dishes with Sonneborn medium [46]. The methodology employed in these toxicity tests was made according to Madoni [47]. Briefly, ten organisms were picked from the culture with a micropipette and individually inoculated into the 24-well polystyrene plate (Corning Costar Corporation, USA). Each well containing a total volume of 1.0 mL of EPA medium was diluted with five BC elutriate concentrations (6.25%, 12.5%, 25%, 50%, 100% plus one negative control with EPA medium [48]) from each of the four BCs and incubated for 24-h at 25 ± 1 °C in darkness. These dilutions were done in accordance with the Mexican Norm NMX-AA-087-SCFI [49]. The test was conducted with five replications for each BC. After 24 h period, the ciliates were counted to determine mortality.

2.3.2. *Lecane quadridentata* 48 h Acute Toxicity Test

We used *Lecane quadridentata* collected from Lake Chapala, Mexico [33]. These strains have been continuously cultured in EPA medium [48] for more than 20 years in our UAA laboratory and fed *Nanochloris oculata* (UTEX strain LB2194). Asexual eggs were collected and incubated at 25 °C in Petri dishes with EPA medium. EPA medium had pH 7.4–7.8 and its hardness was 80–100 mg L^{-1} $CaCO_3$. Acute toxicity tests were conducted in 24-well polystyrene plates (Corning Costar Corporation, USA), following the protocol of Pérez-Legaspi and Rico-Martínez [33]. Briefly, ten 24 h old neonates were placed in each well containing a total volume of 1.0 mL of EPA medium diluted with five BC elutriate concentrations (as mentioned previously) and incubated for 48-h at 25 ± 1 °C in a 16:8 light:darkness cycle. These dilutions were done according to the Mexican Norm [49]. The test was conducted with five replications for each BC. After 48 h, the number of dead animals was recorded and the data analyzed statistically to establish significant differences between negative control (with EPA medium [48]) and elutriate samples.

2.3.3. *Daphnia magna* 48 h Acute Toxicity Test

We used the *Daphnia magna* acute toxicity protocol detailed in the Mexican Norm NMX-AA-087-SCFI [49]. Briefly, this technique consisted of 48 h exposure of 24 h old neonates of *D. magna* to a control and 5 different concentrations determined through a range toxicity test [50]. In the control, 10 neonates were placed in 100 mL of EPA medium in a 250 mL glass beaker [48]. The same was done for each replication except that, besides the EPA medium, the beakers contained the corresponding test concentration (as mentioned previously). Light intensity was kept between 400 to 1000 lux, as determined by an illuminometer (Kyoritan 140 Electrical Instruments), and temperature was kept at 20 ± 1 °C.

2.3.4. *Moina macrocopa* 48 h Acute Toxicity Test

We used the *Daphnia magna* acute toxicity protocol detailed in the Mexican Norm NMX-AA-087-SCFI [49]. The only difference was to substitute *D. magna* with *M. macrocopa*.

2.3.5. *Paramecium caudatum* Growth Inhibition Test

To assess the sublethal toxicity of the four BCs to *Paramecium caudatum*, we performed the protocol of Miyoshi et al. [51], with slight modifications. Briefly, this test starts with the placement of five *P. caudatum* organisms in a well of a 24-well polystyrene plate, with a negative control (EPA medium) and five dilutions of the biochar elutriate (100%, 50%, 25%, 12.5%, and 6.25%) in a final volume of 2 mL. We add Sonneborn medium [46] at 1 g L^{-1} at the start of the test. Then, the plate was placed in a bioclimatic chamber with a 16:8 light:darkness cycle at 25 °C for 96 h. At the end of the 96 h exposure

time the total number of organisms was counted in each well to obtain the percentage of inhibition of the population applying the following formula:

$$\%I = \frac{Nc - Nt}{Nc} * 100 \quad (1)$$

with:

N = total number of *P. caudatum* organisms alive after 96-h
t = treatment
c = control

2.3.6. Chronic Five-Day Toxicity Tests (Growth Inhibition) with *Lecane quadridentata*

Since no lethal toxicity was found with the four BC:soil 1:8 mixes, and to assess the sublethal toxicity of the four BC:soil 1:8 blend elutriates, we performed the 5-day chronic toxicity tests with *L. quadridentata* using the protocol of Hernández-Flores and Rico-Martínez [52].

2.3.7. Chronic Seven-Day Toxicity Test (Growth Inhibition) with *Moina macrocopa*

Since no lethal toxicity was found with the four BC:soil elutriates, we used the protocol of the 7-day Chronic Test with the cladoceran *Ceriodaphnia dubia* [53] to assess the sublethal toxicity with slight modifications: (a) Instead of using *C. dubia,* we substituted this cladoceran with *Moina macrocopa*; (b) instead of using the yeast, cereal leaves, and tetramin (YCT) food we used the micro algae *Pseudokirchneriella subcapitata* to feed *M. macrocopa*.

2.4. Statistical Analysis for the 48h Acute Toxicity Test

Data were analyzed through ANOVA and Tukey HSD test (n = 5 replicates) to establish significant differences from controls to obtain NOEC and LOEC values. To determine r^2 values (correlation coefficient) and to conduct regression to calculate LC_{50} and LC_{10} values with the corresponding toxicants, the software Statistica 7.0 (Stat-Soft Inc., Tulsa, OK, USA, 1993) was employed.

3. Results

3.1. Physical and Chemical Parameters of the Biochars

BC yields of the four samples ranged from 16%–30% ± 5% on a dry weight basis, which is in the same range as other biomass feedstocks and pyrolysis systems which operate at equal temperatures around 700 °C [54]. Cornelissen et al. [10] compared Kon-Tiki flame curtain kiln produced BC with traditional low-temperature kilns and retorts. With regard to their investigations, BC from traditional low-temperature kilns has been in the same order of magnitude, whereas the percental output ratio in contrast to low-temperature retort kilns (typically around 30%–40% on a dry weight basis) has been lower. The BC, as in the present case, corresponds to these insights.

The following interpretation of the BC characteristics is based on the values presented in Table 3. Water holding capacity (WHC) of the four BCs ranged from 165%–254% on dry matter basis, with the lowest value being for BC 3 and the highest value being for BC 4. Hence, all produced chars hold excellent properties to improve water holding capacity if added to the soil. Specific surface (based on Brunauer–Emmet–Teller [BET] theory) of the BCs were in the range of 54–305 m^{-2} g, which is in the same order of magnitude found in literature. Low BET values mean larger pores and thus less water is retained. As BET values increase, pores become smaller and water is better retained [55]. Surprisingly, the WHC of the four BCs was higher when BET values were low.

Table 3. Analyses of four BCs based on four different feedstocks generated with a Kon-Tiki flame curtain kiln in Aguascalientes, Mexico at Universidad Panamericana. Analyzed by an European Biochar Certificate (EBC) accredited laboratory following the EBC analytical methods and compared to the EBC thresholds for premium and basic BC quality [1].

Biochar (BC)		**BC 1**		**BC 2**		**BC 3**		**BC 4**		**EBC Threshold**	
Parameter	***Unit***	***FM***	***aDM***	***FM***	***aDM***	***FM***	***aDM***	***FM***	***aDM***	***Premium***	***Basic***
Water holding capacity (WHC)	mass-%		165.8		200.0		149.1		254.2		
Bulk density	kg m^{-3}	385		504		567		368			
Specific surface (by *BET*)	m^{-2} g		305		140		280		54		
Particle density	g cm^{-3}		1.64		1.59		1.76		1.58		
Total water content	mass-%	28.4		39.8		35.9		60.7			
Ash content 550 °C	mass-%	8.6	12	8.7	14.5	11.4	17.7	5.1	13.1		
Hydrogen	mass-%	0.69	0.96	1.10	1.82	0.56	0.87	0.67	1.70		
Total carbon (TC)	mass-%	63.9	89.2	47.0	78.1	51.5	80.4	30.9	78.7	>50	>50
Total inorganic carbon (TIC)	mass-%	0.7	1.0	0.8	1.3	0.9	1.3	0.4	1.0		
Nitrogen	mass-%	0.22	0 0.3	0.39	40.66	0.5	0.79	0.46	1.17		
Oxygen	mass-%	0.8	1.1	5.1	8.5	3.3	5.2	3.1	7.9		
Carbonate CO_2	mass-%	2.62	3.66	2.77	4.6	3.12	4.87	1.51	3.84		
Organic carbon	mass-%	63.2	88.2	46.2	76.8	50.6	79.1	30.5	77.7		
H/C (molar ratio)		0.13	0.13	0.28	0.28	0.13	0.13	0.26	0.26	<0.6	<0.6
H/Corg (molar ratio)		0.13	0.13	0.28	0.28	0.13	0.13	0.26	0.26	<0.7	<0.7
O/C (molar ratio)		0.01	0.009	0.08	0.082	0.05	0.05	0.08	0.08	<0.4	<0.4
pH value ($CaCl_2$)		7.8		8.2		8.5		8.3		≤10	≤10
Electric conductivity	µS cm^{-1}	336		566		617		580			
Salt content	g kg^{-1}	1.77	2.48	2.99	4.96	3.26	5.09	3.06	7.79		
Salt content	g L^{-1}	0.68	0.95	1.51	2.50	1.85	2.88	1.16			
Phosphorous	mg kg^{-1}		470		1400		2300		7.79		
Magnesium	mg kg^{-1}		1800		2500		2500		2.94		
Calcium	mg kg^{-1}		36,000		41,000		51,000		2300		
Potassium	mg kg^{-1}		4000		11,000		9800		2900		
Sodium	mg kg^{-1}		350		1000		910		32,000		
Iron	mg kg^{-1}		460		760		830		12,000		
Silica	mg kg^{-1}		6100		10,000		9200		1400		
Sulfur	mg kg^{-1}		170		680		2100		1000		
Arsenic	mg kg^{-1}		<0.8		<0.8		<0.8		<0.8	<13	<13
Lead	mg kg^{-1}		3		3		<2		3	<120	<150
Cadmium	mg kg^{-1}		<0.2		<0.2		<0.2		<0.2	<1.0	<1.5
Copper	mg kg^{-1}		7		13		15		37	<100	<100
Nickel	mg kg^{-1}		<1		1		<1		1	<30	<50
Mercury	mg kg^{-1}		<0.07		<0.07		<0.07		<0.07	<1.0	<1
Zinc	mg kg^{-1}		61		28		21		53	<400	<400
Chromium	mg kg^{-1}		<1		1		<1		<1	< 0	<90
Boron	mg kg^{-1}		15		29		21		51		
Manganese	mg kg^{-1}		560		350		360		460		
Total PAH (EPA-16)	mg kg^{-1}		4.8		5.3		0.7		8.0	<4	<12
pH QW (source water pH 8.1)		n.a.		10.8		13.2		10.5			

[1] Abbreviations: aDM, absolute dry matter; FM, fresh matter; QW, quenched water; BET, Brunauer–Emmet–Teller method; EBC, European Biochar Certificate; PAH, polycyclic aromatic hydrocarbon; EPA, Environmental Protection Agency; n.a., not applicable.

Total carbon content (TC) of the BCs were in the range of 78%–89% in dry matter, complying with the EBC threshold of >50% for both premium and basic quality. The lowest value being for the BC 4 with 78% and the highest value being for the BC 1 with 89%. H contents of the four sample BCs were 0.87%–1.82%, O contents were 1.1%–8.5%. Based on these values, H/C, O/C, and H/C_{org} ratios on molar basis were calculated with H/C of 0.13–0.28 and O/C of 0.01–0.08 as well as H/C_{org} ratios equivalent to H/C, whereby the high aromaticity and consequential high recalcitrance and inertia was confirmed, referring to the given threshold by the EBC. An electron micrograph of BC 1 is presented in Figure 2.

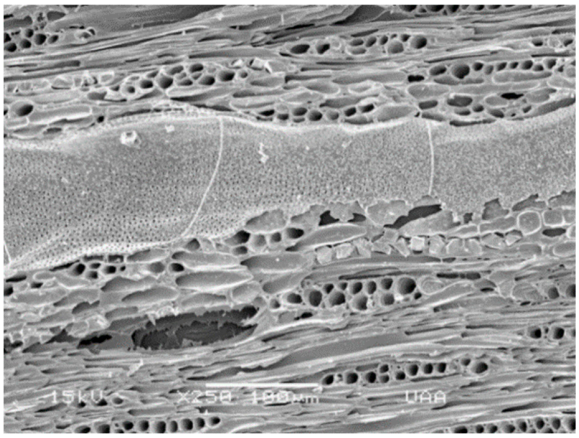

Figure 2. Structure of biochar (BC) 1 from manchineel tree and pine (60/40) mixed wood produced with Kon-Tiki flame curtain kiln in Aguascalientes. Electron micrograph: 15 kV zoom × 250, Universidad Autónoma de Aguascalientes, November 2017.

Compared to the EBC thresholds, all heavy metal contents indicated uncritical biomass feedstock, with values far below the limits. Only zinc and copper showed a slight presence, but still far below the thresholds.

The most relevant toxic compounds in BC are considered to be the PAH-16, as it is known that they are carcinogenic if entered in the food chain and can affect plant growth negatively. Values for the PAHs detected in each type of BC produced are presented in Table 4.

Table 4. Values for the polycyclic aromatic hydrocarbons (PAHs) in four BCs [2].

Biochar (BC)	**BC 1**	**BC 2**	**BC 3**	**BC 4**
PAH	**mg kg^{-1}**	**mg kg^{-1}**	**mg kg^{-1}**	**mg kg^{-1}**
Naphtalin	2.5	2.6	0.6	3.3
Acenaphthylen	<0.1	<0.1	<0.1	<0.1
Acenaphthen	<0.1	<0.1	<0.1	<0.1
Fluoren	0.7	0.3	<0.1	0.5
Phenanthren	0.6	1.1	0.1	1.6
Anthracen	0.1	0.3	<0.1	0.3
Fluoranthen	0.4	0.4	<0.1	1.0
Pyren	0.5	0.4	<0.1	1.0
Benz(a)anthraren	<0.1	0.1	<0.1	0.1
Chrysen	<0.1	0.1	<0.1	0.2
Benzo(b)fluoranthen	<0.1	<0.1	<0.1	<0.1
Benzo(k)fluoranthen	<0.1	<0.1	<0.1	<0.1
Benzo(a)pyren	<0.1	<0.1	<0.1	<0.1
Indeno(1,2,3-cd)pyren	<0.1	<0.1	<0.1	<0.1
Dibenzo(a,h)anthracen	<0.1	<0.1	<0.1	<0.1
Benzo(g,h,i)perylen	<0.1	<0.1	<0.1	<0.1
Total PAH	**4.8**	**5.3**	**0.7**	**8.0**

[2] Toluol extraction, DIN EN 15527 (FR-JE02).

Concentrations in all BCs are far below the permitted EBC thresholds, both for PCDD/Fs and PCBs, but still contain substantial content to endanger living organisms. Table 5 shows the sum of PCDD/F and PCB values calculated based on the toxicity equivalency quotient (TEQ). The total analysis is illustrated in Table A1 attached to the Appendix A.

Table 5. Condensed values for the polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and dl-PCBs in four BCs plus EBC threshold [3].

Sum of Substances	Unit	BC 1	BC 2	BC 3	BC 4	EBC
Σ $WHO_{(2005)}$ $PCDD_{(7)}/F_{(10)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} DM	0.40	0.39	0.39	0.40	20
Σ $WHO_{(2005)}$ $PCDD_{(7)}/F_{(10)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} 88%DM	0.35	0.34	0.34	0.35	0.75
Σ $WHO_{(2005)}$ $PCB_{(12)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} DM	0.041	0.040	0.040	0.040	0.35
Σ $WHO_{(2005)}$ $PCDD_{(7)}/F_{(10)}$ + $PCB_{(12)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} 88%DM	0.036	0.035	0.035	0.036	1.25
Σ $WHO_{(2005)}$ Indicator $PCB_{(6)}$ ***[excl.** LOQ]*	µg kg^{-1} 88%DM	0.400	0.230	0.018	0.024	10

[3] Abbreviations: $WHO_{(2005)}$, values based on World Health Organization toxic equivalency factor from 2005; $PCDD_{(7)}$, 7 EBC required polychlorinated dibenzo-*p*-dioxins; $PCDF_{(10)}$, 10 EBC required polychlorinated dibenzofurans; TEQ, toxicity equivalency quotient; LOQ, limit of quantitation; $PCB_{(12)}$, 12 EBC required polychlorinated biphenyls; $PCB_{(6)}$, 6 EBC required indicator polychlorinated biphenyls; DM, dry matter.

3.2. *Paramecium caudatum* 24 h Acute and Sublethal Toxicity Tests

No significant lethal or sublethal toxicity was found. In the lethal tests, no LC_{50} values could be determined as only one ciliate was dead in one BC elutriate. In the sublethal tests no growth inhibition was detected after 96 h in each biochar treatment with no dilution (elutriate at 100%) (Table 6).

Table 6. Results of the growth inhibition tests with *Paramecium caudatum*. $n = 5$.

Treatment	% Inhibition
Control	0
Soil	1.99
BC 1	2.65
BC 2	2.65
BC 3	0
BC 4	1.32

3.3. *Lecane quadridentata* 48 h Acute and Sublethal Toxicity Tests

Lethal toxicity was detected when *L. quadridentata* was exposed to all four BC (Table 7). We observed many particles of BC in the stomach and digestive apparatus of *L. quadridentata* (Figure 3). We obtained LC_{50} value in the range of 8.3%–25.1% of effective concentration for *Lecane quadridentata*. The confidence limits (CL95%) were close to the LC_{50}, and CV values were in the range of 3.8%–6.8% and are far below 25% as the maximum allowed threshold. Detected order of susceptibility: BC 3 > BC 4 > BC 1 > BC 2 (lowest to highest toxicity). No sublethal tests were conducted with *L. quadridentata* with the BC elutriates since all BC resulted in acute toxicity.

Table 7. Acute toxicity values of *Lecane quadridentata* to BCs [4].

Parameter	BC 1	BC 2	BC 3	BC 4
LC_{10}	10.76	4.16	13.36	10.37
LC_{50}	19.95	8.36	25.14	20.36
NOEC	25	<6.25	6.25	<6.25
LOEC	50	6.25	12.50	6.25
95% CL LC_{50}	12.36–32.21	5.92–11.83	17.78–35.48	15.85–20.40
CV	3.8	5.7	6.5	6.8
r^2	0.3855	0.7001	0.6876	0.8110

[4] Abbreviations correspond to the following: BC 1–4, Biochar 1 to 4; LC_{10}, lethal concentration where 10% of animals die; LC_{50}, lethal concentration where 50% of animals die; NOEC, no observed effect concentration; LOEC, lowest observed effect concentration; CV, coefficient of variation; 95% CL LC_{50}, confidence limits for the LC_{50} values; r^2, correlation coefficient. LC, NOEC, LOEC, 95% CL, and CV are all in percentages of dilution of each elutriate.

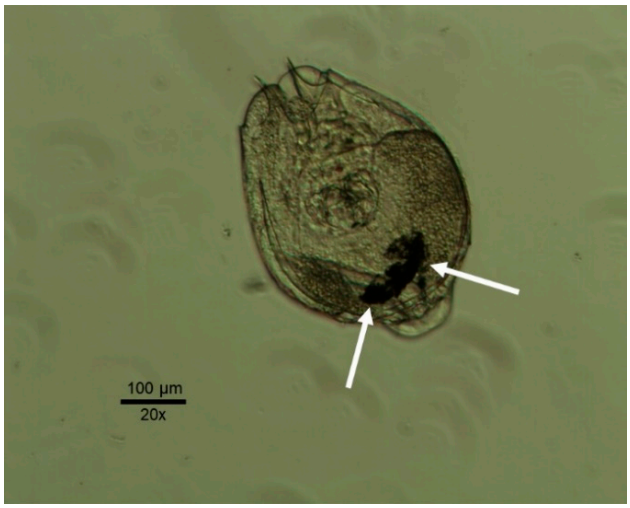

Figure 3. Photograph of dead *Lecane quadridentata*. Digestive tract filled with BC particles as indicated by the arrows.

3.4. *Daphnia magna* 48 h Acute Toxicity Test

We observed many particles of BC in the digestive tract of *D. magna* (Figure 4). When we exposed *D. magna* to the four BC elutriates without dilution (100% sample) we found in BC 1, 4%, in BC 2, 20%, in BC 3, 44%, and in BC 4, 2% mortality (n = 5 in all cases). We decided not to conduct any more experiments with *D. magna* since this is an exotic species that has never been found in Mexican reservoirs, and we decided to substitute this cladoceran species with *M. macrocopa*.

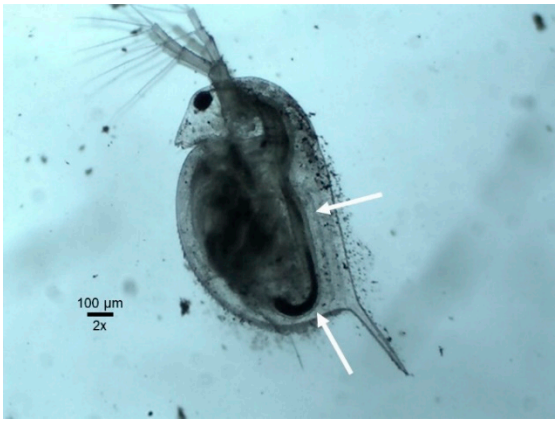

Figure 4. Photograph of dead *Daphnia magna*. Digestive tract filled with BC particles as indicated by the arrows.

3.5. *Moina macrocopa* 48 h Acute Toxicity Test

We observed the digestive tract of *M. macrocopa* completely filled with BC particles (Figure 5). Table 8 shows the results of the acute tests with *M. macrocopa*. Low lethal toxicity levels were found with this cladocerans species which allow one to calculate LC_{50} values only for two BC's. We obtained LC_{50} value of effective concentration only for BC 1 and BC 2, with 134.87 and 306.33, respectively.

Figure 5. Photograph of dead *Moina Macrocopa*. Digestive tract fully filled with BC particles as indicated by the arrows.

Table 8. Acute toxicity values of *Moina Macrocopa* to BCs [5].

Parameter	BC 1	BC 2	BC 3	BC 4
LC_{10}	ND	ND	ND	ND
LC_{50}	ND	134.87	306.33	ND
NOEC	ND	50	50	ND
LOEC	ND	100	100	ND
95% CL LC_{50}	ND	36.16–503.03	37.59–2495.74	ND
CV	ND	ND	ND	ND
r^2	ND	0.25	0.23	ND

[5] Abbreviations correspond to the following: BC 1–4, Biochar 1 to 4; LC_{10}, lethal concentration where 10% of animals die; LC_{50}, lethal concentration where 50% of animals die; NOEC, no observed effect concentration; LOEC, lowest observed effect concentration; CV, coefficient of variation; 95% CL LC_{50}, confidence limits for the LC_{50} values; r^2, correlation coefficient. LC, NOEC, LOEC, 95% CL, and CV are all in percentages of dilution of each elutriate.

Due to the low levels of lethal toxicity found in BC 1 and BC 4, we decided to perform growth inhibition tests with this cladoceran species. Results of the sublethal growth inhibition tests are shown in Table 9. No sublethal toxicity was detected at the 100% (no dilution) elutriate sample for BC 1 and BC 4 where no EC_{50} values were calculated.

Table 9. Reproductive test with Moina macrocopa (7 days). $n = 5$.

Treatment	Mean Value *r*
Control	0.408
BC 1	0.401
BC 4	0.401
Soil	0.406

3.6. Soil–Biochar Mixture Elutriate Tests

No lethal or sublethal toxicity was found in the soil–biochar mixture experiments. Not a single animal was dead in the lethal tests with any of the two species used (*M. macrocopa* and *L. quadridentata*). In the sublethal tests, there was no significant difference among any treatment with the control in the chronic parameters used. Table 10 shows the growth inhibition sublethal tests with *L. quadridentata*, *M. macrocopa,* and *P. caudatum*.

Table 10. Results of the growth inhibition and reproductive tests with *Lecane quadridentata* (Lecane), *Moina macrocopa* (Moina), and *Paramecium caudatum* (Paramecium) exposed to the BC:soil (1:8) elutriate. $n = 5$.

Treatment	*Lecane* Mean Value *r*	*Moina* Mean Value *r*	*Paramecium* % Inhibition
Control	0.371	0.408	0
BC 1 + soil	0.356	0.400	1.99
BC 2 + soil	0.358	0.402	0.66
BC 3 + soil	0.356	0.402	2.65
BC 4 + soil	0.359	0.402	0.66
Soil	0.358	0.406	1.32

4. Discussion

4.1. Toxicity of Biochar Elutriates to Aquatic Invertebrates

This experiment was conducted to investigate the toxicity on four aquatic invertebrate species exposed to four different BCs. We discovered that the respective BCs, despite complying with international certification standards such as the EBC, can induce adverse effects to non-target organisms in the form of acute toxicity, as was the case with *Lecane quadridentata*, *Moina macrocopa,* and *Daphnia magna*. The ciliate *Paramecium caudatum*, in contrast, did not show any chronic or lethal toxicity when exposed to the BCs.

Acute toxicity was only detected if the organisms were exposed to the pure BC elutriate. When the organisms were exposed to elutriate obtained from a BC–soil mixture in ratio 1:8, no chronic and no lethal effects to all tested species were observed. The results show that the application rates have a decisive influence on the soil biota. If users follow standards that regulate BC additions to the soil (e.g., BBodSchG), the potentially harmful effects on rotifers and cladocerans can be most widely diminished. Nonetheless, the compliant use of certified BC does not guarantee 100% safety, particularly near sensitive habitats or with regard to BC utilization in animal feed.

In the living environment, the toxicants are mixed, blended and, in many cases, occur at low concentrations that may be regarded as non-adverse. Mejía-Saavedra et al. [56] showed that one toxicant, even at no observable effective concentration (NOEC), can cause an increase in the toxicity of the other (synergistic effect). With regard to the present results, it remains unclear if a single hazardous substance evidenced in the BCs or the sum of hazardous substances is responsible for the detected toxicities in rotifers and cladocerans.

Based on the concentration of pollutants known to be hazardous to aquatic invertebrates (e.g., PAHs, PCDD/Fs, and heavy metals (especially zinc, copper, and manganese)) [16,33,56–58], the four

BCs could be classified in their potential danger to each other in a direct comparison. The result was the following order and its toxicity potential: BC 3 < BC 2 < BC 1 < BC 4 (from low to high toxicity potential). Thus, BC 4 is expected to generate the potentially lowest LC_{50} values, causing the highest toxicity. BC 3 instead is expected to tendentially have the highest LC_{50} values, causing the lowest toxicity to the organisms. Interestingly the BC with the highest concentrations of hazardous substances, which undeniably was BC 4, did not provoke the highest toxicological impact on the organisms. Instead, BC 2, which is probably a "cleaner" and with regard to EBC thresholds (e.g., PAHs) an uncritical BC, had the lowest LC_{50} values both for *L. quadridentata* and *M. macrocopa*. The EBC analysis did not indicate a very particular high concentration of any specific contaminant for BC 2, nor for the other BCs, which could be an explanation for the incongruous order of toxicity.

This result is an indication that maybe there are synergistic responses among the entire mixture of hazardous substances and that this is the trigger producing the observed toxicity. Future research shall focus on the explanation of the witnessed digestion processes and the assessment of susceptibility of every single toxicant and the hazardous mixtures of toxic substances.

Summarized, *Lecane quadridentata* basically showed the highest susceptibility to the BCs, followed by *Moina macrocopa* and *Daphnia magna*.

4.2. Toxic Mechanism, Actuator, and Relevance to the Environment

Apparently, the mechanism of toxicity is the digestion of BC particles, whereby gastric juices liberate toxins, which are present in the BC. This mechanism is supported by the images of dead animals that show digestive tracts with abundant (in some cases) BC traces. As no feed was applied, the only particles contained in the elutriate and found in the digestive tract can arise from the BC. Another indicator, which subscribes the digestion hypothesis, is that ciliates did not absorb BC particles. In fact, ciliates did not experience any harm from the BCs.

Except for ciliates, the results show that rotifers and cladocerans, which habitat in intercellular soil water, are capable of digesting micro-particles of BC and thus release bounded and mobilize immobilized hazardous substances contained therein. Rotifers have an activity of phosphatases, ß-N-acetylhexosaminidases, and lipases [59]. In the case of *D. magna*, many digestive enzymes have been found: galactosidases, esterases, trypsin, and cellulases [60]. Based on the ingestion mechanism triggered by rotifers and cladocerans, potentially toxins could enter the food chain, accumulate biologically and, at higher concentrations, potentially provoke carcinogenic, mutagenic, or reprotoxic effects to higher organisms.

Naturally, PCDD/Fs and PAHs are generated during pyrogenic oxidation of hydrocarbon compounds and are ubiquitous and persistent pollutants in the environment [61,62]. Volcanic eruptions, as well as forest and vegetation fires, will release PCDD/Fs and PAHs on a natural basis [63], which will most likely degrade in the soil. But the man-made application of BC and associated toxins, in quantitative terms, is much higher and more frequent. Hence, naturally driven degradation process may take a long time until complete dissolution and will restrict, significantly, specific soil biota, as demonstrated within this study.

High concentrations of Zn and Cu (Table 3) could be an indication for increased zinc accumulation by the feedstock, as other sources of contamination can probably be excluded. As far as the authors know, there are rather no documentations known that indicate an increased zinc uptake by the used wood species. As mesquite is known for its tendency to accumulate heavy metals above average, in contrast to other trees, it was expected to find higher contents in BC 2 and BC 3, nevertheless this assumption was not confirmed by the present study.

Among the PAH-16 (Table 4) used as benchmarks by many environmental authorities in many countries, benzo (a) pyrene is considered to be the most crucial. Especially fodder producers require a maximum content of benzo (a) pyrene below 0.1 mg kg^{-1}, no matter if the total PAH-16 content is below the maximum threshold of (<4 ± 2 mg kg^{-1}). Concentrations of benzo (a) pyrene in all samples were below 0.1 mg kg^{-1}. Even though BC 1, 2, and 3 (4.8 mg kg^{-1}, 5.3 mg kg^{-1}, 0.7 mg kg^{-1},

respectively) qualify for EBC premium quality, BC 4 (8.3 mg kg^{-1}) was only permissible for basic quality (<12 ± 4 mg kg^{-1}). The highest content in all samples was shown by naphthalin in a range from 2.5–3.4 mg kg^{-1}. This is probably explicable due to improved naphthalin emergence at high temperatures in pyrosynthesis above 700 °C. BC 4 additionally had an outlier in phenanthren content with 1.6 mg kg^{-1}. Basically, PAHs in BC are very hydrophobic and hardly bio-accessible [7], but could potentially be liberated by the enzymatic and mechanistic processes.

PCDD/Fs and PCBs belong to the POP substances. They are (P) persistent (not biodegradable), (O) organic, and (P) pollutant. Furthermore, they belong to the CMR substances. Their human toxicological effects may; therefore, be (C) carcinogenic, (M) mutagenic, and (R) reprotoxic. Most of the toxicological effect is shown by disorders of the immune and nervous system, the respiratory tract, the thyroid gland and, for example, the digestive tract. Dioxins, furans, and biphenyls may be produced as undesirable by-products within combustion processes in the presence of chlorine and organic carbon, in particular at temperatures of 300–400 °C, whereas at a temperature level of 900 °C, the chlorine-based pollutants are destroyed. Concentrations in all BCs were far below the permitted EBC thresholds, both for PCDD/Fs and PCBs (c.p. Table A1); however, a there was a potential coherence between the toxicity potential of these POP substances and the detected mortality of rotifers and cladocerans.

5. Conclusions

The original hypothesis was proved by the experimental test results. BC, despite complying with international certification standards such as the EBC, can induce adverse effects to non-target organisms in the form of acute toxicity. The rotifer *L. quadridentata* showed substantial toxicological response to the four tested BCs, with LC_{50} values in the range of 8.3%–25.1% of effective concentration. *Daphnia magna* and *M. macrocopa* showed reduced lethal toxicity when compared with the rotifer. Only *Paramecium caudatum* did not show any negative response to the exposed BCs. If BC is applied soundly, adhering to the recommended limit masses, no serious adverse effects are to be expected. The present study provides a potential toxicity mechanism to benthic invertebrates when BC is applied to soil. As a result of this study, we recommend to not adhere only to the international certification thresholds concerning BC, but also consider country-specific rules of application meticulously, especially with regard to BC quantities.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Values for polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and dl-PCBs in four BCs plus EBC threshold [6].

Substance	Unit	BC-1	BC-2	BC-3	BC-4	EBC
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	ng kg^{-1} $_{DM}$	<0.1	<0.1	<0.1	<0.1	
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	ng kg^{-1} $_{DM}$	<0.15	<0.15	<0.15	<0.15	
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	ng kg^{-1} $_{DM}$	<0.15	<0.15	<0.15	<0.15	
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	ng kg^{-1} $_{DM}$	<0.15	<0.15	<0.15	<0.15	
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	ng kg^{-1} $_{DM}$	<0.15	<0.15	<0.15	<0.15	
1,2,3,4,6,7,8,-Heptachlorodibenzo-*p*-dioxin	ng kg^{-1} $_{DM}$	0.57	0.39	0.25	0.55	
Octachlorodibenzo-*p*-dioxin	ng kg^{-1} $_{DM}$	1.6	1.6	0.9	1.5	
2,3,7,8-Tetrachlorodibenzofuran	ng kg^{-1} $_{DM}$	0.17	0.12	0.17	0.17	
1,2,3,7,8-Pentachlorodibenzofuran	ng kg^{-1} $_{DM}$	<0.1	<0.1	<0.1	<0.1	
2,3,4,7,8-Pentachlorodibenzofuran	ng kg^{-1} $_{DM}$	<0.1	<0.1	<0.1	<0.1	
1,2,3,4,7,8-Hexachlorodibenzofuran	ng kg^{-1} $_{DM}$	0.13	<0.1	<0.1	0.12	
1,2,3,6,7,8-Hexachlorodibenzofuran	ng kg^{-1} $_{DM}$	<0.1	<0.1	<0.1	<0.1	
1,2,3,7,8,9-Hexachlorodibenzofuran	ng kg^{-1} $_{DM}$	<0.1	<0.1	<0.1	<0.1	
2,3,4,6,7,8-Hexachlorodibenzofuran	ng kg^{-1} $_{DM}$	<0.1	<0.1	<0.1	<0.1	
1,2,3,4,6,7,8-Heptachlorodibenzofuran	ng kg^{-1} $_{DM}$	0.21	0.14	0.16	0.25	
1,2,3,4,7,8,9-Heptachlorodibenzofuran	ng kg^{-1} $_{DM}$	<0.1	<0.1	<0.1	<0.1	
Octachlorodibenzofuran	ng kg^{-1} $_{DM}$	<0.2	0.2	<0.2	0.2	
Σ $WHO_{(2005)}$ $PCDD_{(7)}/F_{(10)}$ (TEQ) ***[excl.** LOQ]*	ng kg^{-1} $_{DM}$	0.04	0.02	0.02	0.04	
Σ $WHO_{(2005)}$ $PCDD_{(7)}/F_{(10)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} $_{DM}$	0.40	0.39	0.39	0.40	**20**
Σ $WHO_{(2005)}$ $PCDD_{(7)}/F_{(10)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} $_{88\%DM}$	0.35	0.34	0.34	0.35	**0.75**
3,3′,4,4′-Tetrachlorobiphenyl	ng kg^{-1} $_{DM}$	3	1.6	1.3	1.6	
3,4,4′,5-Tetrachlorobiphenyl	ng kg^{-1} $_{DM}$	<0.2	<0.2	<0.2	<0.2	
2,3,3′,4,4′-Pentachlorobiphenyl	ng kg^{-1} $_{DM}$	13	6.5	6.4	7.5	
2,3,4,4′,5-Pentachlorobiphenyl	ng kg^{-1} $_{DM}$	29	15	14	18	
2,3′,4,4′,5-Pentachlorobiphenyl	ng kg^{-1} $_{DM}$	<3	<3	<3	<3	
2,3′,4,4′,5′-Pentachlorobiphenyl	ng kg^{-1} $_{DM}$	<2	<2	<2	<2	
3,3′,4,4′,5-Pentachlorobiphenyl	ng kg^{-1} $_{DM}$	<0.3	<0.3	<0.3	<0.3	
2,3,3′,4,4′,5-Hexachlorobiphenyl	ng kg^{-1} $_{DM}$	4.2	2.3	<2	2.4	
2,3,3′,4,4′,5′-Hexachlorobiphenyl	ng kg^{-1} $_{DM}$	<2	<2	<2	<2	
2,3′,4,4′,5,5′-Hexachlorobiphenyl	ng kg^{-1} $_{DM}$	<2	<2	<2	<2	
3,3′,4,4′,5,5′-Hexachlorobiphenyl	ng kg^{-1} $_{DM}$	<0.3	<0.3	<0.3	<0.3	
2,3,3′,4,4′,5,5′-Heptachlorobiphenyl	ng kg^{-1} $_{DM}$	<3	<3	<3	<3	
Σ $WHO_{(2005)}$ $PCB_{(12)}$ (TEQ) ***[excl.** LOQ]*	ng kg^{-1} $_{DM}$	0.00169	0.00087	0.00074	0.00100	
Σ $WHO_{(2005)}$ $PCB_{(12)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} $_{DM}$	0.04111	0.04029	0.04022	0.04042	**0.35**
Σ $WHO_{(2005)}$ $PCB_{(12)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} $_{88\%DM}$	0.03617	0.03546	0.03540	0.03557	
Σ $WHO_{(2005)}$ $PCDD_{(7)}/F_{(10)}$ + PCB (TEQ) ***[incl.** LOQ]*	ng kg^{-1} $_{DM}$	0.43845	0.42713	0.43065	0.43693	
Σ $WHO_{(2005)}$ $PCDD_{(7)}/F_{(10)}$ + $PCB_{(12)}$ (TEQ) ***[incl.** LOQ]*	ng kg^{-1} $_{88\%DM}$	0.38583	0.37588	0.37897	0.38450	**1.25**
2,4,4′-Trichlorobiphenyl	µg kg^{-1} $_{88\%DM}$	0.080	<0.055	<0.050	<0.050	
2,2′,5,5′-Tetrachlorobiphenyl	µg kg^{-1} $_{88\%DM}$	0.130	0.075	0.074	0.078	
2,2′,4,5,5′-Pentachlorobiphenyl	µg kg^{-1} $_{88\%DM}$	0.082	0.044	0.047	0.047	
2,2′,3,4,4′,5′-Hexachlorobiphenyl	µg kg^{-1} $_{88\%DM}$	0.050	0.028	0.026	0.031	
2,2′,4,4′,5,5′-Hexachlorobiphenyl	µg kg^{-1} $_{88\%DM}$	0.054	0.03	0.028	0.032	
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	µg kg^{-1} $_{88\%DM}$	<0.020	<0.020	<0.020	<0.0020	
Σ $WHO_{(2005)}$ Indicator $PCB_{(6)}$ ***[excl.** LOQ]*	µg kg^{-1} $_{88\%DM}$	0.400	0.230	0.018	0.024	**10**

[6]. Abbreviations: $WHO_{(2005)}$, values based on World Health Organization toxic equivalency factor from 2005; $PCDD_{(7)}$, 7 EBC required polychlorinated dibenzo-*p*-dioxins; $PCDF_{(10)}$, 10 EBC required polychlorinated dibenzofurans; TEQ, toxicity equivalency quotient; LOQ, limit of quantitation; $PCB_{(12)}$, 12 EBC required polychlorinated biphenyls; $PCB_{(6)}$, 6 EBC required indicator polychlorinated biphenyls; DM, dry matter.

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