CENTRO DE CIENCIAS BÁSICAS

DEPARTAMENTO DE QUÍMICA

THESIS

# PHYSICOCHEMICAL ASSESSMENT AND TOXICOLOGICAL RISK EVALUATION IN AMENDING AGRARIAN SOILS WITH BIOCHAR GENERATED FROM WOODY RESIDUES IN THE STATE OF AGUASCALIENTES, MEXICO.

*Apreciación fisicoquímico y evaluación del riesgo toxicológico de la aplicación de biocarbón como enmienda de suelo generado a partir de los residuos leñosos del estado de Aguascalientes, México.*

PRESENTS

**M.Sc. Felix Flesch**

TO OBTAIN THE DOCTORAL DEGREE IN BIOLOGICAL SCIENCES

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Aguascalientes, 20 January 2020

UNIVERSIDAD AUTONOMA DE AGUASCALIENTES

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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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## Declaration of authorship

I declare that this thesis has not been already accepted in substance for any degree and is not being submitted in candidature for any degree.

I affirm that the substance of this thesis is entirely the result of my own investigation and that due reference and acknowledgement is made where necessary to the work of other researchers.

Place, Date and Signature: 11 February 2020, Mülheim,

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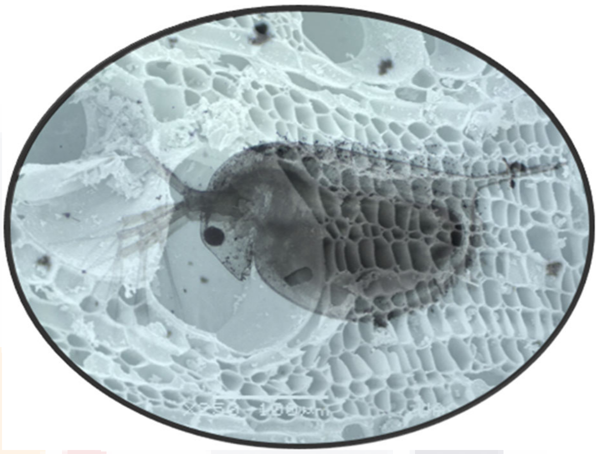
## Dedication

To my beloved son Lino and my beautiful, sweet, loving girl, life partner and best friend Sandra



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## Abbreviations

| | |
|---|---|
| AC | Activated Carbon |
| ACS | Accredited Control System |
| $a_{DM}$ | absolute dry matter |
| ASTM | American Society for Testing and Materials |
| BC | Biochar |
| BET | Brunauer-Emmett-Teller theory |
| C | Carbon |
| CAPEX | Capital Expenditures |
| CE | *Spanish* Concentración Effectiva (Effective Concentration) |
| $CO_2$ | Carbon Dioxide |
| $C_{org}$ | Organic Carbon |
| CV | Coefficient of Variation |
| DM | Dry Matter |
| EBC | European Biochar Certificate |
| EAT | Earnings after Tax |
| EBT | Earnings before Tax |
| EBIT | Earnings before Interest and Tax |
| EBITDA | Earnings before Interest, Tax, Depreciation and Amortization |
| EC | Effective Concentration |
| EPA | Environmental Protection Agency |
| EUR | Currency EURO |
| FC | Field Capacity |
| FM | Fresh Matter |
| g/g | Gravimetric/Gravimetric Proportion |
| GHG | Greenhouse Gas |
| GPS | Global Positioning System |
| HGB | *German* Handelsgesetzbuch (German Commercial Code) |

| | |
|---|---|
| HHA | Heterocyclic Aromatic Amines |
| HTT | Highest Treatment Temperature |
| IBI | International Biochar Initiative |
| IFRS | International Financial Reporting Standards |
| INAGUA | Instituto del Agua del Estado de Aguascalientes |
| INEGI | National Institute of Statistics, Geography and Informatics |
| IPCC | Intergovernmental Panel on Climate Change |
| IRR | Internal Rate of Return |
| KPI | Key Performance Indicator |
| $kg_{FM}$ | Kilogram Fresh Matter |
| $LC_{10}$ | Lethal Concentration where 10% of the test animals die |
| $LC_{50}$ | Lethal Concentration where 50% of the test animals die |
| LOEC | Lowest Observed Effect Concentration |
| NAVSTAR | NAVigation System with Timing And Ranging |
| NOEC | No Observed Effect Concentration |
| NPV | Net Present Value |
| OPEX | Operational Expenditures |
| OUPR | Optimum User Performance Ratio |
| PAH | Polycyclic Aromatic Hydrocarbon |
| PAW | Plant Available Water |
| PBP | Payback Period |
| PCB | Polychlorinated Biphenyl |
| PCDD/F | Polychlorinated Dibenzodioxin and Furan |
| pH | pH-value |
| P&L | Profit and Loss Account |
| SOC | Soil Organic Carbon |
| SWC | Soil Water Capacity |
| TOTEX | Total Expenditures |
| UBA | *German* Umweltbundesamt (Environmental Bureau of Germany) |

| | |
|---|---|
| UAA | Universidad Autonoma de Aguascalientes |
| UP | Universidad Panamericana |
| USD | Currency US Dollars |
| uFC | Usable Field Capacity |
| v/v | Volumetric/Volumetric Proportion |
| WACC | Weighted Average Cost of Capital |
| WC | Water Capacity |
| $WC_{max.}$ | Maximum Water Capacity |
| WHC | Water Holding Capacity |
| WHO | World Health Organisation |
| WQM | Water Quenched Matter |

# 1 Resumen (español)

Investigaciones recientes, han revelado los múltiples impactos positivos del biocarbón o sustrato con cierto contenido de biocarbón aplicado en suelos: a) el potencial secuestro de emisiones de $CO_2$ asociado para mitigar el cambio climático y b) la gran oportunidad que supone el biocarbón para contribuir hacia una bio-economía regenerativa. En la última década, se ha incrementado la proporción de investigaciones relacionadas con el biocarbón, donde predomina la ejecución de experimentos con cultivos específicos. Estas investigaciones muestran que la aplicación de bio-carbón ofrece oportunidades prometedoras para incrementar sustancialmente la eficiencia del suelo en numerosos campos de aplicación ya que se crean mejoras cualitativas tanto a nivel microbiológico como físico, y se logra cerrar el ciclo de los materiales.

El biocarbón obtenido por conversión termodinámica de biomasa bajo condiciones anaeróbicas, desencadena propiedades particulares cuando es aplicado en el suelo, tales como: a) reducción de gases de efecto invernadero, b) mejoramiento de las propiedades microbiológicas y fisicoquímicas y c) absorción de sustancias perniciosas. El uso de biocarbón en Aguascalientes, un estado con clima semidesértico en México que sufre de escasez crónica de agua, podría incrementar la capacidad hídrica del suelo, incrementar la disponibilidad de agua para las plantas, así como reducir la sobreexplotación de los acuíferos. El biocarbón puede ser generado a partir de una gran variedad de materias primas de biomasa, debido a que Aguascalientes posee cantidades sustanciales de ésta como un potencial inexplotado.

Se realizó un análisis cuantitativo de materias primas adecuadas; el cual contenía 11 especies diferentes de biomasa leñosa y se produjeron 4 tipos diferentes de biocarbón, mediante un horno cortina de llama Kon-Tiki en el estado de Aguascalientes, México. Las características fisicoquímicas de los biocarbones se

compararon contra criterios establecidos por el Certificado Europeo de Biocarbón (European Biochar Certificate, EBC por sus siglas en inglés) para evaluar la calidad y garantizar que su aplicación no supone ninguna amenaza. Todos los biocarbones producidos cumplieron con los requisitos básicos de calidad, tres calificaron con calidad premium. La capacidad de retención de agua varía en un rango de 149 a 254% masa de materia seca con superficie específica (BET) de 54 a 305 g $m^{-2}$, lo que se relaciona con los valores prestablecidos por la literatura y corrobora la probabilidad de incrementar las capacidades hídricas del suelo.

Para evaluar la magnitud del incremento de la capacidad de retención de agua en suelos agrícolas, tres suelos diferentes presentes en Aguascalientes: calcisol, phaeozem y cambisol, se mezclaron en diferentes proporciones con los cuatro tipos de biocarbón producidos localmente bajo condiciones replicables y así la capacidad hídrica máxima ($WC_{max.}$) fue determinada. Pequeñas cantidades de biocarbón (proporción de biocarbón : suelo 1 : 100 [g/g] = 13 $t_{BC}$ $ha^{-1}$) incrementaron la capacidad hídrica máxima en el rango de 1.4 a 8.1 %, a diferencia del control dónde cantidades superiores a 80 $t_{BC}$ $ha^{-1}$ (= ratio 1:15) obtuvieron un incremento en la capacidad hídrica máxima en el rango de 6.6 a 11.8 %. Una evaluación micro-económica de estos datos indica que es benéfico aplicar biocarbón como una estrategia para mejorar el suelo en Aguascalientes.

A pesar de la alta calidad (certificada por el EBC), y la pruebas prometedoras sobre la capacidad hídrica, los biocarbones contienen cantidades significativas de sustancias peligrosas, como hidrocarbonos aromáticos policíclicos, dibenzeno-p-dioxinas y dibencenofuranos policíclicos, bifenilos policlorados y metales pesados, que pueden inducir efectos adversos si es aplicado incorrectamente en el suelo. Comparado con los niveles máximos permitidos de EBC, el contenido de metales pesados indica que la materia prima no es crítica, con valores muy por debajo de los límites. Los compuestos tóxicos más relevantes en el biocarbón se considera que son PAH-16, ya que se sabe que son carcinógenos si entran en la cadena alimenticia y pueden afectar el crecimiento de la planta (negativamente). Valores agregados de PAH-16 detectados en cada tipo de biocarbón producido variaron entre 0.7 y 5.3 mg $kg^{-1}$, un rango no crítico que se encuentra dentro de los valores permitidos. De acuerdo con la Organización Mundial de la Salud (WHO por sus sigals en inglés), las concentraciones agregadas en factor de equivalencia tanto para PCDD/Fs (< 0.35 mg $kg^{-1}$) y PCBs (< 0.4 μg $kg^{-1}$ $88\%_{DM}$) están por debajo de los valores de EBC permitidos. Sin embargo, contienen sustancias que pueden afectar organismos vivos.

Pruebas de toxicidad en las cuales organismos vivos, como los invertebrados acuáticos, son expuestos a contaminantes bajo condiciones de laboratorio, se ha vuelto una herramienta poderosa en los últimos años. El propósito de estas pruebas de toxicidad es obtener una visión apropiada, que pueda informar/concientizar a los que toman decisiones y a quienes las ejecutan sobre los niveles de toxicidad y el riesgo asociado creado por actividades antropogénicas en los ecosistemas. Por lo tanto, dichas pruebas eco-toxicológicas suponen una herramienta sustancial para evaluar el potencial de toxicidad de una mezcla compleja de tóxicos típicamente encontrada en el biocarbón.

Para evaluar la toxicidad de los biocarbones en organismos no-diana, se realizaron pruebas de toxicidad en cuatro especies de invertebrados bénticos y zooplanctónicos: a) el ciliado *Paramecium caudatum*, b) el rotífero *Lecane quadridentata*, y c) los cladóceros *Daphnia magna* y *Moina macrocopa*, utilizando elutriados de biocarbón puro obtenidos del biocarbón previamente mencionado. La respuesta a la toxicidad por parte de los invertebrados a los elutriados de biocarbón en un ambiente controlado de laboratorio ha sido analizada, para estimar las concentraciones letales dónde 50% y 10% de los animales sometidos a la prueba mueren ($LC_{50}$, $LC_{10}$) y definir la concentracion máxima donde no hay difereencias con el control (NOEC por sus siglas en inglés) y la concentración mínima donde hay diferencias con el control (LOEC por sus siglas en inglés).

Subsecuentemente, se calculó el rango de toxicidad debida a la concentración de los cuatro biocarbones en las pruebas ambientales, utilizando las ecuaciones obtenidas de las pruebas de toxicidad.

En pruebas de toxicidad aguda y crónica, no se detectaron efectos agudos en ciliados pero sí significativamente letales para rotíferos y sustancialmente letales para los cladóceros; con valores de $LC_{50}$ debajo del 25% de concentración efectiva (CE) para los rotíferos y $LC_{50}$ debajo del 306% CE para los cladóceros. Esta toxicidad letal puede deberse a la ingestión/digestión de biocarbón y las sustancias tóxicas presentes en él mediante procesos enzimático/mecánico de los cladóceros y rotíferos; el análisis fotográfico muestra el tracto digestivo de los organismos analizados completamente lleno de partículas de biocarbón. Solamente se detectó una toxicidad aguda si los organismos eran expuestos a elutriados de biocarbón puro. Cuando los organismos eran expuestos a elutriados obtenidos de una mezcla biocarbón : suelo en proporción 1:8 (v/v), se observaron efectos no crónicos ni letales en todas las especies analizadas.

Los resultados muestran que la proporción aplicada tiene una influencia decisiva en la biota del suelo. Si los usuarios siguen estándares que regulen la adición de biocarbón en el suelo, los potenciales efectos negativos en rotíferos y cladóceros pueden ser ampliamente reducidos. No obstante, el uso adecuado del biocarbón certificado no garantiza un 100% seguridad, particularmente en hábitats sensibles cuando se utiliza el biocarbón como alimento animal. Esta información indica que el uso de las pruebas toxicológicas en organismos vivos es una herramienta importante para evaluar la toxicidad de los biocarbones en el ambiente, especialmente cuando se aplica a biomasas vulnerables, y los usuarios deben adecuarse lo más cerca posible a los valores cuantitativos establecidos.

# 2 Abstract

Recent research studies reveal the multi-dimensional positive impacts of biochar or biochar based substrate application in soil, the associated $CO_2$ sequestration potential to mitigate climate change and the great chance of biochar to contribute towards a regenerative bio-economy. Pertinent fundamental biochar related research with an increasing rate has been conducted in the last decade, whereby predominantly plot trials with selected crops were executed. Tenor of these investigations is that the application of biochar offers promising chances to sustainably increase soil efficiency in numerous fields of application whereby qualitative microbiological and physical improvements as well as the closing of materials cycles is achieved.

Biochar obtained by thermochemical conversion of biomass under anaerobiosis triggers particular effects when applied to soil, such as: greenhouse gas emissions reduction, improvement of physicochemical and microbial properties, and absorption of pernicious substances. The use of biochar in Aguascalientes, a semidesertic State in Mexico that suffers from chronic water paucity, could increase soil water capacity, improve plant available water and reduce aquifer overdraft. Biochar can be produced from a broad variety of biomass feedstock, whereby Aguascalientes possesses a substantial quantity of untapped biomass potential.

Following a quantitative analysis of adequate feedstock, comprising 11 woody biomass species, four different biochars were generated using a Kon-Tiki flame curtain kiln in the state of Aguascalientes. The biochars physicochemical characteristics were analyzed against criteria set by the European Biochar Certificate to assess quality and increase probability for hazard-free application. All biochars produced fulfilled basic quality requirements, three qualified for premium quality. Water holding capacity ranged from 149 to 254 mass % in dry matter with specific surface (BET) of 54 to 305 g $m^{-2}$, which correlates to literature

default values and corroborates the probability to increase soil water capacities. To assess the magnitude of increasing soil water capacity of agrarian soil three different soils from Aguascalientes, a calcisol, a phaeozem and cambisol have been blended in different ratios with the four locally produced biochars under reproducible conditions and the Maximum Water Capacity ($WC_{max.}$) [also known as Water Holding Capacity, short WHC) of the substrates was determined. Already small quantities of biochar (ratio biochar : soil 1:100 [g/g] = 13 $t_{BC}$ $ha^{-1}$) increased the $WC_{max.}$ in the range of 1.4 to 8.1 percentage in contrast to the control, whereby quantities above 80 $t_{BC}$ $ha^{-1}$ (= ratio 1:15) obtained an augmentation of $WC_{max.}$ in the range of 6.6 to 11.8 percentage. Cost-benefit evaluation of these data indicate that it can be beneficial to apply a biochar based soil amendment strategy in Aguascalientes, particularly in high water cost realms.

Despite the high quality (certified by European Biochar Certificate, short EBC), and the promising $WC_{max.}$ tests the biochars contain substantial quantities of hazardous substances, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and heavy metals, which can induce adverse effects if wrongly applied to the environment. Compared to the EBC thresholds, all heavy metal contents indicated uncritical biomass feedstock, with values far below the limits. The most relevant toxic compounds in biochar 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. Aggregate values for the PAH-16 detected in each type of biochar produced ranged from 0.7 to 5.3 mg $kg^{-1}$, which are uncritical values within the allowed threshold. Based on WHO equivalency factor aggregated concentrations both for PCDD/Fs (<0.35 ng $kg^{-1}$) and PCBs (<0.4 μg $kg^{-1}$ 88%DM) are below the permitted EBC thresholds, however, still contain substantial content to endanger living organisms.

Toxicity tests in which live organisms, such as aquatic invertebrates, are exposed to contaminants under laboratory conditions, has become a powerful tool in the past years. 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. Hence, eco-toxicological tests provide a substantial tool to assess the toxicity potential of a complex mixture of toxicants typically found in biochar.

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 pure biochar elutriates generated from the aforementioned biochar. The toxic responsiveness of the invertebrates to the biochar 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 biochars in the environmental samples, using the equations obtained from the toxicity tests, was calculated. In acute and chronic toxicity tests, no acute toxic effect to ciliates, but significant lethality to rotifers and substantial lethality to cladocerans with $LC_{50}$ values below 25% effective concentration (EC) for rotifers and $LC_{50}$ below 306% EC for 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, as photographic analysis showed digestive tracts of the test organisms fully filled with biochar particles. Acute toxicity was only detected if the organisms were exposed to the pure biochar elutriate. When the organisms were exposed to elutriate obtained from a biochar : soil mixture in ratio 1:8 (v/v), 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 biochar additions to the soil, the potentially harmful effects on rotifers and cladocerans can be most widely diminished. Nonetheless, the compliant use of certified biochar does not guarantee 100% safety, particularly near sensitive habitats or with regard to biochar utilization in animal feed. These data indicate that it is instrumental to use toxicity tests with living organisms to assess biochars toxicity to the environment, especially when applied at vulnerable biomes, and that applicants should stick closely to the quantitative set-point values.

# 3 Introduction

## 3.1 Biochar

Within the landscape of infertile soils (Ferralsols, Acrisols, Lixisols and Arenosols) in central Amazonia, small islands of highly sustainable fertile soils known as Terra Preta do Indio occur in patches averaging approximately 20 ha. In the 16$^{th}$ century, first European Conquistador Francisco de Orellana undertook an expedition from east to west on Amazonas River. He reported from high cultures with prosperous and flowering agriculture, outstanding soil fertility and settlement with more than 100,000 inhabitants. *Terra Preta* soils have on average three times higher soil organic matter (SOM) content, higher nutrient levels and a better nutrient retention capacity than surrounding infertile soils. Radiocarbon dating indicates that these soils were formed between 7,000 and 500 cal yr BP and are of pre-Columbian origin. The *Terra Preta* soils were generated by pre-Columbian native populations by chance or intentionally, adding large amounts of charred residues (biochar), organic wastes, excrements and bones. It still remains a matter of speculation whether these soils were made intentionally or resulted as a by-product of human occupation. What is known, however, is that *Terra Preta* soils have been under continuous agricultural use for centuries. *Terra Preta* soils exhibit approximately three times more soil organic matter, nitrogen and phosphorus and 70 times more charcoal compared to adjacent infertile soils [1–3]. Biochar (BC) plays a decisive role in the formation processes of *Terra Preta*, besides the incorporation of organic matter and nutrients as well as the growth of particular micro-organic flora and fauna.[4] Biochar can be considered as the skeleton of *Terra Preta*.

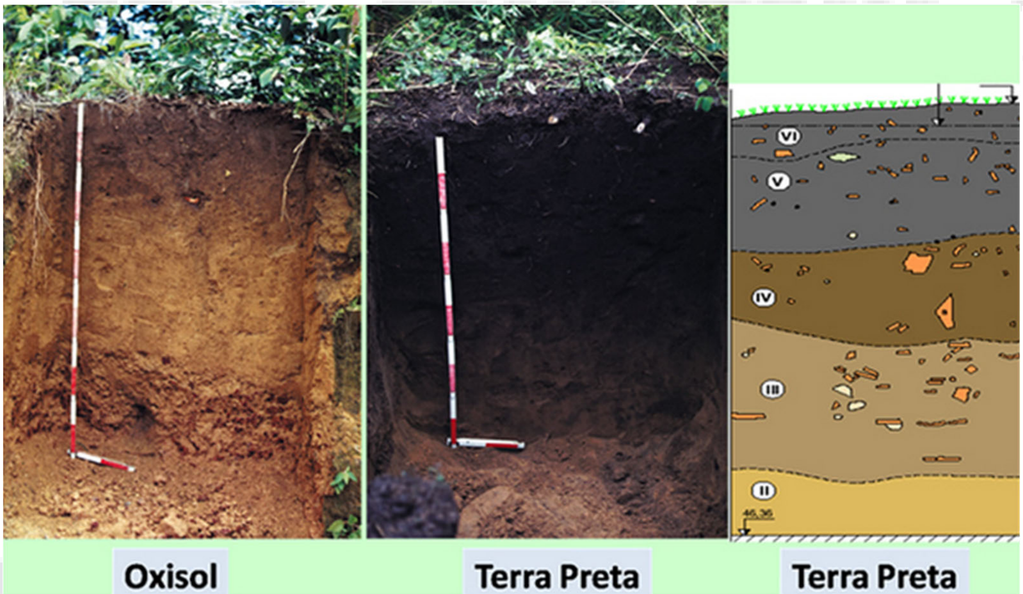


**Figure 1.** Illustration of Oxisol (left) and *Terra Preta* soil (center) typically found in central Amazonia. Terra Preta soil profile (right) containing shards and broken fragments of pottery vessel as indicator for anthropogenic activities.[1]

Biochar is produced by thermochemical conversion of different types of biomass including agricultural waste (invasive plants, crop residues), animal manure and woody biomass (tree cuttings) in an oxygen-limited process (e.g. slow pyrolysis, fast pyrolysis, hydrothermal carbonization, flash carbonization, torrefaction, and gasification) [5,6]. Despite the fact that almost all types of biomass are suitable for biochar production, various studies have shown that by tendency a higher lignin content results in larger wood to biochar efficiency; the same rule applies to the dry matter content, whereby less residual water in the biomass results in larger biochar output [5,7]. Carbon content of biochar is usually above 50 % [8,9] and is typically 70 % – 80 % (except for biochar derived from sewage sludge, paper sludge, manure and bones). In addition to carbon, biochar is composed of oxygen, nitrogen and other elements. Biochar is relatively stable and can be preserved for hundreds of years, due to its alkyl and aromatic compounds [9–11].

Within the last decade, research on the dark, fertile, charcoal-rich anthropogenic Terra preta soils of Amazonia [1,12] 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, adds several advantageous features if applied to soil, such as: (a) Greenhouse gas emissions reduction (e.g. $CO_2$, $CH_4$ and $N_2O$ provoked by soil-biota); (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 [9,10,13–15]. Biochar benefits agriculture, livestock farming, economy and the environment.

The application of biochar to soils is a potentially valuable agricultural practice that affects soil physicochemical properties and improves soil microbial health [6,16]. Moreover, biochar enhances soil fertility, by increasing soil nutrients, such as K and Mg [17–19], cation exchange capacity, porosity, density, moisture as well as regulating soil pH [10,17]. Among many other beneficial properties, the high inner surface area of biochar is of particular importance in regard to water storage [20–22] owing to its high porosity [23] and its large number of micro pores [24]. Application of biochar to the soil has the potential to improve soil properties [25,26] to create a better environment for plant root growth, root penetration, and nutrient and water uptake. Biochar intra- and interpores significantly increase field capacity, permanent wilting point and plant available water[27]. The physical properties of the biochar depend on the characteristics of the biomass feedstock and the conditions of the thermochemical conversion [11,28–30], besides the type of reactor in which pyrolysis takes place [31]. Application of biochar can improve the chemical properties of soil, whereby microbe and enzyme functions which enable complicated biochemical processes are enhanced. These processes stimulate the material cycle and energy flow of inorganic and organic matter in soil [32].

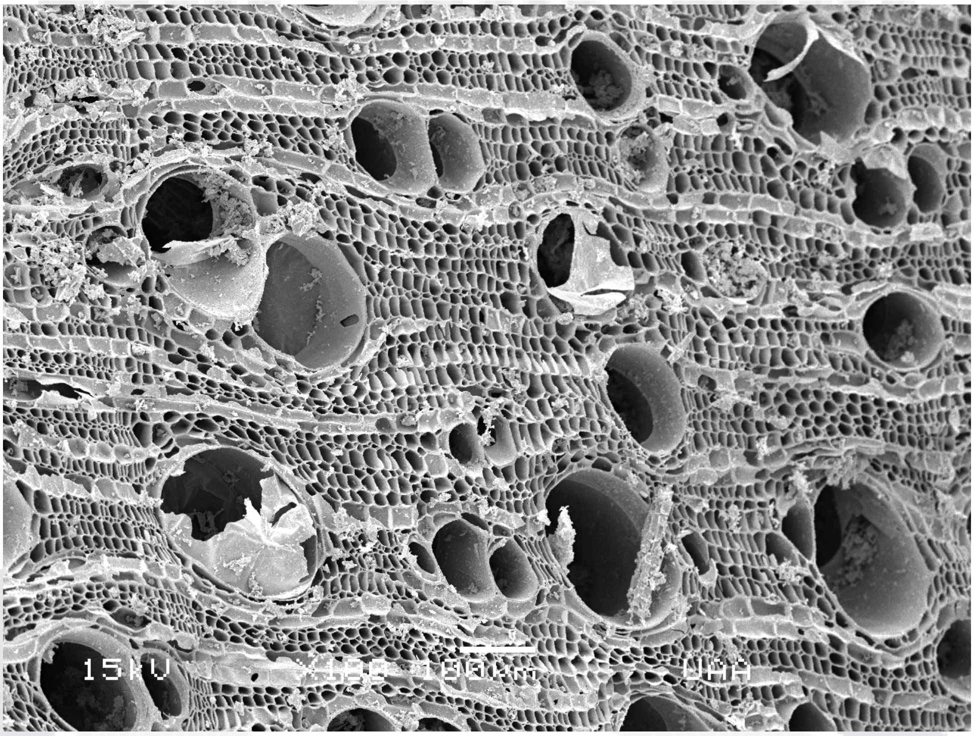


**Figure 2.** Porous honeycomb structure of biochar generated from manchineel tree and pine (60/40) mixed wood produced with Kon-Tiki flame curtain kiln in Aguascalientes. Electron micrograph: 15 kV zoom × 100, Universidad Autónoma de Aguascalientes, November 2017.

Biochar influences physical properties of soil, such as soil porosity, compaction, density, permeability and water content [26,28,32,33]. Adding biochar to the soil improves the biochemical conversion processes, increases the water holding capacity [34] and enhances mineral nutrition for the development and reproduction of microbes [35,36].

Biochar increases soil water, air and nutrient levels [37]. Biochar significantly increased the water holding capacity (WHC) of the sandy soil due to its porous nature [17,37,38]. In particular, biochar changes soil WHC by altering soil porosity and agglomerate level [17]. Soil water content increases with the amount of biochar applied, because of its physical characteristics. At the largest biochar-to-soil ratio (i.e. 2:3), WHC increased up to 56 % [32]. However, increased WHC does not implicitly improves plant available water (PAW), only if intra-particle pore radii of biochar is predominantly above 0.01 mm whereby related capillary forces of the char are smaller than the plant suction capacity. Therefore biochar should be grinded before its application to field, in order to improve intra-particle porosity [39]. In this case a 4-50 % increase in PAW was reported [40].

Biochar research sharply increased within the last ten years; however, biochars can be tremendously different produced from a broad range of different types of biomass and thus serve different purposes and trigger unwanted effects [41–43].

### 3.2 Status Quo on biochar related science

Soil fertility, here defined as the inherent ability of a soil to reliably deliver high and constant crop yields at adequate fertilizer input, and environmental pollution costs, is vulnerable especially in non-temperate Mexico. With accelerating global warming (cp. IPCC) rising temperature and extreme weather events decomposition and loss of soil organic carbon (SOC) will increase, on top of other pathways of human-induced soil degradation [14,44].

In addition, soil productivity needs to be increased to double the food production until 2050 [44]. Thus, agricultural practices that actively increase soil fertility and resilience as well as (different) soil organic carbon stocks are urgently required. Nitrogen fertilizer use in agriculture is often in excess of demand resulting in a low N use efficiency of crop production with considerable room for improvement if N delivery during the respective crop growth stages is optimized. Humankind annually introduces more reactive N into the global N cycle than natural pathways, mainly via the energy-consuming Haber-Bosch process used in N fertilizer production [45]. As a consequence, rising amounts of reactive N are “pumped” through the “N transformation pipeline” into soils, sediments and aquatic ecosystems, increasing N leaching to groundwater and rivers, and nitrous oxide ($N_2O$) emissions to the atmosphere where it contributes to global warming, tropospheric ozone increase, and stratospheric ozone depletion [46]. Ultimately, innovative agricultural strategies are necessary delivering continuous carbon-sequestration, reducing N leaching and GHG emission (per unit of food or bioenergy produced) and enhancing the efficiency of nitrogen use, to ensure positive effects of the agricultural sub-system towards climate protection. Biochar is considered one of these strategies.

The current state of knowledge regarding biochar can be resumed as follows. Average yield increases with pure biochar application range from significant but moderate (on average +10%) in temperate soils [47], to good e.g. in drought-prone soils where it may have improved plant water supply (e.g. in Germany [48] and Italy [49,50]), up to dramatically positive in acidic weathered tropic soils (e.g. maize in Zambia: [51]). No yield effect findings, e.g. when soils are already fertile, have been reported, too. In the case of Mexico, unfortunately biochar application is rarely to not considered as an agricultural beneficial practice [52].

A range of environmental concerns can be addressed by biochar or biochar-compost use in soils. Considerably reduced nitrate leaching was observed in apple orchards over 1.5 years [53] or with Riesling grapes grown in sandy soil amended with biochar-compost. The reduction of $N_2O$ emissions is a frequent finding that was significant in a recent meta-analysis [54]; reductions were even observed when biochar was mixed with compost at high water saturation [15]. Anderson et al. [55] and Kolton et al. [56] reported that growth in biochar-amended soil media promoted plant growth-promoting bacteria; likewise, Terra preta soils have a higher microbial biodiversity [57,58]. Thus, biochar use in soils can have positive effects directly or indirectly via the four pathways 'improved soil water supply and soil aeration', 'improved nutrient retention and use efficiency' (including reduced GHG emissions), 'soil microbiota' and 'soil organic carbon build-up'. Effects may partly be achieved by soil organic matter formation accelerated by, and in addition to, the stable pyrogenic carbon delivered by the biochar amendment itself. Several soils containing pyrogenic carbon are quite fertile (anthropogenic as well as natural) and developed larger stocks of non-pyrogenic soil organic carbon [1,59,60]. Liang et al. [61] investigated the decomposition of labile 13C-labelled organic matter in Terra preta and adjacent non-Terra preta soils, and observed significantly reduced total C mineralization, more rapid incorporation of litter-derived (labile) C into the stable organo-mineral fraction, corroborating the synergistic C sequestration of labile, non-biochar-derived C in addition to the stable, biochar-derived C. This may be connected to a shift in the microbial community towards a greater abundance of fungi [12] which has also been observed several times in biochar-amended soils when residues are incorporated [62]. Pyrogenic biochars are mostly very stable in soils, with much longer (decennial to centennial) residence times than any other SOC fraction [60,63–65], and they are much more stable than other C amendments such as un-carbonized residue, or hydro-char produced via hydrothermal carbonization, a brown-coal-like material [66–68]. If biochar promotes the formation of soil organic carbon stocks besides just the pyrogenic carbon, as suggested by the results of e.g. Liang et al. [61] or Jindo et al. [69], its true potential will not always become immediately visible. Thus, it clearly requires long-term field studies, where the indications for such changes in soil functions, C and N pools and C and N cycling and biodiversity are dedicatedly investigated. Furthermore, the young field of soil-biochar research just starts to connect the multitude of different biochar types, properties and pre- and post-treatment options ('biochar systematics') to its respective potential and (desired) functionality in soils and animal husbandry.

Although the existence of *Terra preta* soils already suggests a beneficial combination of biochar and nutrient-rich organic materials/wastes, most research projects started out with pure biochar amendments [47]. Until recently, more results were available for tropical and subtropical than temperate soils and the results are highly dependent on the applied type of biochar and amended soil. Biochar use in composting may also offer several co-benefits for improving the quality of the compost or composting process [69–72]. The subsequent plant growth was significantly improved with biochar-composts with increasing biochar concentrations in the mixture [73], due to nutrient loading of the biochar [74], e.g.

by N retention [70]. Biochar has been proved to adsorb $NH_3$ which was subsequently plant-available when the loaded biochar was incorporated into soil [75]. One of the largest economic but scientifically nearly unexplored potentials is the use of biochars in animal husbandry, with subsequent delivery of the biochar-treated manures, slurry or bedding material to soils.

However, biochar is also a valuable energy carrier worth currently ~300 to 1,000 EUR/t, depending on its quality and post-treatment. For example, medical activated carbon (AC) is very expensive (e.g. for use in animal husbandry), but can likely be substituted by even more effective post-treated EBC-certified biochar, which is not only better characterized than medical AC, but also considerably cheaper. Another example for a novel effective biochar implementation strategy may be the use of biochar-based clay-mineral composite fertilizers that significantly increased grain yield at considerably reduced N input rates [76]. The amount of biochar, a few hundred kilograms per hectare, was much lower than in almost all field trials set up in Europe so far [76]. Thus, well designed biochar products plus cascading use implementation strategies will likely enable economically feasible, value-generating biochar use without further subsidies, enabling society as a whole to 'harvest' long-term ecologic benefits [77]. In summary, to date implementation of biochar use in agriculture is often hampered by (a) lack of predictability of beneficial effects due to limited mechanistic understanding, (b) mismatch of biochar type and its intended function in soils or land management system, (c) lack of proven evidence on the positive micro-economic impacts of biochar application and (d) lack of knowledge exchange between farmers and scientists. If we are able to optimize economically as well as ecologically the beneficial use of biochar in agriculture, we may have the long-term potential to turn soil management strategies from being part of the problem to being part of the solution [78].

### 3.3 Toxicological potential of biochar

Reports concerning dioxins, furans and polycyclic aromatic hydrocarbons in the environment and charred foods constantly meet high media interest and unsettle consumers. Everyone knows the black on the grill meat or the charred on the bread. Cited in a technical jargon, these compounds are the heterocyclic aromatic amines (HAA[1]) and polycyclic aromatic hydrocarbons (PAH). For example, the PAHs form when fat or marinade drips from the meat into the embers. However, there are also other origins. Dioxins and furans, however, arise in different thermochemical processes and occur in particular as feed contaminants as an unwilled by-product in the production of feed and food. In addition to local sources leading to impurities of feed and food, the diffuse pollution of the environment with dioxins, furans and PAHs still plays a major causative role. This chapter provides short insight into the world of these pollutants, defines and classifies them, explains their origin, classifies their toxicological relevance with regard to biochar. A special emphasize in this work is set to the relation of PAHs and PCDD/Fs to biochar, due to the fact that the biochar topic experiences increasing attention in the scientific community within the last decade and as a consequences thereof new developed standards in order to guarantee hazard-free application to the environment touch the present topic.

Oleszczuk et al. [79] state, that pollutants in biochar can result toxic to organisms during environmental application of biochar. When toxic potencies of biochar 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 biochar containing compounds that are bioavailable and phytotoxic [80]. 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 biochar, especially when used in the vicinity of sensitive

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[1] HAA are generally produced during the roasting process, e.g. also when frying in the pan. Carcinogenic amines are formed especially during very hot and long crickets.

habitats, such as water bodies or nature reserves. Concerning these arguments, the harmlessness (respectively the quality) of biochar cannot be assessed solely by analyzing physicochemical parameters.

PAHs are a large chemical substance group that has been the focus of science and public attention for decades because of its problematic properties for humans and the environment. PAHs consist of multi-membered rings of carbon and hydrogen atoms (usually benzene rings), which are joint by shared edges [81].

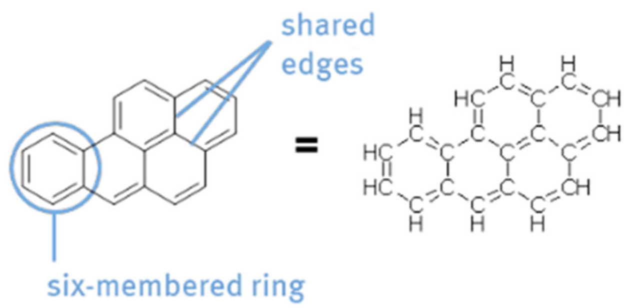


**Figure 3.** The structure of PAHs, using the example of benzo[a]pyren. Adapted [81]

The atomic PAHs group is divided into "lighter" and "heavier" PAHs, with the lighter PAHs con-sisting of two to three rings and the heavier PAHs of four to seven rings. The smallest compounds of the group of substances consist of two rings (for example naphthalene), the largest of seven rings (e.g. coronene). Due to the variety of combinations of the rings, there are many different PAHs present. It is assumed, that more than about 10,000 different compounds exist. PAHs are solid at room temperature. The lighter PAHs are volatile, hence they are easily released into the gaseous state. As the number of rings increases, the molecular weight also increases and thus the volatility decreases. PAHs are nonpolar substances; hence they dissolve badly in water, but good in fats and oils. Also, PAH adsorb well to dust or soil particles. Again, this depends on the molecular size [43,81].

Many PAHs occur in variable matrices. That is why chemical agents often identify "representatives" of a group of substances. Back in 1977, the US Environmental Protection Agency (EPA) added 16 PAHs to the list of "priority pollutants" within the US Clean Water Act. These 16 PAHs are included in the list because they have been classified as highly toxic and readily chemically detectable, contain a wide range of possible structures and are found to be proportionally abundant in aquatic bodies. In chemical analyzes, the totality of all 16 compounds is usually determined to assess the PAH contamination hazard of products. Benzo [a] pyrene serves as the lead compound for the highest hazard potential, meaning that it is representative of all other PAHs [82].

PAHs belong to the PBT substances. These are substances that are both persistent (i.e., degrading poorly or not at all degrading in the environment), as well as bio-accumulative (i.e., accumulating in organisms) and toxic (i.e., produce adverse effects). This combination of properties is considered particularly critical in ecotoxicology, the science that deals with the impact of chemicals on the living environment. Once released into the environment, such substances remain for a very long time, ac-cumulate and can develop their toxic effect over a longer period of time. In addition, many PAHs also have a carcinogenic effect and are therefore among the "CMR" substances (C) carcinogenic, (M) mutagenic and (R) reprotoxic). PAHs can be absorbed into the body through various routes, such as through the respiratory tract, through smoke or contaminated dusts (by inhalation), through food (orally), or through dermal contact. The fact that PAHs are considered ubiquitous, meaning they are found almost everywhere in the environment, makes them in combination with their particular properties, a serious problematic group of substances. “You can find PAHs in every corner of the Milky Way, "says Douglas Hudgins of NASA's Ames Research Center. PAHs always occur as a mixture of many hundreds of individual compounds. Depending on the source, the composition may differ, resulting in a particular "profile" of single PAHs that differs in type and content of each PAH from other sources [83].

Every combustion process of organic material or petrogenic sources, be it wood, coal, oil, diesel or tobacco triggers the generation of PAHs. The lower the temperature during combustion, the more incomplete oxidation occurs and the more PAHs are potentially generated. But PAHs can occur in different pyrolysis phases, such as in carbonization, reduction and aromatization, when char is mainly generated in high absence of oxygen at low temperature (250°C), whereas gas reduction accelerates the generation of polycycles within pyrosynthetic phase where gases and tar are mainly produced at high temperature (up to 750°C) and augmented oxygen environment. The following Figure 4 illustrates the different phases of PAHs generation in pyrolysis. It is assumed that PAH formation in biochar occurs predominantly via gas phase pyrosynthesis which occurs principally at higher temperature, hence, in order to generate PAH-narrowed biochars, any condensation phases of gas and tar onto the end product should be avoided during production [43]. This is the case for instance when using a Kon-Tiki flame curtain kiln (cp. chapter 7.2) [7].

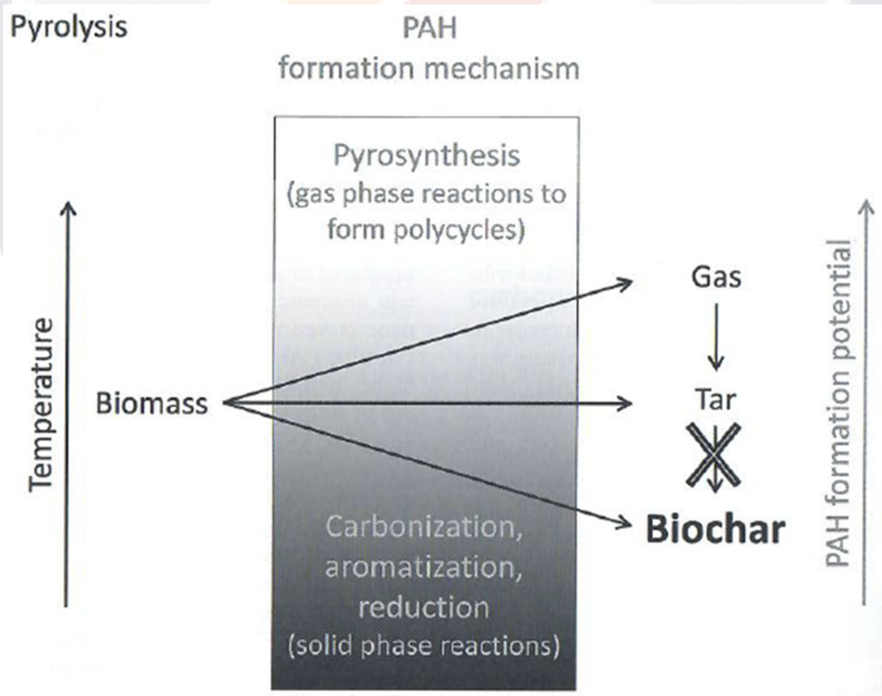


**Figure 4.** Main products of biomass pyrolysis and presumed major PAH formation processes associated with it.[43]

Even though PAHs are considered potentially toxic, also they can be utilized under suitable conditions by soil microorganisms as carbon and energy source and become partially or fully mineralized [84,85]. PAHs with two and three aromatic rings are considered to be readily biodegradable. In contaminated site assessment, 27 bacterial strains are known which are able to completely break down PAHs with more than 3 rings, such as fluoranthene, fluorene, pyrene and benzo [a] anthracene, or to co-metabolize without gaining energy. However, only a very limited number of bacterial strains have been isolated, which are even able to use PAK with five or more rings as a growth substrate [84]. By adding biochar to soil mobility and thus the availability of higher molecular weight aromatics can be reduced [86].

Besides its hazard-potential attributable to its potential contaminates biochar on the other hand, due to its strong ad- and absorption potential, because of its high inner surface, is also able to positively affect PAHs availability in soils. In a Germany-wide study with field trials in the Lausitz, it could be shown that soils with the described PAH contamination could significantly reduce mobility and bioavailability of the pollutants when biochar based substrates where applied [87]. As a result, the eco-toxicological potential of PAHs, as well as the risk of uptake into the plant and the potential for displacement into the groundwater, have increasingly diminished.

PCDD/Fs are two groups of chemically similar chlorinated organic compounds. They are among the oxygen-containing derivatives of halogenated hydrocarbons and are in common usage. PCDD/Fs are hydrocarbons in which at least one hydrogen atom is replaced by a halogen (e.g., fluorine, chlorine, bromine, iodine). The structural formula of the dioxins consists of two benzene rings which are bridged by an oxygen atom (so-called "ether bridge"). In the case of the furans, the structural formula is similar, whereas two benzo rings are bridged by an oxygen atom and are connected directly. There are currently 75 diverse isomers known as dioxins and 135 diverse isomers as furans. The following figure illustrates the generic structure of dibenzodioxins and dibenzofurans [88]. PCDD/Fs belong to the POP substances. They are (P) persistent (difficult to not biodegradable), (O) organic (in organisms enriching) and (P) pollutant (semi-volatile). 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.

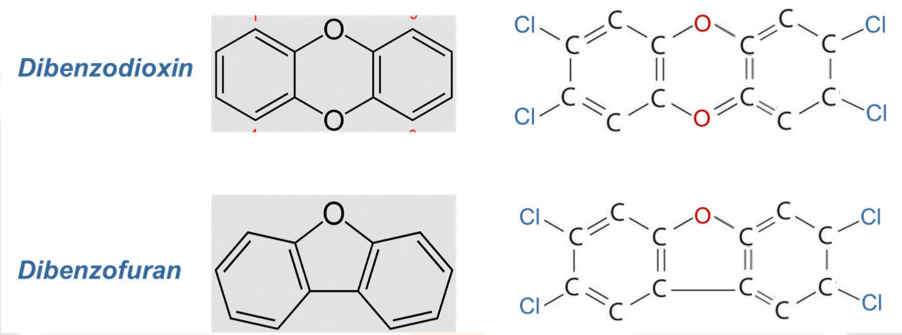


**Figure 5.** Generic structure formula of Dibenzodioxin (TCDD - 2,3,7,8-Tetrachlordibenzodioxin[2]) and Dibenzofuran (2,3,7,8-Tetrachlordibenzofuran). Own illustration [88].

Dioxins first gained worldwide recognition due to the chemical accident in Seveso (Italy) in July 1976. Since that day, the public is aware of the potential danger of dioxins and it is reported more intensively on dioxin disasters, however, dioxin-like substances were already produced and used in the 1940s, especially in agriculture. Some long term consequential damages are due to these applications recognized today. Dioxins may be produced as undesirable by-products within the chlorine chemistry and thus be included as impurities in chemicals and products, e.g. in pentachlorophenol and other organochlorine pesticides. In addition, dioxins are produced in combustion processes in the presence of chlorine and organic carbon, in particular at temperatures of 300 to 400 °C, whereas at a temperature level of 900 °C, the chlorine-based pollutants are destroyed.

[2] The most toxic dioxin is the 2,3,7,8-tetrachloro-dibenzo p-dioxin (2,3,7,8-TCDD), which has been known since the accident at Seveso in July 1976 and since then is also known as "Seveso Poison".

The intake can occur inhalative (dust, smoke), oral (food) and dermal. However, due to their ubiquitous availability, most PCDD/Fs reach humans via the air path. PCDD/Fs are planar and hydrophobic, lipophilic and quickly accumulate in adipose tissue as they enter the food chain. Traces of polychlorinated dioxins and furans are found all over the world. Via the food chain dioxins accumulate in living organisms, in vertebrates especially in the liver as the detoxification organ of the metabolic cycles. Humans absorb dioxins mainly from animal foods (fish, meat, eggs, and dairy products). The World Health Organisation (WHO) determines the Tolerable Daily Intake of TCDD equivalent at 1-10 pg l-TEQ/kgBW. The following table illustrates the maximum thresholds of TCDD for different animal products set by the European Union. Important to mention is, that due to the high toxicity potential of PCDDs, the limits are at picogram.

**Table 1.** Thresholds of TCDD for different animal products set by the European Union in $pg/g_{Fat}$.

| Animal Product | Threshold [$pg/g_{Fat}$] |
|---|---|
| Pork meat | 1.0 |
| Chicken | 1.7 |
| Milk Products / Eggs | 2.5 |
| Fish | 3.5 |
| Liver | 4.5 |

**Source:** Ordinance (EU) Nr. 1259/2011; Federal Institute for Risk Assessment Germany

Humans also get in contact with contaminated products being exposed to sediments or organic substances such as sewage sludge. As persistent organic pollutants, they are hardly degraded in the environment. Despite their persistent structure, dioxins and furans are photolytically degradable.

The collective term "dioxins" is usually used with a few exceptions for polychlorinated dioxins and furans (PCDD / Fs). However, PCDD/Fs should not be confused with polychlorinated bi-phenyls (PCBs). These belong to another, similarly constructed group of substances. Due to the same human toxicological effect PCDD/Fs and PCBs are often evaluated together and wrongly used in the same context. Considering aforementioned toxicity potential, scientific statement on environmental safety and proven ecological advantages and disadvantages and explanation of underlying processes for biochar application are urgently needed.

### 3.4 Quality assurance and environmental risk mitigation

Within the last six years, considerable progress has been made with regard to standardization, pyrolysis techniques, quality, and sustainability control of biochar. In 2012 the European Biochar Foundation established an accredited control system (ACS) for biochar 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 [89]. Together with the United States (US) induced International Biochar Initiative (IBI) [90], EBC led to considerable improvements in biochar 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, biochar, 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 [7,89,91,92]. Potential contamination of soils when using biochar 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 biochar is generally limited, as the hazardous substances contained in biochar are strongly bound, due to the high adsorption potential caused by the porous structure and increased redox potential [43,93].

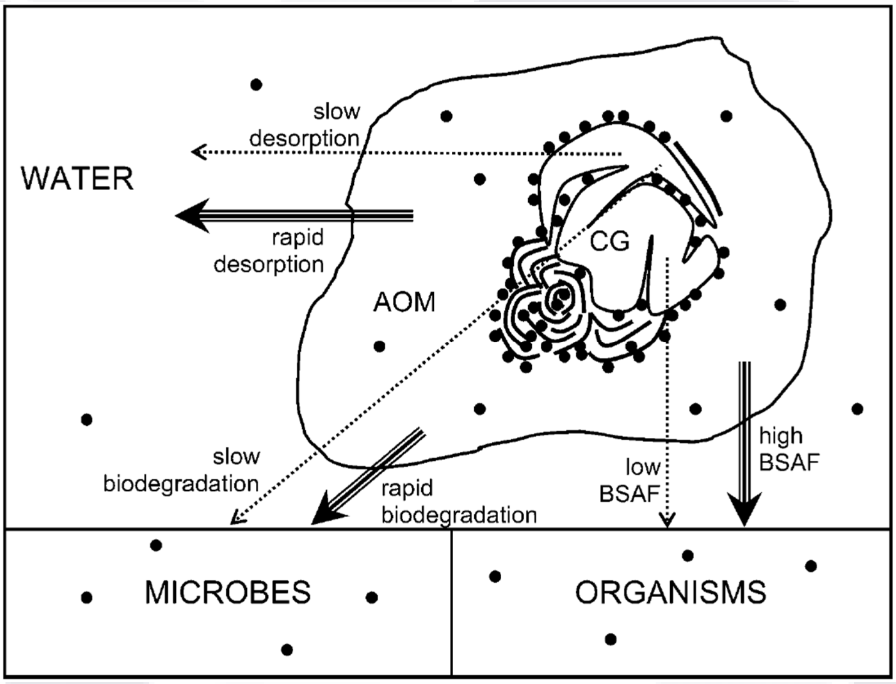


**Figure 6.** The link between strong sorption of contaminants (black dots) to biochar (BC), and slow desorption (release), slow biodegradation and low uptake (risk).[94]

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 [95,96]. The concentration of PAHs is strongly dependent on feedstock, temperature, and design of the pyrolysis unit [93]. However, even if the strong adsorption capacity of biochars limits the noxiousness, mineralization and metabolization processes propelled by microfauna could potentially trigger a bioavailable release of pollutants.

## 3.5 Toxicity testing with live organisms

Toxicity tests permit an evaluation of the degree to which a chemical substance has an adverse effect in live organisms, either acute or chronic [97]. The author hypothesizes that the aforementioned toxicological risk of biochar 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 [98]. 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 [99]. Eco-toxicological tests provide a substantial tool to assess the toxicity potential of a complex mixture of toxicants typically found in biochars. 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 [100]. 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 [101–103]. This is why several aquatic invertebrate species are most likely adequate as a suitable bioassay approach to examine the toxicity potential of biochar 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 [104]. The biota contamination by such elements deserves attention, because of cumulative effects within trophic networks [105]. Transportation of heavy metals through trophic networks often commences with the assimilation of these by bacteria and protists [106]. Various heavy metals and organic pollutants, such as antifouling agents, pesticides, PAHs, and PCBs, which are traceable in biochars, have shown reproducible biological responses when tested in rotifers [107]. 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 biochars.

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 [108,109]. Although Mexico has embraced the D. magna test, this European cladoceran species has never been found in Mexican reservoirs nor terrestrial water bodies [110]. As the contamination of reservoirs involves deposition of pollutants in sediments [97,111], 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 biochars, it is expedient to work with elutriates.

# 4 Justification

There is an increasing evidence that biochar use in soils assists in the build-up of stable (humic) soil carbon pools and hence beneficially effects soil fertility and its resilience to climate change threats [41]. In particular, biochar can significantly increase soil water and usable field capacity [17,112], one of the mayor challenges for Aguascalientes, a semidesertic state in Mexico that suffers from chronic water paucity [113]. Surpassing extraction of water from wells, both for agriculture and residence, since 1990 exceeding natural groundwater recharge rates creates severe problems all over the state. Naturally provided geological faults facilitate the situation. However, anthropogenic activities in particular deep drilling to reach annually declining water tables constantly leads to increasing aquifer overdraft and is the main reasons for steadily deterioration of groundwater [110]. The application of biochar at agrarian and communal irrigated land is presumably not a panacea, but certainly can contribute to relieve the water scarcity burden.

As far as the author knows, only few biochar related trials have been conducted in Mexico, whereas research studies related to biochar in Aguascalientes possess a scientific character with predominantly laboratory scale. Biochar can be generated from a various number of different feedstock, whereas agricultural residues and greenery waste from landscape management are of vocal interest, because they are mainly untapped and free of rivalry. From an applied material flow management approach, these biomasses are usually untapped potentials or are even contemplated as waste, which is disposed of bounding communal financial resources and causing environmental burden contemporaneously.

From a resource efficiency and circular economic point of view it is not beneficial not to valorize these potentials, especially with regard to the potential of adding value to the region [114]. Considering these arguments, a local investigation that envisages the quantitative and qualitative determination of potential feedstock for biochar generation constitutes a reasonable approach. The same holds true for the qualitative assessment of the biochar itself, taking as a basis international standards and certification requirements.

Besides the positive effects of biochar when added to the soil, contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibeno-dioxins and furans (PCDD/Fs) can be co-generated or heavy metals can be concentrated with biochar thus be present in its matrix, bound in a physical (e.g. non-covalent) manner [115]. The hazardous substances potentially can exert unwanted toxic or chronic effects unintentionally to non-target organisms. Potential negative side effects associated with the use of biochar in soil amendment shall be foreclosed where practitioners deploy larger quantities, especially in areas with high potential environmental risk. It is important to reduce solubility, mobility, toxicity and bioavailability of potential contaminants in the environment to a minimum. Eco-toxicological test using live organisms such as aquatic invertebrates provide a substantial tool to assess the toxicity potential of a complex mixture of toxicants typically found in biochar. Toxicity test with aquatic invertebrates that assess the toxicity potential of biochar are rarely found, are rather conducted applying *Daphnia magna* only and concentrate on a single contaminant instead of contemplating the entire chemical cocktail.

Although *Daphnia magna* is a cosmopolitan species and well established organism for eco-toxicological test in many countries, the species has never been found in Mexican reservoirs [116]. Indigenous species such as *Lecane quadridentata* or *Paramecium caudatum* are most likely adequate as a suitable bioassay approach to assess the toxicity potential of biochar whereby both aquatic invertebrate species are existent within the soil porosphere (aquatic and edaphic sphere; both benthic). As eco-toxicological test with indigenous aquatic invertebrates to assess the environmental risk that biochar can provoke on the environment have not been conducted in Mexico, nor in other countries, the present work possesses a substantiated scientific character of novelty.

In this context, the present work contributes to increase the state of scientific knowledge with respect to physical, chemical and biological mechanisms involved in biochar application as well as the response of soil biota community and associated risk. The present work contributes to better assess bioavailability of hazardous substances contained in biochar and adjust application strategies in biochar use. Furthermore, the investigation supports the development and implementation of new techniques to evaluate biochar, both much more cost effective and faster.

Considering, that current international biochar market prices trigger new beneficial business opportunities all over the globe, the present work contributes to develop tailor-made feedstock valorisation and biochar application strategies along communal and agrarian value chains in Aguascalientes. Application of biochar to soil is a promising method to remove surplus $CO_2$ concentrations from the atmosphere and sequester carbon in the long run, which is palpably the largest challenges of mankind these days.

# 5 Hypothesis

Based on the above-mentioned rationales the following research hypotheses subdivided into quantitative, qualitative, toxicological and agronomic realm is tested:

1. Quantitative: Within the state of Aguascalientes suitable quantities of woody biomass, which are unused at present, are available for biochar production.
2. Qualitative: The biochar generated complies with the requirements of the European Biochar Certificate (EBC) and possesses high water saving potential and significantly can improve soils water capacity.
3. Toxicological: Despite the high quality (certified by the EBC), the biochars contain substantial quantities of hazardous substances, which can induce adverse effects to non-target organisms if wrongly applied to the environment.
4. Monetary: The monetized water saving potential exceed the costs of biochar production.

# 6 Main objectives

## 6.1 General objectives

1. Determine the quantity and quality of feedstock in the state of Aguascalientes, which are suitable for the production of biochar.
2. Produce biochar on practitioners scale from identified biomass and analyse physicochemical characteristics based on international certification standards.
3. Test the biochar on its eco-toxicological potential by applying aquatic invertebrate toxicity test with protozoa.
4. Conduct a cost-benefit-calculation for biochar application in agriculture in Aguascalientes based on the water saving potential.

## 6.2 Specific objectives

1a) Conduct a material flow analysis of potentially untapped biomass streams within the state of Aguascalientes.

1b) Define the wood species, determine cost of collection and treatment.

2a) Generate four different biochars from different feedstock (species or compositions) using a locally assembled pyrolysis kiln.

2b) Manufacture a pyrolysis kiln.

2c) Determine the physicochemical characteristics of the produced biochars based on EBC requirements, with special emphasize on toxicants such as PAHs, PCDD/Fs and heavy metals.

2d) Define the maximum water capacity enhancement of three different soils when biochar is added.

2e) Generate elutriates from the biochars in different concentrations.

3a) Assess acute and chronic toxicity of biochars to non-target organisms using four benthic and zooplanktonic invertebrate species, the ciliate *Paramecium caudatum*, the rotifer *Lecane quadridentata*, and the cladocerans *Daphnia magna* and *Moina macrocopa*

3b) Determine $LC_{10}$, $LC_{50}$, NOEC and LOEC values.

4a) Check economic performance of biochar application based on the local feedstock, pyrolysis kiln and maximum water capacity.

# 7 Materials and Methods

## 7.1 Study site, Feedstock and Sample Preparation

Aguascalientes is a state in Central Mexico, whose capital is also called Aguascalientes and has around 800,000 inhabitants; the eponymous municipality of Aguascalientes is one of eleven municipalities in the state with a total surface of 1,173 $km^2$. According to NAVSTAR GPS the state of Aguascalientes ranges from 22°27′ to 21°38′ N and from 101°53′ to 102°52′ W [117]. Aguascalientes suffers from chronic paucity of water [118] and lacks natural methods for soil-water improvement and water saving. At the same time the state holds substantial untapped biomass potentials for biochar generation, which could be of higher interest for the semi-desert state as biochar addition to soil can increase water absorbance and water holding capacity (WHC) [17,33,38,119]. The current disposal of potentially unrecognized biomass is a burden for the municipality and causes multiple negative environmental effects. For these reasons, converting untapped biomass potentials into biochar and applying this to the semi-desertic soils of Aguascalientes could help to alleviate the urging water deficit of that region and relieve communal expenses. This research evaluates, both quantitatively and qualitatively, the biomass and biochar potential of Aguascalientes as well as the expected effects on soil WHC if applied to the field.

Data concerning quantity and woody species composition was acquired and analyzed from the municipal composting site in the municipality of Aguascalientes and the sanitary landfill San Nicholas (cp. Figure 7). Further samples were collected from forest residues in the northern municipality of Rincón de Romos. The aim was to: a) define the biomass potential in the state of Aguascalientes using data from the aforementioned spots; b) to produce four types of biochar from different plant material and to analyze the quality of these types of biochar based on the requirements requested by the EBC. Figure 7 illustrates the study related locations within the state of Aguascalientes.

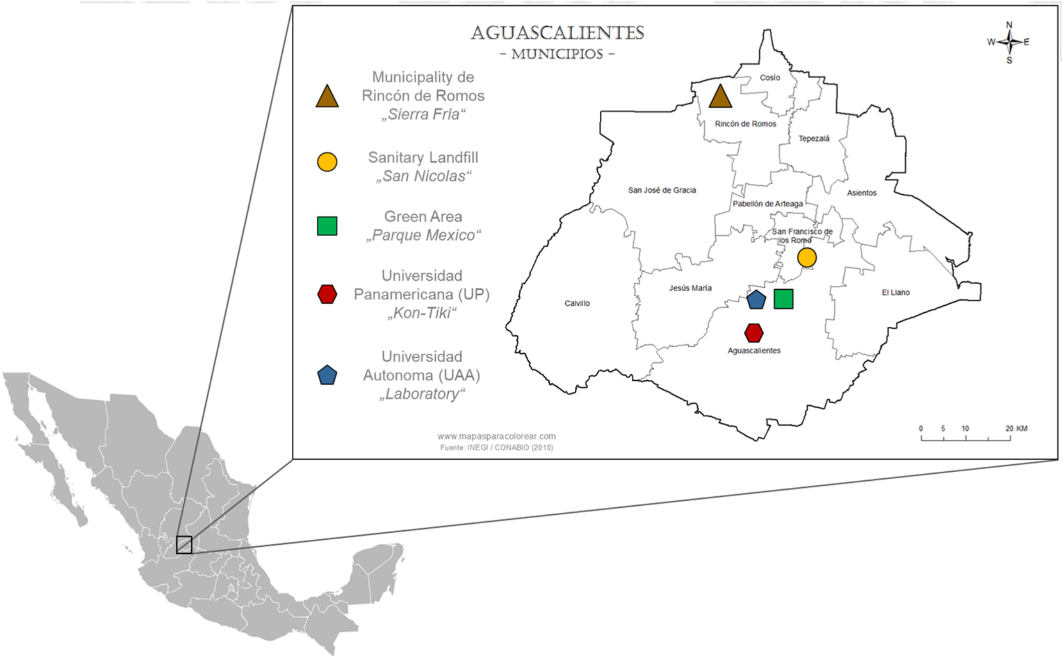


**Figure 7.** Location of three feedstock sampling sites, pyrolysis, and the laboratory site in the state of Aguascalientes, Mexico. Brown dot: Municipality of Rincón de Romos—"Sierra Fria"; yellow dot: Sanitary Landfill—"San Nicolas"; green dot: Green Area—"Parque Mexico"; Red dot: Universidad Panamericana—"Kon-Tiki flame curtain kiln"; blue dot: Universidad Autónoma de Aguascalientes—"Laboratory".

Aguascalientes holds considerable untapped biomass potential, which on the one hand is not even recognized and valorized and on the other hand is disposed of, burden municipal households and causing multiple negative environmental effects. For these reasons, converting untapped biomass potentials into biochar and applying this biochar to the semidesertic soils of Aguascalientes could help to buffer the urging water deficit of that region and relieve communal expenses or even provide a new business opportunity. This study evaluates both, quantitatively and qualitatively, the biomass and biochar potential for Aguascalientes as well as the application spectrum, effects and opportunities. 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 (Figure 7).

This study attempts to assess ways of biomass use for biochar 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.

Different biomass samples have been collected and transported to the Kon-Tiki pyroliser at Universidad Panamericana (cp. chapter 7.2), Campus Bonaterra which is located in the south of the municipality. There, the material has been weighted, measured in size and moisture content has been determined. Here, a conventional humidity measuring device GANN®, series *Hydromette, type S* was used. Afterwards, oversized woody branches and trunks have been chopped, cleaved and broken into smaller pieces so they fit into the pyroliser. Four different samples, constituting different woody compositions, have been generated from the detected types of woody biomass. Figure 8 illustrates the four different biomass samples collected from the different collection spots.

The first sample (see Figure 8) consists of a mixture of 65% manzanita (85.1 $kg_{FM}$)[3] and 35% pine (46.4 $kg_{FM}$) with an average humidity of 7.8% for manzanita and 6.9% for pine (cp. Table 2). A total mass of 122 $kg_{DM}$[4] has been applied to the pyrolising process. The branch diameter ranges from 0.5 to 8 cm for both types of wood. The maximum length of wood pieces was 33cm. The collection took place in the forests of Rincón de Romos, where the material is typically encountered in form of a left over from illegal wood theft and forest management.

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[3] $kg_{FM}$ = kilogram fresh matter
[4] $kg_{DM}$ = kilogram dry matter


**Figure 8.** a) Sample 1 – Municipality of Rincón de Romos "Sierra Fria" – Forest Loppings; b) Sample 2 – Municipal Landfill "San Nicolas" – Mixed Loppings; c) Sample 3 – Municipal composting and Green Area "Parque Mexico" – Trunk Wood; d) Sample 4 – Municipal composting and Green Area "Parque Mexico" – Wood Chips.

**Table 2.** Woody composition of sample 1, Rincón de Romos.

| | **Timber Species** | |
|---|---|---|
| Common Name | Scientific Name | Content (%) |
| Manzanita | *Arctostaphylos pungens* | 65 |
| Pine Tree | *Pinus lumholtzii* | 35 |

The second sample collected from the Municipal composting site consists of a multilateral mixture of different species of wood, which are mainly branches. Table 3 illustrates the composition of Sample 2.

**Table 3.** Woody composition of Sample 2, municipal composting.

| **Timber Species** | | |
|---|---|---|
| **Common Name** | **Scientific Name** | **Content (%)** |
| Velvet Mesquite | *Prosopis velutina* | 18 |
| Ash Tree | *Fraxinus excelsior* | 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 |

The branch diameter ranges from 0.5 to 10 cm for all types of branches. The average humidity was detected to 18.7 %. A total mass of 220.8 $kg_{FM}$ (179.4 $kg_{DM}$) has been applied to the pyrolising process.

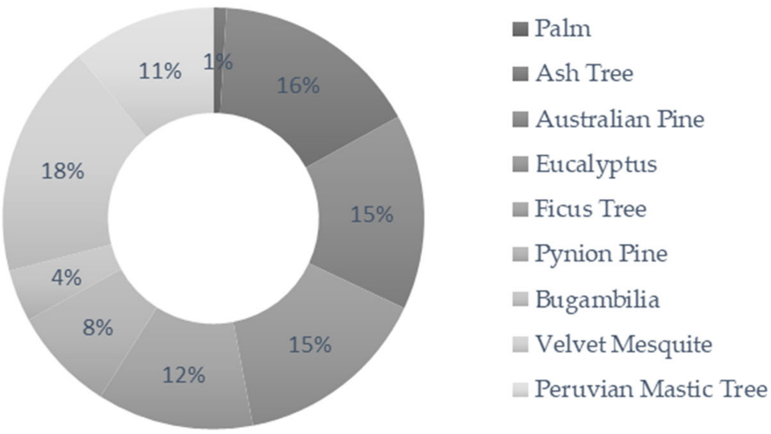



**Figure 9.** Pie chart – Woody composition of Sample 2, municipal composting.

The third sample was also collected from the Municipal composting site and consists even of a multilateral mixture of different species of wood, but is composed of thicker trunks (Table 4 and Figure 10). The trunks have been chopped into logs, with a diameter range from 4.5 to 12 cm in final size. The maximum length was measured at 33 cm. Basically, the trunk wood was expected to have a much slower charring, due to its higher density and lignin compound, and hence has been separated from the branches. Figure 10 illustrates the composition. A total mass of 113.8 $kg_{FM}$ (82.7 $kg_{DM}$) has been applied to the pyrolising process.

**Table 4.** Woody composition of Sample 3, municipal composting.

| Tree Species | | |
|---|---|---|
| **Colloquial Name** | **Scientific Name** | **Content (%)** |
| Velvet Mesquite | *Prosopis velutina* | 38 |
| Ash Tree | *Fraxinus excelsiur* | 24 |
| Manzanita | *Arctostaphylos pungens* | 21 |
| Australian Pine | *Casuarina equisetifolia* | 17 |

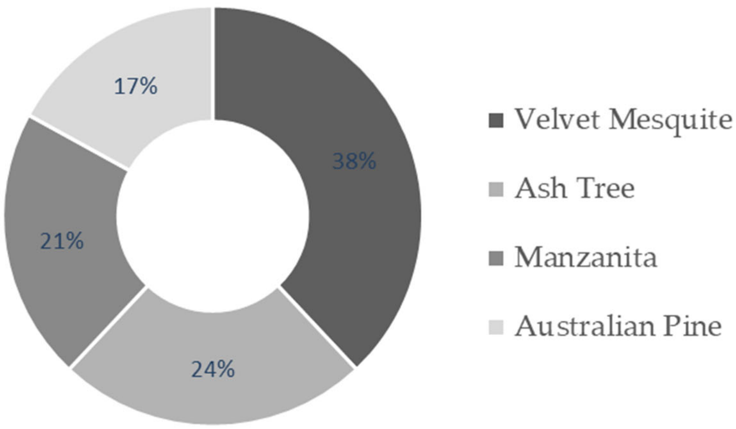



**Figure 10.** Pie chart – Woody composition of Sample 3, municipal composting.

The fourth sample was also collected from the Municipal composting site. Its woody composition is similar to sample 2 but the sample has been shredded (chopped) to grain size 0.5 to 8 cm long and 0.1-2 cm thick. The humidity was measured at 45%. Exactly 40 $kg_{FM}$ have been applied to the pyrolysis process. Due to the high residual moisture, the ignition was initiated by using 22 $kg_{FM}$ of chamomile wood branches from Rincón de Romos (cp. to sample 1). After having a sound firebed, the wood chips have been applied to the kiln. The municipal composting occasionally needs to shredder woody biomass material to overcome lack of space or even to provide wood chips similar to bark mulch for greenery activities within the city garden management. Currently the demand does not meet the supply, hence this kind of woody biomass has been selected to become a valuable sample.

### 7.2 Production technology Kon-Tiki flame curtain kiln

The properties of biochar depend on the raw materials [120,121], the thermochemical conversion conditions and the used pyroliser respectively [11,29,122]. Due to the fact, that Aguascalientes is considered a state in transition, high-end and state-of-the-art pyrolising technologies demanding high investments do not seem to be adequate, because the local market situation currently does not afford large-scale production plants. Furthermore, laboratory scale pyrolisers such as ovens or muffles are not suitable for practical biochar production in this study since the scope is beyond laboratory scale. Quantities of woody biomass in the range of 50 to 200 kg of input material, were used because this amount represents a transportable and collectable daily quantity collected by municipals employees, farmers and forest workers using their own basic equipment (e.g. pick-up truck). Therefore, the goal of this study in the long-run, is to multiply the production and "democratize" the knowledge among the public, if the produced biochars fulfill the quality requirements and meet monetary market conditions. An economically affordable type of pyroliser, which can be produced locally, complying to the quality requirements and has proven low greenhouse gas emissions is the Kon-Tiki flame curtain kiln pyroliser [7,123].

The Kon-Tiki kiln is short in processing time compared to traditional kilns (hours instead of days), easy to operate and cost-effective. The average yield of the Kon-Tiki "technology" was measured at rates between 15 % and 25 % on a dry matter basis, which corresponds to other high temperature pyrolysis technologies [123]. Based on all these arguments the decision was taken to produce a Kon-Tiki kiln locally to conduct the intended biochar production tests. The produced pyroliser is a metal cone (wall thickness 3 mm), not having an apex but a plane lateral surface used in upright position. The lateral surface has a diameter of 82 cm and the top surface holds 142 cm. The height is 1 m. The center of the lateral surface is equipped with a one inch tube inlet for water quenching covered with a metal sieve. The entire pyroliser is edged in a steel rack including a tilting mechanism in order to tip out the char. The construction plan was based on successfully used predecessors provided by the Ithaka Institut for Carbon Management from Switzerland. The Kon-Tiki kiln built in Aguascalientes at Universidad Panamericana is shown in Figure 11.

a) b) c) d)

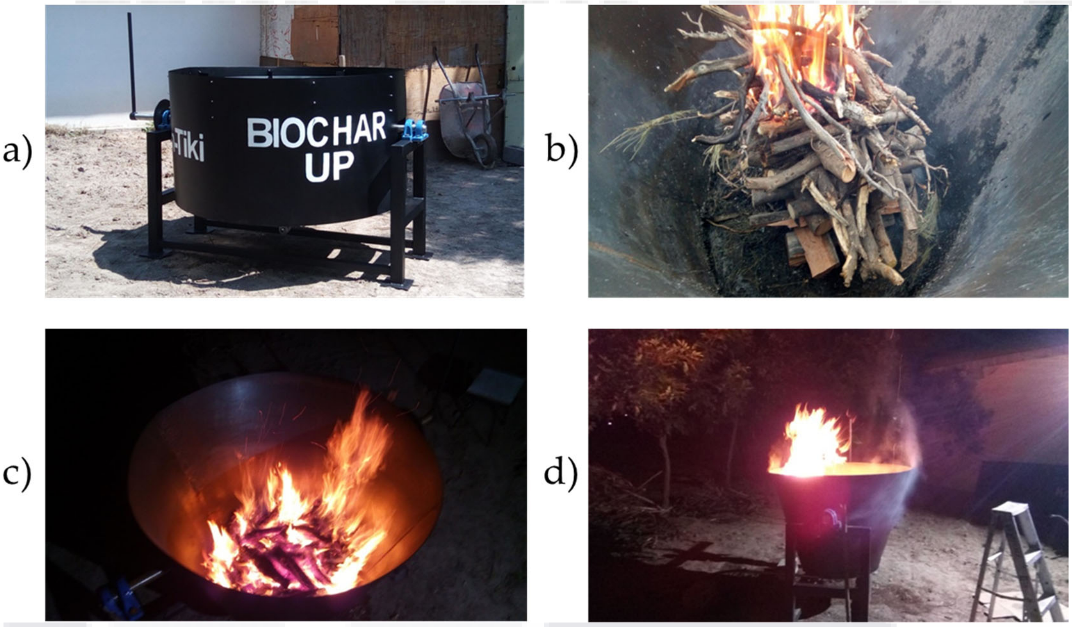



**Figure 11.** a) Kon-Tiki kiln; b) Ignition pyramide (S.1 Camomille and Pine); c) Pronounced firebed, 45min. after ignition; d) Typical turning flame, approx. 2h after ignition, indicating complete combustion of pyrolysis gases.

The collected feedstocks were used to generate four different biochars. 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.

### 7.3 Biochar Analysis and Elutriate Preparation

The four biochars 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 [124].

The biochar elutriates were prepared for acute toxicity testing based on procedures described in the American Society for Testing and Materials Guide E 1391 [125] and US EPA-U.S. Army Corps of Engineers [126] with slight modifications (see also US

EPA 823-B-01-002 [127]). After grinding biochar to powder (grain size <1 mm) using a ceramic mortar and pistils, the biochars were mixed in a 1:4 (v/v) ratio of biochar 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 6,000 rpm for 20 min. The aqueous fraction (elutriate sample) was pipetted and stored in beakers at 20 ± 1 °C (Figure 12).

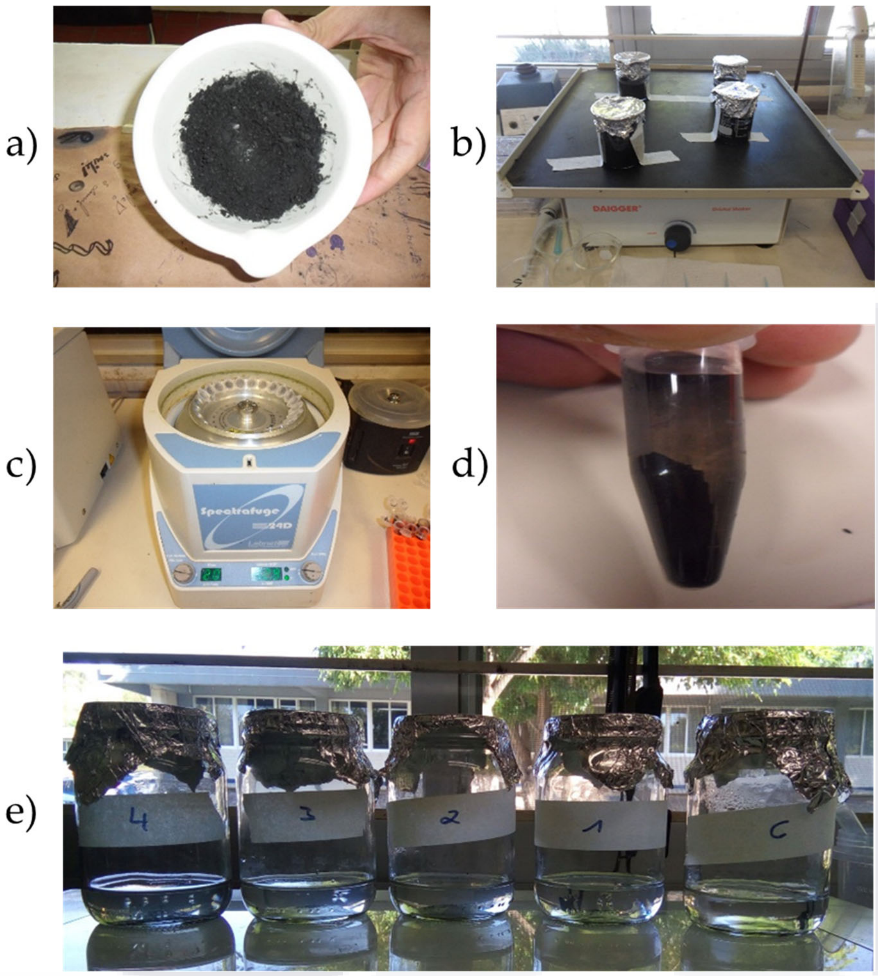



**Figure 12.** Illustration of elutriate preparation procedure: a) Grinding biochar samples to powder in mortar vessel using a pestle; b) Mixing biochar powder with EPA and rotary shaking; c) Centrifugation; d) Pipetting of aqueous fraction from Eppendorf tube; e) Final storage of elutriates in beakers.

## 7.4 Soil: Biochar Water Capacity Test

Terra Preta soils evidently contain up to 50 t $ha^{-1}$ of black carbon whereby these soils are highly fertile when compared to the adjacent soils [4]. This knowledge led to the idea of biochar being applied to soil to both sequester carbon and improve physicochemical characteristics in and biological activities of soil such as water storage capacity or penetrability by plant roots and fungal hyphae [128]. In the semidesertic state of Aguascalientes, agriculture suffers from permanent water paucity [118]. Biochar potentially can help to increase the soil water capacity (SWC) of agrarian soils and hence subdue the impacts of water shortage [34]. To assess the magnitude of increasing SWC of agrarian soil in Aguascalientes three different soils have been blended with the four locally produced biochars under reproducible conditions and the maximum water capacity ($WC_{max.}$) was defined, following the protocol of Reuter [129] with slight modifications oriented at DIN 19683 [130]. In brief, $WC_{max.}$ is measured as the amount of water retained in an amended soil that has been saturated and then allowed to freely drain for a specific amount of time. Figure 13 and Figure 14 show the classification of the $WC_{max.}$ within the different soil parameters.

<table>
<tr><td>Water tension</td><td>< 60 [hPa]</td><td>60 – 300 [hPa]</td><td>300 – 15,000 [hPa]</td><td>> 15,000 [hPa]</td></tr>
<tr><td>pF-value</td><td>< 1.8</td><td>1.8 – 2.5</td><td>2.5 – 4.2</td><td>≥ 4.2</td></tr>
<tr><td>Equivalent pore diameter</td><td>> 50 [μm]</td><td>50 - > 10 [μm]</td><td>10 - > 0.2 [μm]</td><td>≤ 0.2 [μm]</td></tr>
<tr><td>Pore description</td><td>wide coarse</td><td>narrow coarse</td><td>medium</td><td>fine</td></tr>
<tr><td>Soil water</td><td>fast seepage</td><td>slow seepage</td><td>plant available water (PAW)</td><td>not PAW</td></tr>
<tr><td rowspan="3">Soil parameter</td><td rowspan="2">Air capacity (AC)</td><td colspan="2">Usable Field Capacity (uFC)</td><td>Permanent Wilting Point (PWP)</td></tr>
<tr><td colspan="3">Field Capacity (FC) = Water Capacity (WC)</td></tr>
<tr><td colspan="4">Water Holding Capcity (WHC) = maximum Water Capacity ($WC_{max.}$)<br>Total Pore Volume (TPV)</td></tr>
</table>

**Figure 13.** Parameters of soil hydraulic balance. Adapted from DIN 4220:14 [131].

The water tension, also known as matrix potential, is an important parameter of the soil water balance and characterizes the suction tension caused by capillary and adsorption forces to which the soil water is adhered, and thus corresponds to the force or bond strength with which the soil matrix holds water in the soil porosphere. Due to the large range of values that the water tension in the soil can inherit (0 to over 15 bar = 1,500 kPa = 15,000 cm water column), it is usually given in the form of the decimal logarithm based on the unit ´cm water column´ (pF value) ( e.g. pF 2 = 60 cm water column = 0.06 bar). The water tension is primarily a function of the soil type and the pore volume [132].

The field capacity (FC), also known as field water capacity, storage moisture or water capacity, characterizes the maximum amount of water in the soil hold by capillary and adsorption forces, which, contrary to gravity, remains in undisturbed storage above the groundwater level and is accessible by plants. The size of the FC is primarily dependent on the grain size distribution, the soil structure and the content of organic soil and is conventionally stated as the water content two to three days after sufficient water saturation. The water tension at field capacity fluctuates approximately between pF values of 1.8 and 2.5 usable field capacity.

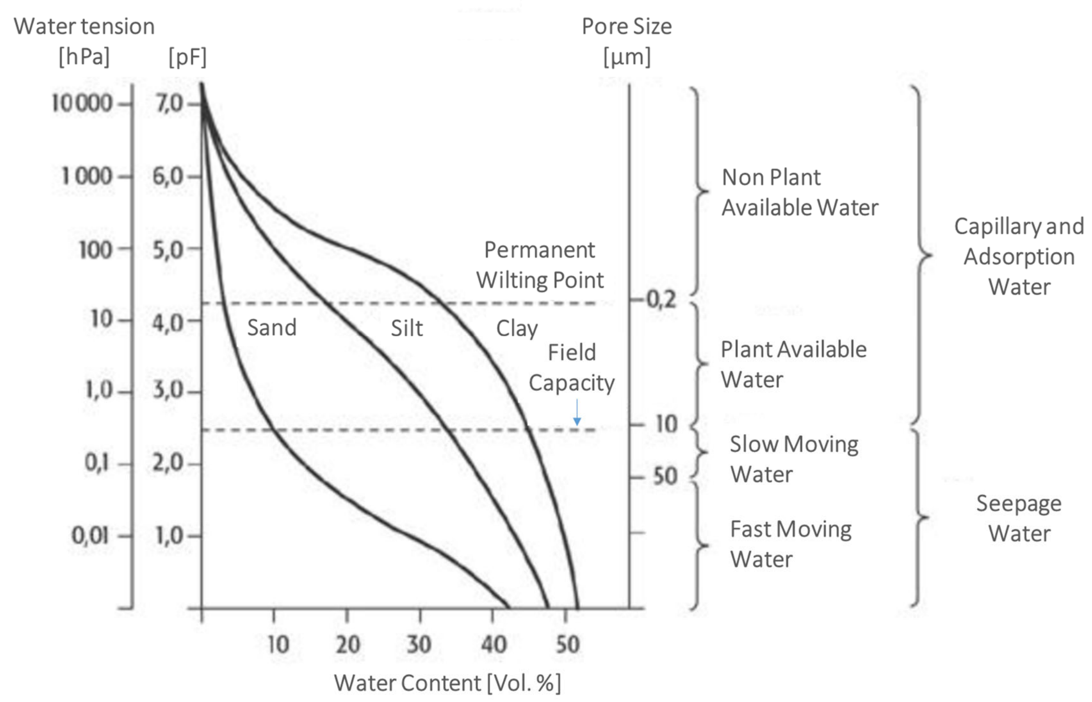



**Figure 14.** Water tension curves of typical sand, silt and clay soil [132].

The usable field capacity (uFC) results as the difference between the water content at field capacity (pF about 1.8 to 2.5) and at the permanent wilting point (pF = 4.2) from the pF curve. Utile FC is usually regarded as the water reserve of a soil that can be used by plants. The uFC is greatest for clay and silt soils, for sandy soils the uFC is limited by relatively low water content in field capacity and in clay soils by relatively high water content at permanent wilting point (PWP) [132,133].

The three soil types were obtained from different location within the state of Aguascalientes not exceeding a profundity of 10 cm (crumb approach). Figure 15 illustrates the types of soil obtained and the corresponding coordinates of the sampling site.

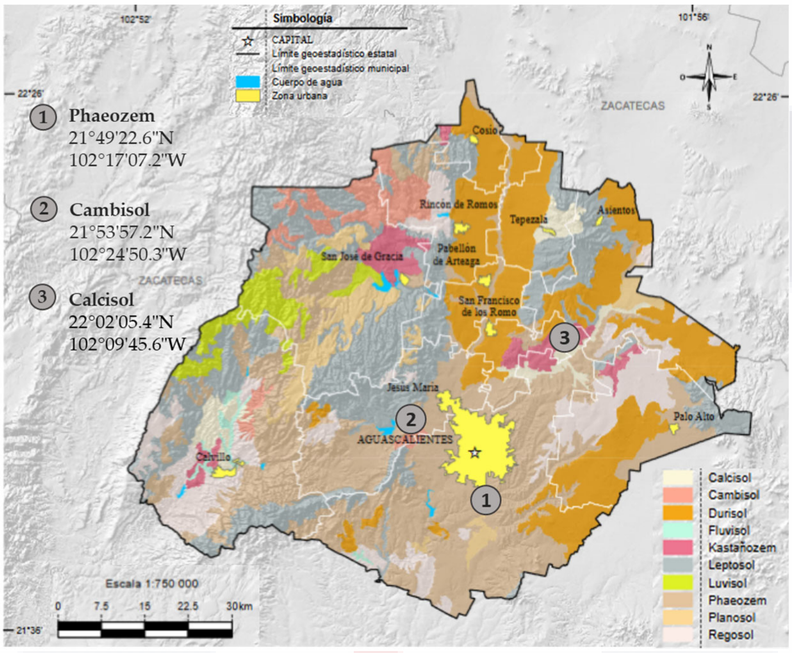



**Figure 15.** Location of three soil sampling sites and soil type distribution in the state of Aguascalientes, Mexico.

Source: INEGI. https://www.inegi.org.mx/temas/edafologia (access November 2019) altered through own illustration.

After sampling, to get homogeneous and comparable sample material, first the soils were sieved to separate bigger stones, non-mineralized organic matter and other impurities. Here a conventional metal screen with mash size 0.8 cm was used. After definition of fresh matter bulk-density, subsequently the soil moisture was determined. Therefore, 50 g of each soil has been placed into an aluminium bowl and inserted into a drying oven for 24 h at 105 °C. After cooling in a glass-dome desiccator for 1 h the samples were scaled again to determine the dry matter bulk-density. The delta between the two measurements indicates the soil moisture. Figure 16 illustrates the three soil types and Table 5 the corresponding physical soil texture, moisture and bulk density.

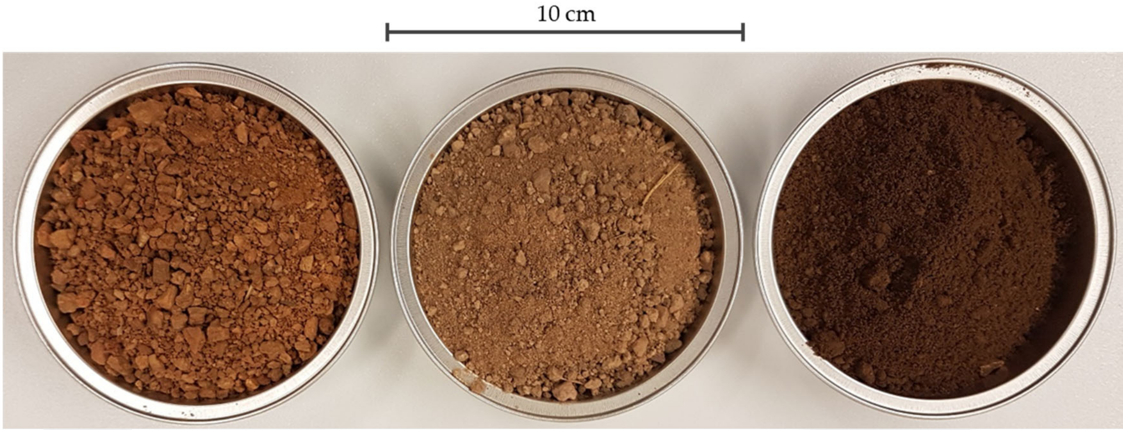



**Figure 16.** Three soil types: *Left)* Cambisol; *Centre)* Phaeozem; *Right)* Calcisol.

**Table 5.** Soil types and corresponding soil texture.

| *Soil type/Texture* | **Cambisol** | **Phaeozem** | **Calcisol** |
|---|---|---|---|
| Clay [%] | 31 | 26 | 14 |
| Silt [%] | 33 | 24 | 8 |
| Sand [%] | 36 | 50 | 78 |
| Soil moisture [%] | 5.2 | 4.0 | 8.5 |
| Bulk density [$kg_{FM}$ $cm^{-3}$] | 1,272 | 1,366 | 1,100 |

A funnel (ø 100 mm, h 106 mm) equipped with a pleated filter (VWR 516-0298) was mounted above a plastic cup (volume 1,000 $cm^{-3}$) and filled with naturally moist soil substrate (soil + biochar) corresponding to 100 g. The soil was not artificially compacted. A gravimetric approach was used, as application limits of biochar follow a gravimetric approach too, e.g. 30 $t_{aDM}$ $ha^{-1}$ [134,135]. Subsequently 100 mL $H_2O$ was applied to the substrate at room temperature 20°C (+/- 1°C). After 30 min. drip time (or if gravity driven drainage has removed any excess water [assumed soil suction of -33 kPa) the throughput again was applied onto the substrate. This procedure was repeated twice and in the last stage after 60 min. the leaked amount of water was weighted. The $WC_{max.}$ was determined using the following equation:

$$WC_{[100\%\,max.]} = \frac{Addition_{[ml]} - Eluate_{[ml]} + Soil\ moisture_{[ml]} - Filter\ Absorbence_{[ml]}}{Weighed\ Substrate\ Portion_{[g]}}\ x\ 100$$

$WC_{[100\%\ max.]}$ = maximum soil water capacity at 100%

$Addition_{[ml]}$ = water application into the funnel

$Eluate_{[ml]}$ = water freely drained from the substrate

Soil $moisture_{[ml]}$ = moisture contained in the substrate

Filter $Absorbence_{[ml]}$ = water absorbed by the pleated filter

Weighed Substrate $Portion_{[g]}$ = Weight of the substrate applied to the filter

Figure 17 exemplarily shows the test set-up and conduction.

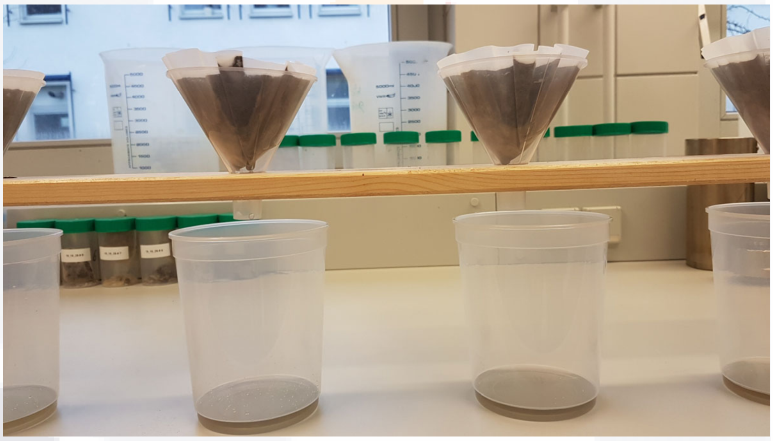


**Figure 17.** Plastic funnel equipped with pleated filter attached to a wood rack including sub-positioned plastic beaker.

For the preparation of the substrates (100 g per sample) air-dried soil substrate and absolutely dry biochar (24 h at 105° C in a drying oven) was used. The biochar was ground to 0.1 to 0.2 mm particle size following the protocol of Briggs et al. [40]. The $WC_{max.}$ was determined separately for each sample. Due to the different densities of the soils, different gravimetric additions of the biochars resulted. In order to ensure a comparability of the individual soils among each other, the biochar soil ratio in percentage was defined as the benchmark. The substrates were mixed according to the following table.

**Table 6.** Biochar concentration in substrate mixtures for the three soils.

| | Biochar Soil Ratio | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|---|
| Soil type | Percentage Biochar in TopSoil | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.4% |
| Cambisol | Biochar Content [t $ha^{-1}$] | 0 | 12.5 | 17 | 25.5 | 42 | 85 | 170 |
| | Soil Content Sample [$g_{FM}$] | 100.0 | 99.0 | 98.7 | 98.0 | 96.7 | 93.3 | 86.6 |
| | Biochar Content Sample [$g_{DM}$] | 0.0 | 1.0 | 1.3 | 2.0 | 3.3 | 6.7 | 13.4 |
| Phaeozem | Biochar Content [t $ha^{-1}$] | 0 | 13.5 | 18 | 27.5 | 45.5 | 91 | 182 |
| | Soil Content Sample [$g_{FM}$] | 100.0 | 99.0 | 98.7 | 98.0 | 96.7 | 93.3 | 86.7 |
| | Biochar Content Sample [$g_{DM}$] | 0.0 | 1.0 | 1.3 | 2.0 | 3.3 | 6.7 | 13.3 |
| Calcisol | Biochar Content [t $ha^{-1}$] | 0 | 11 | 14.5 | 22 | 37 | 73 | 147 |
| | Soil Content Sample [$g_{FM}$] | 100.0 | 99.0 | 98.7 | 98.0 | 96.6 | 93.4 | 86.6 |
| | Biochar Content Sample [$g_{DM}$] | 0.0 | 1.0 | 1.3 | 2.0 | 3.4 | 6.6 | 13.4 |

The substrate parts (soil/biochar) were manually mixed in a beaker. The beaker was inserted into a friabilator (type MZ 2000-Fria) and turned at 36.5 rpm for 2 minutes before the final substrate was placed into the funnel. The test was conducted with four replications for each soil type with each biochar. In total 336 samples were conducted.

## 7.5 Toxicity Tests

Acute toxicity tests with each of the four biochar elutriates using the ciliate *Paramecium caudatum*, the rotifer *Lecane quadridentata,* and two cladocerans: *Daphnia magna* and *Moina macrocopa* were performed. 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* (Figure 18).

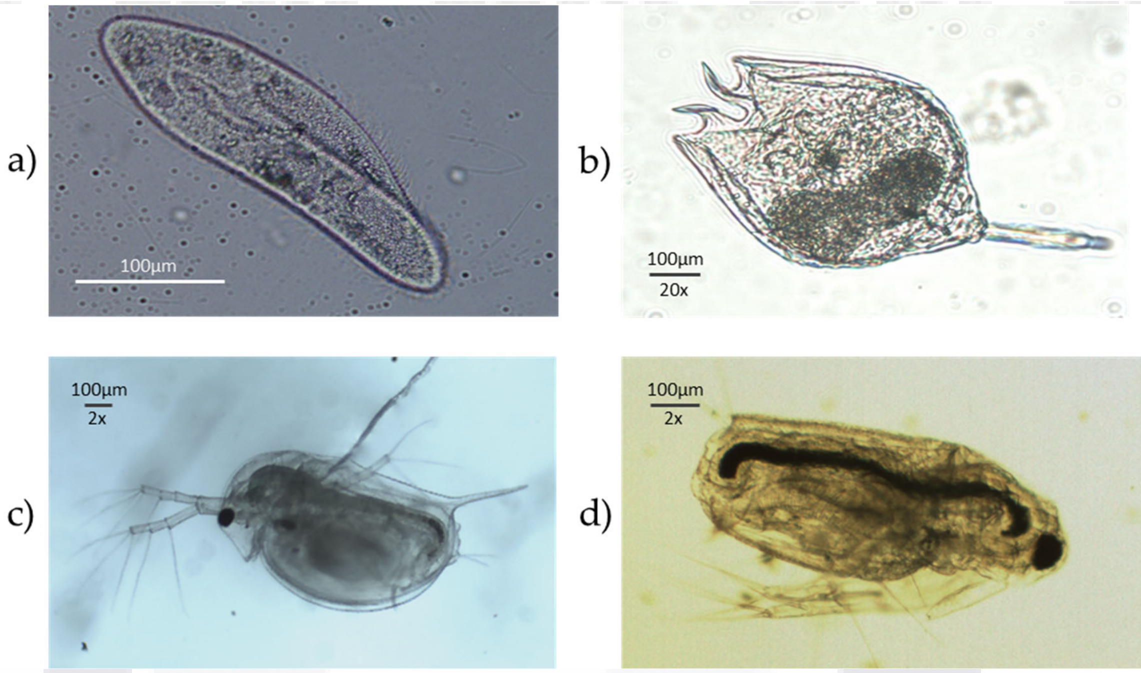



**Figure 18.** Photographs of test organisms. a) *Paramecium caudatum*; b) *Lecane quadridentata*; c) *Daphnia magna*; d) *Moina macrocopa*. Source: own images Flesch UAA 2018.

The four different species have been selected based on the idea of conducting the acute toxicity test along the trophic chain from ciliates via rotifers towards cladocerans. Furthermore, it seemed meaningful to conduct the test both with uni- and multicellular organisms, as their metabolisms strongly differ, and hence distinguished inferences can be drawn. Table 7 briefly presents the taxonomy and characteristics of the organisms employed in the tests.

**Table 7.** Condensed taxonomy, cellular structure, nutrition, habitat, size and sex of the four test organisms [113,136,137].

| Organism | Order | Genus | Phylum | Cellular Structure | Nutrition | Habitat | Size | Sex |
|---|---|---|---|---|---|---|---|---|
| *Paramecium caudatum* | Peneculida | Paramecium | Ciliophora | unicellular | bacteriophage<br>eukariotic cells (yeast) | marine, fresh and brackish water<br>benthic zone | 120 - 330 µm | asexual |
| *Lecane quadridentada* | Ploima | Lecane | Rotifer | multicellular | algophage | fresh and brackish water<br>benthic zone | 150 -400 µm | m/f |
| *Daphnia magna*<br>*Moina macrocopa* | Cladocera | Daphnia | Arthropoda | multicellular | algophage<br>bacteriophage<br>detrituphage | holartic<br>fresh and brackish water<br>pelagic and benthic zone | 1.5 - 5 mm | m/f |

Finally, *L. quadridentata* and *M. macrocopa* were exposed to an elutriate generated from a soil–biochar mixture, to assess the acute toxicity if biochar 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 biochar ($V_{soil}/V_{biochar}$). 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 biochar to the soil with a specific bulk density of 250 kg $m^{-3}$. This value refers to the maximum amount of biochar that is allowed to be applied to acreage, for example, in Germany, predetermined by the German Fertilizer Application Ordinance (DüV) [135] and the German Federal Soil Protection Act (BBodSchG) [134]. As far as the authors know, México does not specify the application of biochar in agriculture, which is the reason to refer to German thresholds. Based hereupon, 880 mL of leptosol was mixed with 110 mL of biochar 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 biochar.

### 7.5.1 *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 [137]. Selected *P. caudatum* specimens were cultivated in Petri dishes with Sonneborn medium [138]. The methodology employed in these toxicity tests was made according to Madoni [139]. 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 biochar elutriate concentrations (6.25%, 12.5%, 25%, 50%, 100% plus one negative control with EPA medium [140]) from each of the four biochars 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 [141]. The test was conducted with five replications for each biochar. After 24 h period, the ciliates were counted using a binocular electron microscope (type Leica DMLS) to determine mortality. Table 8 and Figure 19 illustrate the test set-up.

**Table 8.** Toxicity test set-up, EPA and eluate proportion.

| Concentration | Control | 6.25% | 12% | 25% | 50% | 100% |
|---|---|---|---|---|---|---|
| Drop Organisms [µl] | 50 | 50 | 50 | 50 | 50 | 50 |
| Eluate [µl] | 0 | 62 | 120 | 250 | 500 | 950 |
| EPA [µl] | 950 | 888 | 830 | 700 | 450 | 0 |

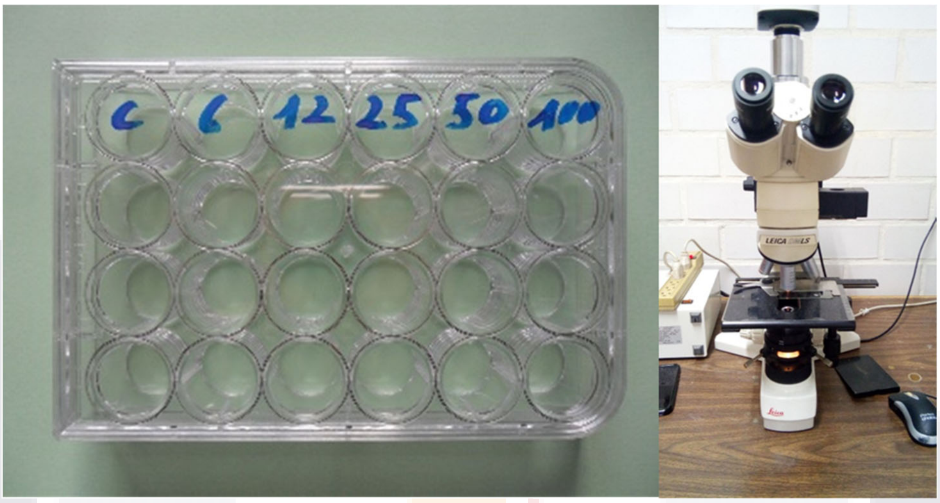



**Figure 19.** Test equipment. Left) 24-well polystyrene plate (Corning Costar Corporation, USA; Right) Leica DMLS binocular electron microscope.

### 7.5.2 *Lecane quadridentata* 48 h Acute Toxicity Test

*Lecane quadridentata* organisms were collected from Lake Chapala, Mexico [142]. These strains have been continuously cultured in EPA medium [140] for more than 20 years in the 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 [142]. 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 biochar 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 [141]. The test was conducted with five replications for each biochar. 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 [140]) and elutriate samples. The test set-up and used equipment are identical to the *Paramecium caudatum* toxicity test.

#### 7.5.3 *Daphnia magna* 48 h Acute Toxicity Test

The *Daphnia magna* acute toxicity protocol detailed in the Mexican Norm NMX-AA-087-SCFI [141] was applied. 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 [143]. In the control, 10 neonates were placed in 100 mL of EPA medium in a 250 mL glass beaker [140]. The same was done for each replica 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. The test set-up and used equipment are identical to the *Paramecium caudatum* toxicity test.

#### 7.5.4 *Moina macrocopa* 48 h Acute Toxicity Test

The *Daphnia magna* acute toxicity protocol detailed in the Mexican Norm NMX-AA-087-SCFI [141] was applied. The only difference was to substitute *D. magna* with *M. macrocopa*. The test set-up and used equipment are identical to the *Paramecium caudatum* toxicity test.

#### 7.5.5 *Paramecium caudatum* Growth Inhibition Test

To assess the sublethal toxicity of the four biochars to *Paramecium caudatum*, a growth inhibition test following the the protocol of Miyoshi et al. [144] was performed, 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. Sonneborn medium [138] was added 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:

$$\%\ \mathrm{I} = \frac{Nc - Nt}{Nc} * 100$$

where:

*N* = total number of *P. caudatum* organisms alive after 96-h

*t* = treatment

*c* = control

### 7.5.6 Chronic Five-Day Toxicity Tests (Growth Inhibition) with *Lecane quadridentata*

Since no lethal toxicity was found with the four biochar:soil 1:8 mixes, and to assess the sublethal toxicity of the four biochar:soil 1:8 blend elutriates, the 5-day chronic toxicity tests with *L. quadridentata* using the protocol of Hernández-Flores and Rico-Martínez [145] was performed. Technically the test is similar to the test by Miyoshi, however instead of adding Sonneborn medium *Nannochloropsis oculata* at $1X10^5$ cells/mL were added.

### 7.5.7 Chronic Seven-Day Toxicity Test (Growth Inhibition) with *Moina macrocopa*

Since no lethal toxicity was found with the four biochar:soil elutriates, the protocol of the 7-day Chronic Test with the cladoceran *Ceriodaphnia dubia* [146] was used to assess the sublethal toxicity with slight modifications: (a) Instead of using *C. dubia,* this cladoceran was substituted with *Moina macrocopa*; (b) instead of using the yeast, cereal leaves, and tetramin (YCT) food the micro algae *Pseudokirchneriella subcapitata* to feed *M. macrocopa* was used.

### 7.5.8 Statistical Analysis for the 48h Acute Toxicity Test

Data were analyzed through ANOVA Ducans MRT test 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.

## 7.6 Economic Evaluation of Biochar Use

Based on the gained insights from the biochar physicochemical analysis, the water capacity trials and the technology examination, this chapter concentrates on the economic performance evaluation of biochar when used in amending soil. This economic pre-feasibility analysis is done according to the individual characteristics of each biochar and to the local circumstances present in the study area (cp. chapter 7.1). Primarily, a simple static cost-benefit-analysis was applied, with the aim to evaluate rather small-scale generation and application of biochar. Based on these insights of the cost-benefit-analysis, secondly a Financial Statement was developed, with the aim to evaluate a rather medium to large-scale commercialisation of biochar generation in Aguascalientes.

### 7.6.1 Cost-Benefit-Analysis

In accordance to the 2nd hypothesis, that biochars produced from local feedstock significantly can improve soil water capacity and the 4th hypothesis that monetized water saving potential exceed the costs of biochar production three different scenarios with biochar application for water saving have been determined. The three scenarios are:

a) Green area at Universidad Panamericana (aquifer water, drinking water),
b) Green areas of the Municipality of Aguascalientes (treated effluent),
c) Agrarian cultivation of corn (aquifer water).

Every scenario holds several different calculation pattern, such as the provenance and/or seasonal availability. In particular the most crucial variables are:

- the amount of water applied to a specific terrain [$m^3$/ha/a] and
- the price for water [USD/$m^3$].

In a first step, the production cost of biochar with a locally assembled Kon-Tiki flame curtain kiln were determined, distinguished into operational expenditures (OPEX), financial (or capital) expenditures (CAPEX) and total expenditures (TOTEX). Afterwards, the optimum user performance ratio (OUPR) of each biochar in each soil tested (see chapter 8.3.3), has been set in contrast to the TOTEX in each of the three scenarios in order to define the payback period (PBP). The PBP serves as an indicator, to assess the economic risk but not the profitability of an investment.

However, PBP based on a static cost-benefit-analysis allows for a sound categorisation and initial assessment of the project, which is decisive for any subsequent profound and dynamic analysis. Based on the gained insights from the cost-benefit-analysis the profitability of biochar production in Aguascalientes was assessed using a financial statement model.

#### 7.6.1.1 Green area at Universidad Panamericana

The Universidad Panamericana (UP) irrigates approx. 2.5 ha of lawn, bush and shrub area with water extracted from a well. In average, UP applies about 16,000 $m^3$ of water per hectare and year. The price for water is set by INAGUA to 1.14 USD/$m^3$. The extraction and distribution requires about 70,000 kWh of electricity per year, which causes additional water cost of 0.31 USD/$m^3$. Hence, the levelized cost of water (LCoW) accounts for 1.45 USD/$m^3$ and a total water costs for irrigation of about 58,000 USD/a. UP urgently strives for water saving options. Table 9 shows the calculation of the LCoW.

**Table 9.** LCoW calculation for Scenario UP.

| Parameter | Value | Unit |
|---|---|---|
| Pump capacity | 75 | PS |
| | 55 | kW |
| Flow rate | 8.9 | l/s |
| | 256 | $m^3/d$ |
| | 39,986 | $m^3/a$ |
| Operation time | 8 | h/d |
| | 3 | d/week |
| | 156 | d/a |
| | 1,248 | h/a |
| Irrigation | 2.5 | ha |
| | 15,994 | $m^3/ha/a$ |
| Water price | 1.14 | USD/$m^3$ |
| Water costs | 45,584 | USD/a |
| | 18,234 | USD/ha/a |
| Energy demand | 68,796 | kWh/a |
| Energy price | 0.18 | USD/kWh |
| Energy costs | 12,383 | USD/a |
| | 0.31 | USD/$m^3$ |
| LCoW | 57,967 | USD/a |
| | 1.45 | USD/$m^3$ |

#### 7.6.1.2 Irrigated areas at Parque Ecológico Línea Verde of the Municipality of Aguascalientes

The municipality of Aguascalientes irrigates approx. 20.5 ha of green park area, in the Parque Ecológico Línea Verde, with treated waste water. In average, the municipality applies about 4,500 $m^3$ of water per hectare and year. The water is supplied mainly using trucks with different capacities (see Table 10). The treated waste water is supplied from different treatment plants, mainly from the treatment plant "La Ciudad". The transport are the only cost occur for this type of irrigation water. The levelized cost of water (LCoW) accounts for 3.37 USD/$m^3$ and a total water cost for irrigation of about 310,000 USD/a. As the distribution costs are high, the municipality steadily is striving for alternatives to save water and improve distribution. Table 10 shows the calculation of the respective LCoW.

**Table 10.** LCoW calculation for Scenario Green areas.

| Parameter | Value | Unit |
|---|---|---|
| Truck capacity | 8 - 20 | $m^3$ |
| Truck use rate | 70 - 30 | % |
| Water price range | 4.3 - 1.2 | USD/$m^3$ |
| LCoW (Weighted ø) | 3.37 | USD/$m^3$ |
| Irrigation | 20.4 | ha |
| | 4,500 | $m^3$/ha/a |
| | 91,800 | $m^3$/a |
| | 309,366 | USD/a |

### 7.6.1.3 Agrarian cultivation of corn in Aguascalientes

Improving water efficiency in Aguascalientes agriculture is an urgent need as the state suffer from chronic water paucity. However, any technology or measurement are evaluated against the water price. Based on the example of corn, in average a farmer applies about 6,730 $m^3$ of water per hectare and year. The water usually extracted from wells. The price for water is set by INAGUA to 0.0073 USD/$m^3$. The extraction and distribution requires about 29,000 kWh of electricity per year, which causes additional water cost of 0.12 USD/$m^3$. Hence, the levelized cost of water (LCoW) account for 0.13 USD/$m^3$ and a total water costs for irrigation of about 884 USD/ha/a. Table 11 shows the calculation of the LCoW.[5]

[5] The data were confirmed in personal interviews with farmers.

**Table 11.** LCoW calculation for Scenario Agrarian cultivation.

| Parameter | Value | Unit |
|---|---|---|
| Irrigation | 1.0 | ha |
| | 6,730 | $m^3$/ha/a |
| Water price | 0.0073 | USD/$m^3$ |
| Energy demand | 28,948 | kWh/a |
| Energy price | 0.03 | USD/kWh |
| Energy costs | 835 | USD/a |
| | 0.12 | USD/$m^3$ |
| LCoW | 884 | USD/a |
| | 0.13 | USD/$m^3$ |

### 7.6.2 Financial Statement

Provided, that the cost-benefit-calculation delivers positive results, an up-scaling of the production unit seems worthwhile. The biochar production capacity of a Kon-Tiki is limited to 30-50 t/a, which means that neither a commercialized production size can be reached nor economy of scale effects can be exploited. In order to accomplish a substantial contribution to water saving management in Aguascalientes, a larger biochar production system was evaluated. Here, a standardized charcoal kiln from the company Alfacharcoal, which meets the requirements of the EBC, was subjected to an economic performance evaluation using a financial statement. The use of an industrial steel kiln, such as the Alfacharcoal kiln, in contrast to the small-scale re-torts or kilns such as Kon-Tiki, offers even higher carbon efficiency rates as of ca. 35% and utilizes less ignition fuel, especially because of its four chamber principal. Similar to the Kon-Tiki, the pyrolysis gases are completely combusted to provide heat for the charring process, hence no $CH_4$ is emitted to the atmosphere.

The financial statement will be based on the assumptions presented in Table 12. All assumptions are based on the local prices. The financing structure assumes a typical 80:20 (Loan : Equity) share. Loan period is set to 10 years at 6.5% interest rate. Mexico's current inflation rate is about 4.9% p.a. and corporate tax is about 30%.

**Table 12.** Financial and Techno-Economic assumptions of the Financial Statement.

Financial Input Data

| Parameter | Value | Unit |
|---|---|---|
| Retort | 1 | x |
| Retort Price | 348,000 | USD |
| Equipment | 38,400 | USD |
| Installation | 27,500 | USD |
| Contingency | 20% | |
| Equity | 20% | |
| Dividend | 12% | p.a |
| Dept financing | 80% | |
| Loan interest rate | 6.5% | p.a |
| Loan period | 10 | a |
| Insurance | 2.0% | p.a. of Inv. |
| Inflation rate | 4.9% | p.a. |
| Corporate tax | 30% | p.a. |

Techno-Economic Input Data

| Parameter | Value | Unit |
|---|---|---|
| Input | 12.5 | t/d |
| Output | 4.4 | t/d |
| In-/Out ratio | 35% | |
| Feedstock price | 22 | USD/t |
| Sales price BC | 200 | USD/t |
| Manager (M) | 1 | person |
| Foreman (F) | 2 | person |
| Operator (O) | 12 | person |
| Salary M | 16,400 | USD/a |
| Salary F | 6,800 | USD/a |
| Salary O | 2,304 | USD/a |
| Accrued Liabilities | 2.5% | p.a. of EBIT |
| Maintenance | 1.5% | p.a. of Inv. |

Within a company (or a project), all relevant financial information is presented in an easily comprehensible manner in the form of a financial statement. The financial statement consists mainly of three financial accounting-related calculations, accompanied by a management discussion and analysis. The aim of the financial statement is to be able to evaluate companies or their projects based on balance sheet figures. Valuation variables can be quite different. For example, the internal rate of interest, the return on equity or the net debt can help to provide economic assessments about the company. Above all, the liquidity, especially in the early years and later for dividend payouts and payment of re-investments, is crucial. The asset balance consists of three parts:

I. The standardized balance sheet; with the aim of simplifying the balance sheet and thereby making it analysable (cover ratios).
II. The profit and loss account; used to calculate and judge the operating business.
III. The cash flow statement; to calculate the liquidity as well as the financial strength and profitability [147].

The preparation of a financial statement can be carried out retrospective and prospective. In contrast to the retrospective determination where key figures can be analysed based on these annual financial statements, the prospective calculation serves as a financial plan based on planed-, profit and loss accounts for assessing the future ability to meet financial obligations. Companies should therefore always be examined for their solvency. The sheets prepared in this study is prepared prospectively [147]. Figure 20 illustrates the structure of a Financial Statement.

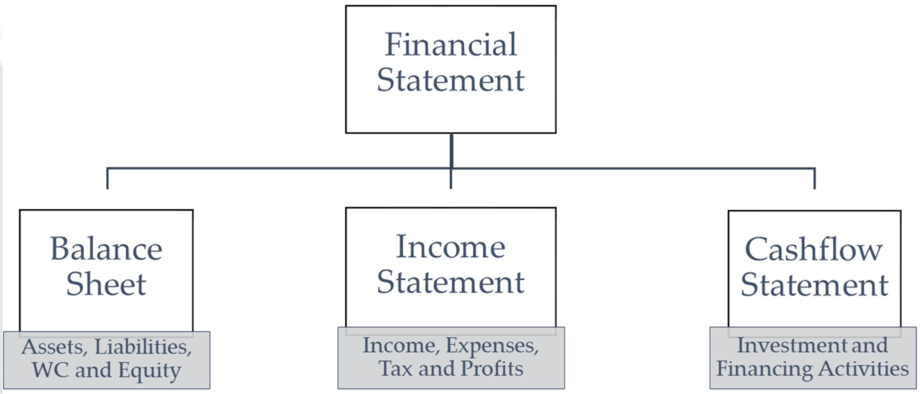



**Figure 20.** Financial Statement set-up.

7.6.2.1 Standardized Balance Sheet

A standardized balance sheet is used by analysts to make comparisons of companies or individual projects comparable and more easily analysable. In doing so, the company's trading balance is structured. The structural preparation means that the large number of listed balance sheet ac-counts of assets and liabilities are restructured into consolidated balance sheet accounts such as fixed assets and current assets or in equity and debt. This makes the balance sheet easier and more comprehensible and enables direct determination of economic indicators. There are principles for the restructuring of the balance sheet, but no generally binding rules. The fixed capital is determined by adding the fixed assets, consisting of property, plant, and equipment (technical equipment and machinery) as well as land and net working capital. In addition, the standardised balance sheet shows the development of equity and debt capital as well as cash. The standardized balance sheet is always linked to the profit and loss account as well as the cash flow statement. The model follows a "roller". The calculations cover periods of up to 30 years.

#### 7.6.2.2 Profit and Loss Account

The Business Encyclopaedia of BPB defines the profit and loss account (P & L) as part of the annual financial statements [148]. By recording and offsetting all income and expenses incurred in a fiscal year, the profit or loss is reported as a net profit or loss for the year. The profit and loss account, therefore, has the task of making the success of the individual success sources identifiable ac-cording to the type and amount, thereby providing an insight into the state of the annual result, thus supplementing the balance sheet. For this purpose, the German Commercial Code (HGB) requires the non-balanced comparison of all types of expenses and income. The income statement thus describes the operational performance of the company.

The profit and loss account is based on the requirements of the IFRS. The gross margin is calculated by offsetting the sales revenues with the material costs. The sum of gross margin, personnel costs, other expenses and administrative costs is the gross profit (EBITDA - Earnings before Interest, Tax, Depreciation and Amortization). Gross profit minus non-cash depreciation results in earnings before interest and tax (EBIT). The sum of EBIT and interest result reveals the taxable income (EBT - Earnings before Tax) which, after deduction of corporation tax, shows the profit or loss carried forward (EAT).

#### 7.6.2.3 Cash Flow Statement

The inadequate liquidity orientation of a balance sheet prevents an insight into the liquidity situation of the company. Impaired payment bottlenecks and liquidity gaps can only be recognized in a timely manner if a cash flow statement is derived from the balance sheet and income statement. The German Commercial Code (HGB) currently does not provide for an autonomous, time-related accounting for the financial position that presents the financial flows that are significant for the assessment of the dynamic aspect of the financial situation. Together with the information provided by the annual financial statements, the cash flow statement serves a better assessment of the company. The evaluation of

- the ability to generate payment surpluses,
- the ability to meet payment obligations and to use equity,
- the impact of investment and financing on the financial situation,
- the reasons for the divergence between net profit for the year and net cash flow from operating activities,

comprise the focus of the activity. The cash flow statement can either be created by direct input of the payment flows or derivatively from the annual financial statement data. A cash flow statement, as a period-based accounting for the financial situation, breaks down the relevant financial statements according to factual aspects and divides them into new, independent areas. The cash flow statement is based on the provisions of IFRS, whereby the cash flows are divided into the following areas:

- operating activities,
- investment activities,
- financing activities.

By subdivision, each activity area can be enriched in its information content, which allows a differentiated analysis. The EBITDA, which is the descriptive value of current business, is cleaned up from taxes and changes in net working capital, the result is the cash flow from operating activi-ties, also known as net OPEX. Investments in property, plants and equipment represent the cash flow from investing activities, also known as CAPEX. The sum of operating and investing cash flow results in the free cash flow. The free cash flow shows how much liquidity is freely available in the financial year after investing. The free cash flow must be able to repay loans and pay dividends. Equity and debt capital are in-flows in the company. The repayment and interest on borrowed capital are outflows. The total cash inflow and outflow is the cash flow from financing activities. The sum of cash flow from financing activities and free cash flow is called Total Cash Flow. The total cash flow indicates the amount of money remaining at the end of the period (financial year) after payment of all liabilities. This shows how much additional funding (in this case equity) exists to postpone the cooperative. A negative total cash flow leads to a reduction in cash. A positive total cash flow leads to an increase in cash.

#### 7.6.2.4 Interpretation

Based on the financial statement, different information (results) can be obtained for various target groups using indicators and values. Some information, such as total cash flow or working capital, is primarily used for company management and decision-making, therefore for the internal representation. Other key figures, such as the Internal Rate of Return (IRR) or the Flow to Equity, are used for the external presentation and promotion of the company. Another key indicator for assessing credit worthiness is, for example, the level of debt service and interest coverage. The calculation model serves two target groups. The target groups of active and passive participants. The group of active participants is the operators and owners. The group of passive participants is the borrower. Based on a performance chart, key figures are presented at a glance. The passive group of investors and borrowers is particularly interested in the internal rate of return and the net asset value according to the WACC based free cash flow discounting, the value of equity and its average growth rate.

# 8 Results and Discussion

## 8.1 Biomass and Biochar Potential

### 8.1.1 Biomass Potential of the Municipal Composting Site

The municipal composting in Aguascalientes receives about 2,400 $m^3$ (approx. 216 $t_{DM}$) woody biomass per year, including different wood types such as: manzanita, ash tree, bougainvillea, ceder, eucalyptus, ficus tree, jawbone, pepper tree, peruvian mastic, mesquite, palm tree, rose pepper tree, she-oak tree and various other types of garden shrubs and bushes (cp. Table 3 and Table 4). The woody biomass is mainly coming from the cities greenery and landscape management as well as from citizens that bring their garden waste to the composting site. The Municipal Composting is searching for alternative utilization pathways to utilize the woody material, because composting woody material is quite difficult and long-lasting, as mentioned by the composting operators.

### 8.1.2 Biomass potential of the Municipal Landfill

The municipal landfill of Aguascalientes receives about 1,000 t of waste per day. These data have been communicated in a personal interview with the landfill operator in August 2017. Approximately 60 % (600 t) of the municipal solid waste is organic. Thereof approximately 40 % (240 t) is considered dry and woody material and 60 % (360 t) is considered wet and cellulosic material. The wet material is not suitable for incineration rather for fermentation, due to the high water content. It is expected, that from the 240 t of dry material only 50 % will be utile, due to high contamination with plastics, sand, dust, metals, glass and other non-organic compounds. Hence, the municipal landfill holds an untapped woody biomass potential of circa 120 t per day. An annual potential of 43,800 t of fresh woody material, having a 25 % of residual moisture, is expected. The physical constitution and species composition of the biomass identified is very similar to the biomass found in the Municipal Composting.

### 8.1.3 Biomass potential of the Municipality of Rincón de Romos

The municipality of Rincón de Romos is located in the north of the state of Aguascalientes. In contrast to the other municipalities it owns considerably more forest area, where plenty of mesquite, stone oaks, manzanita and pine tree grow. The overall agricultural and forestal area (Sierra Fría) comprises a total territory of approx. 18,840 ha whereof approx. 62 % (12,246 ha) account for forest and are predominantly vegetated with manzanita and pine tree [149]. These types of trees, in contrast to mesquite, which is a protected species, can be harvested in a certain sustainable manner. Especially due to the fact, that a lot of illegal cutting of timber takes place and plenty of woody left-overs such as branches occur, a valorization of this untapped potential seems substantial. According to collection activities undertaken in December 2017 by order of the current mayor, each hectare holds an annual collection potential within the range of 2 to 6 t of woody dry matter. Considering these numbers circumspective, on a yearly basis, an untapped woody biomass potential of 25,000 to 75,000 t of dry matter can be assumed. In comparison to the previous described two areas of interest, this potential exceeds the amount identified on the landfill and the composting site together, if a progressive approach seems realistic. However, the material in contrast to the landfill and composting site, needs to be collected and concentrated on a certain spot. On the one hand the collection will cause costs, but on the other hand will provide new jobs. In the case that collection and transport costs would exceed biochar market values, the Kon-Tiki flame curtain kiln could be mounted on a pick-up truck and brought to the biomass instead of transporting the biomass to the kiln.

The identified woody biomass potential totaled up to 58,066 tons of dry matter per year, encompassing 11 different wood species. Table 13 illustrates the total biomass potential and provenance based on a conservative approach.

**Table 13.** Untapped woody biomass potential within the study area. Conservative values in fresh and dry matter (values based on 2017).

| Biomass (B) | Municipal Composting | Municipal Landfill | Rincón de Romos | Total |
|---|---|---|---|---|
| B - fresh matter [m³/a] | 2,400 | | | 2,400 |
| B - dry matter [m³/a] | 1,920 | | | 1,920 |
| B - fresh matter [t/a] | 288 | 43,800 | 33,333 | 77,421 |
| B - dry matter [t/a] | 216 | 32,850 | 25,000 | 58,066 |

### 8.1.4 Biochar yields

Biochar yields of the four samples ranged from 16 to 30 % on a dry weight basis (Table 14), which is in the same range as other biomass feedstocks and pyrolysis systems which operate at equal temperatures around 700 °C [5]. A comparison of produced biochar generated with Kon-Tiki flame curtain kiln and with traditional low-temperature kilns and retorts showed that, biochar from traditional low-temperature kilns have been in the same order of magnitude, whereas the percentual output ratio in contrast to low temperature retort kilns (typically around 30–40% on a dry weight basis) has been lower [7]. The biochar, as in the present case, corresponds to these insights.

**Table 14.** List of the experimental runs including total biomass input on dry matter basis, biochar yields on fresh water quenched basis, water content of biochar and input/output ratio.

| Biochar No. | Biomass Input [$kg_{DM}$] | Biochar Output [$kg_{WQM}$] | Biochar $H_2O$ Content [% $_{abs. DM}$] | Biochar Output [$kg_{DM}$] | In/Out Relation [% $_{of Input}$] |
|---|---|---|---|---|---|
| 1 | 122.0 | 54.4 | 45.3 | 29.8 | 24.4 |
| 2 | 179.4 | 47.8 | 39.8 | 28.8 | 16.0 |
| 3 | 82.7 | 31.1 | 35.9 | 19.9 | 24.1 |
| 4 | 44.4 | 34.2 | 60.7 | 13.4 | 30.3 |

As expected, biochar yields are diverse (16 to 30 %), basically because of distinct biomass feedstocks (bulk density, humidity, size of feedstock [logs or chips], etc.) but also due to variation of biomass application to the kiln (frequency of firing) and alternating weather conditions (rain, wind, temperature, air moisture) that occurred during operation. However, this study aimed doing investigations under ordinary and not laboratory conditions, allowing environmental interferences. Biochar 4 shows the highest and Biochar 2 the lowest output ratio. Due to the fact, that the input biomass of Biochar 4 holds the highest residual moisture, lowest bulk density and cellulosic structure, whereas the input biomass of Biochar 2 holds a comparable low residual moisture, high bulk density and high lignin structure, the opposite was expected. The main factors influencing the biochar yield generated with flame curtain kilns are the water content, the particle size and the bulk density of the feedstock [7]. Duration of complete pyrolysis of the core of larger diameter wood pieces such as Biochar 3 is much longer than for higher surface low diameter feedstocks such as Biochar 4 (wood chips standard size – P30). Such differences in pyrolysis duration explain higher carbon losses and thus lower yields of wood logs compared to chipped material or hemi-cellulosic material.

Based on the mean in/output relation of the samples and the identified biomass potentials the biochar potential of the study area was calculated. Table 15 illustrates the result. The calculated biochar potential sums up to 13,701 t per year, which is a substantial mass.

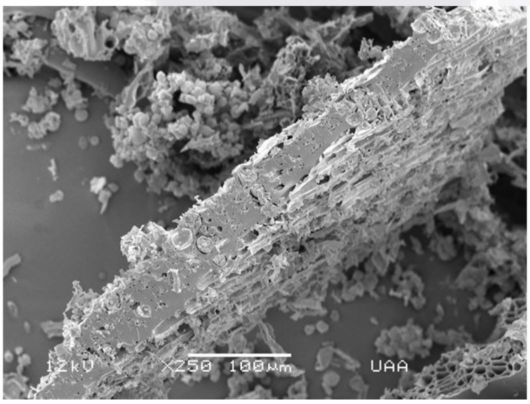
**Table 15.** Biomass to Charcoal Potential in the study area based on the identified biomass quantities and the calculated mean carbon efficiency factor (In/Output ratio).

| Biomass (B) Biochar (BC) | Municipal Composting | Municipal Landfill | Rincón de Romos | Total |
|---|---|---|---|---|
| B - dry matter [t/a] | 216 | 32,850 | 25,000 | 58,066 |
| In/Output ratio [% $_{\text{of input}}$] | 23 | 23 | 24 | |
| BC - [t/a] | 50 | 7,556 | 6,096 | 13,701 |

Simply allocating market values of at least 60 USD/t (calorific value equivalent for lignite, cp. API2 price index) of biochar could generate 822,000 USD of additional turnover for the study area.

## 8.2 Physical and Chemical Parameters of the Biochars

The following interpretation of the biochar characteristics is based on the values presented in Table 16. Water holding capacity (WHC) of the four biochars ranged from 165% to 254% on dry matter basis, with the lowest value being for Biochar 3 and the highest value being for biochar 4. Hence, all produced chars hold excellent properties to improve water holding capacity if added to the soil (Figure 21-24). Specific surface (based on Brunauer–Emmet–Teller [BET] theory) of the biochars were in the range of 54 to 305 $m^2 g^{-1}$, which is in the same order of magnitude found in literature. Tendentially, low BET values mean larger pores and thus less water is retained. As BET values increase, pores become smaller and water is better retained [150]. This property may not apply, due to a change in intra pores and adhesion forces on the surface of the biochar, whereby broken pores potentially can store more water then closed pores. This is probably the case with the four biochars produced in Aguascalientes, where the WHC is higher, when BET values are low. The following electron micrographs (zoom x 250) of the four biochars illustrate the porous structure and differences among each other. Further images in different resolution (zoom x 1,000) are attached to the appendix. Note the complex porosity and variation in each biochar particle and the different structure of the biochars among each other, even though all these particles were created under equal conditions in the same reactor.

a) 

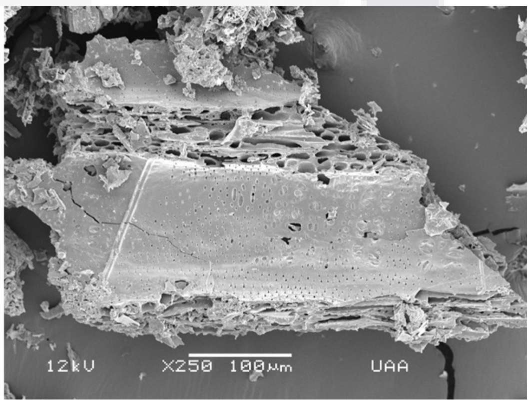

b) 


**Figure 21.** Structure of Biochar **No. 1** particle from different visual angel a) and b). Electron micrograph: 15 kV zoom x **250**, Universidad Autonoma de Aguascalientes, Flesch. F. Nov. 2017.

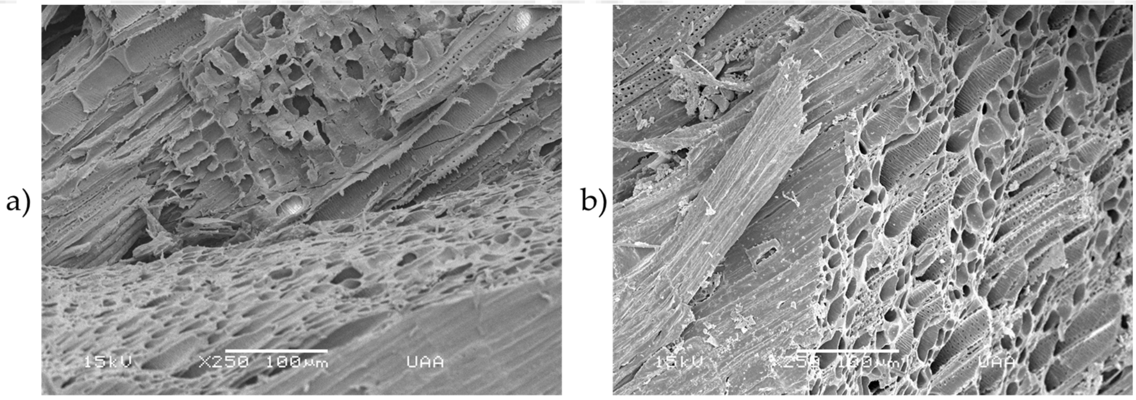



**Figure 22.** Structure of Biochar **No. 2** particle from different visual angel. Electron micrograph: 15 kV zoom x **250**, Universidad Autonoma de Aguascalientes, Flesch. F. November 2017.

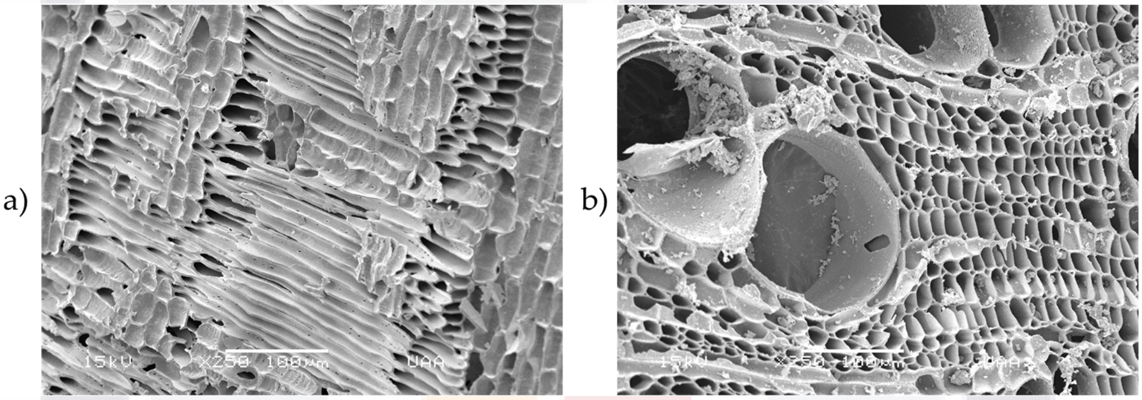



**Figure 23.** Structure of Biochar **No. 3** particle from different visual angel. Electron micrograph: 15 kV zoom x **250**, Universidad Autonoma de Aguascalientes, Flesch. F. November 2017.

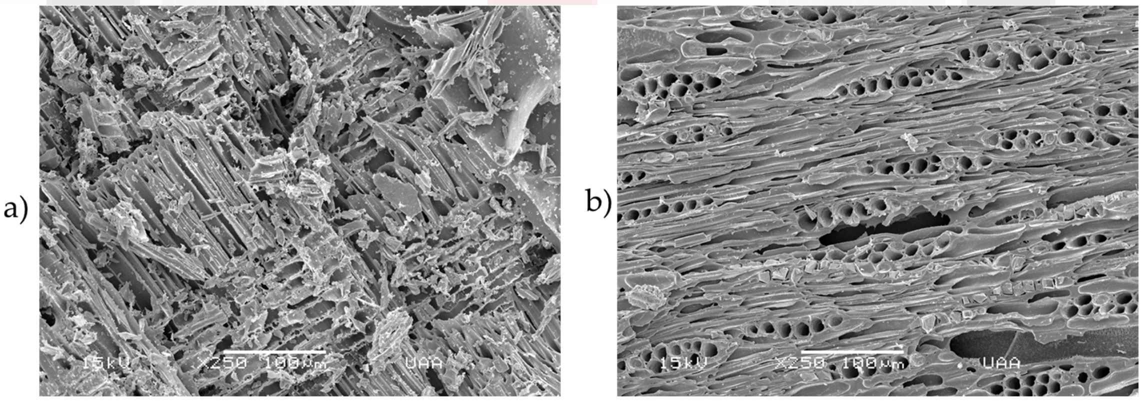



**Figure 24.** Structure of Biochar **No. 4** particle from different visual angel. Electron micrograph: 15 kV zoom x **250**, Universidad Autonoma de Aguascalientes, Flesch. F. November 2017.

**Table 16.** Analyses of four biochars 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 biochar quality.

| **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 | | | |

Total carbon (TC) of the biochars were in the range of 78% to 89% in dry matter, complying with the EBC threshold of >50% for both premium and basic quality. The lowest value being for the biochar 4 with 78% and the highest value being for the biochar 1 with 89%. H contents of the four sample biochars were 0.87% to 1.82%, O contents were 1.1% to 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 to 0.28 and O/C of 0.01 to 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. The ratios comply with the set threshold given by the EBC (Table 16).

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. However, this 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 up-take by the used wood species. As mezquite is known for its tendency to accumulate heavy metals above average in contrast to other trees, it was expected to find higher contents in biochar 2 and 3, nevertheless this assumption was not approved (Table 16).

The most relevant toxic compounds in biochar 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 [123]. This is particularly important if the biochar tends to qualify for applications in animal feed. Among the PAH-16 used as benchmarks by many environmental authorities in many countries (e.g. EPA in the US, UBA in Germany), benzo(a)pyrene is considered to be the most crucial. Especially fodder producers require a maximum content of benzo(a)pyrene below 25 $\mu g\ kg^{-1}$, no matter if the total PAH-16 content is below the maximum threshold of (< 4 ±2 $mg\ kg^{-1}$) [124]. Concentrations of benzo(a)pyrene in all biochars were below 0.1 $mg\ kg^{-1}$ (Table 16). Even though Biochar 1, 2 and 3 (PAH-16 4.8 $mg\ kg^{-1}$, 5.3 $mg\ kg^{-1}$, 0.7 $kg\ kg^{-1}$

accordingly) qualify for EBC premium quality, Biochar 4 (PAH-16 8.3 mg $kg^{-1}$) is only permissible for basic quality (< 12 ±4 mg $kg^{-1}$). The highest content in all biochars is shown by naphthalin in a range from 2.5 to 3.4 mg $kg^{-1}$ (Table 16). This is probably explicable due to improved naphthalin emergence at high temperatures in pyrosynthesis above 700 °C (cp. [115]). Biochar 4 additionally has an outlier in phenanthren content with 1.6 mg $kg^{-1}$. However PAHs in biochar are very hydrophobic and hardly bio-accessible [151]. The dissolution/desorption process of contaminants from biochar into the soil biome is very limited due to the high physical bound and consequently bio-availability is limited to 1 to 10 % of the total content [152]. Values for the PAHs detected in each type of biochar produced are presented in Table 17.

**Table 17.** Values for the polycyclic aromatic hydrocarbons (PAHs) in four biochars.

| **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** |

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

Concentrations in all biochars are far below the permitted EBC thresholds, both for PCDD/Fs and PCBs, but still contain substantial content to endanger living organisms. Table 18 shows the sum of PCDD/F and PCB values calculated based on the toxicity equivalency quotient (TEQ).

**Table 18.** Values for polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and dl-PCBs in four biochars plus EBC threshold.

| 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** |

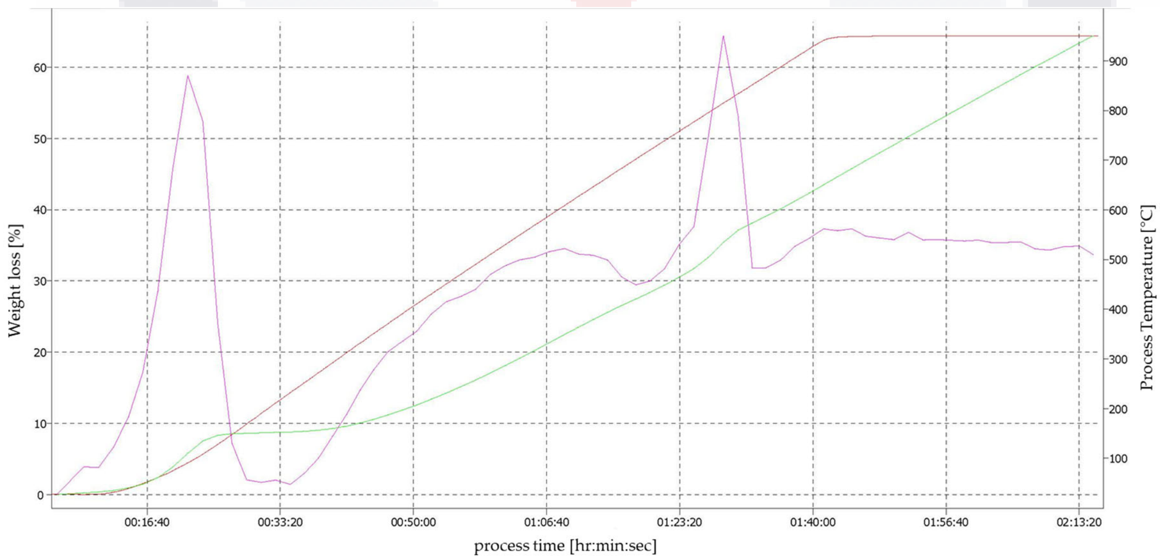
Abbreviations: WHO$_{(2005)}$, values based on World Health Organization toxic equivalency factor from 2005; PCDD$_{(7)}$, 7 EBC required polychlorinated dibenzo-*p*-dioxins; PCDF$_{(10)}$, 10 EBC required polychlorinated dibenzofurans; TEQ, toxicity equivalency quotient; LOQ, limit of quantitation;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

Garcia-Perez [153] conducted a study in 2008 and defined that dioxins may be generated during pyrolysis by two different thermochemical pathways, termed the "precursor" pathway and the "de novo" pathway. Here particular chemicals that contain chlorine form precursor at temperatures above 750 °C, whereas the dioxins themselves form by condensation in the vapor phase at lower temperatures at around 300 °C. When heavy metals are present, the formation is increased. This holds true especially for the presence of copper [7]. The applied feedstock was not measured in chlorine content. However, as the feedstock originated from non-chlorine polluted locations without any particular exposure, there was no pertinent reason to assume atypical chlorine concentration in the feedstock. Copper concentration in the feedstock also have been unobtrusive, with ranges from 7, 13, 15, 37 mg $kg^{-1}$ for Biochar 1 to 4 respectively.

The "de novo" pathway in contrast warrants the presence of both oxygen and solid carbon and takes place between 200 and 400 °C in a catalytic reaction that occurs on particles of fly ash. However, dioxin emissions are less dependent upon chlorine content than they are on process parameters [91]. Garcia-Perez [153] showed that pyrolysis processes that guarantee high temperatures, long vapor residence times in the furnace as well as fast cooling of the biochar are likely to achieve low emissions of PCDD/Fs even while using feedstocks with large contents of chlorine. The Kon-Tiki flame curtain kiln operates at comparably high temperatures. Both flames and vapors are swirled, whereas duration time is prolonged. The fire bed is quenched with water, which ensures a very fast cooling of the biochar. All these features potentially explain the thoroughly low dioxine contents in the biochar.

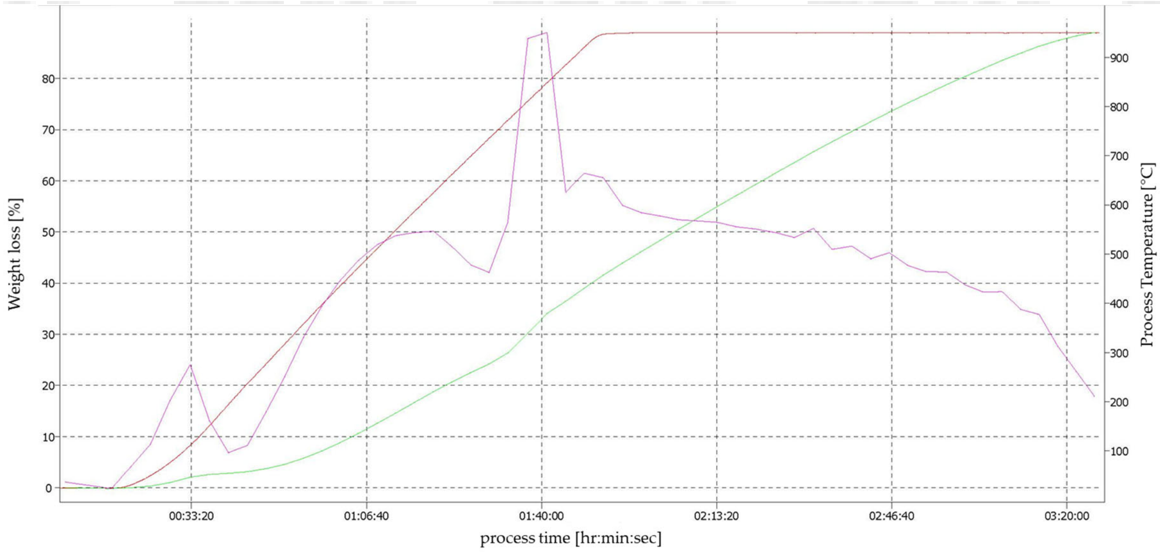
The thermogravimetric analysis TGA-950 demonstrates ordinary properties for all biochars from 0 to 950 °C. The thermal behavior of (bio)chars is studied by measuring the weight loss of a sample in pyrolysis under a nitrogen atmosphere in a thermogravimetric analyzer (TGA). The samples are heated sequentially to eight different temperatures, varying by 100-degree increments, between 200 and 900 °C, with a hold time of 17 minutes at each temperature. The weight loss at each temperature step was recorded to determine the cumulative weight loss as well as low and high temperature loss profiles. TGA analysis helps to assess the stability, the recalcitrance and the ability for biochar to retain carbon in a stable form and hence allow drawing conclusions with regard to residence or aging time when applied to soil. This is a crucial quality characteristic of biochars differing biochar substantially from hydro-char or charcoal. Between 140 °C and 160 °C mainly water and some residual extractive compounds are driven out. Following the reduction curve of weight in correspondence to the temperature, in-between 150 °C and 550 °C a regular and continuous release of volatile organic carbon for all biochar is observed. Concerning the adequate production temperature and associated carbonization of feedstock in the kiln during production, the apparent homogenous weight loss within the indicated temperature range proves a duly operation and generation of biochar. Beginning at 650 °C, up to 850 °C, a peak volume reduction in all samples is noted, which is totally regular as in this temperature range carbonates are oxidized.

Biochar 1 - in contrast to the other three biochars - shows a slightly higher temperature demand to oxidize the carbonate completely, approx. 50 °C higher at around 850 °C instead of 800 °C, which is attributable mainly due to higher total organic carbon content (cp. Table 16) in Biochar 1 in contrast to the other biochars (approx. 10 % more in absolute dry matter +/- 2 %). The highest treatment temperature (HTT) was at 950 °C. The following Figure 25-28 illustrate the biochars thermogravimetric behavior.



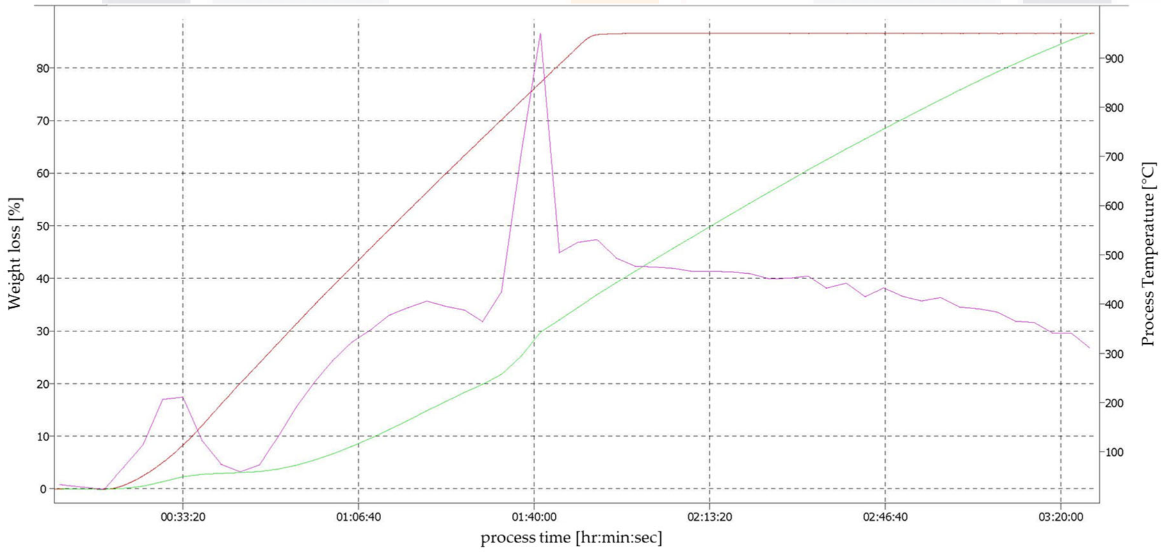

pink curve: weight loss per minute [%]; green curve: accumulated weight loss over pyrolysis time [%]; red curve: process temperature [°C]

**Figure 25.** Thermogravimetric analysis chart of **biochar No. 1** with 1.14 g sample.



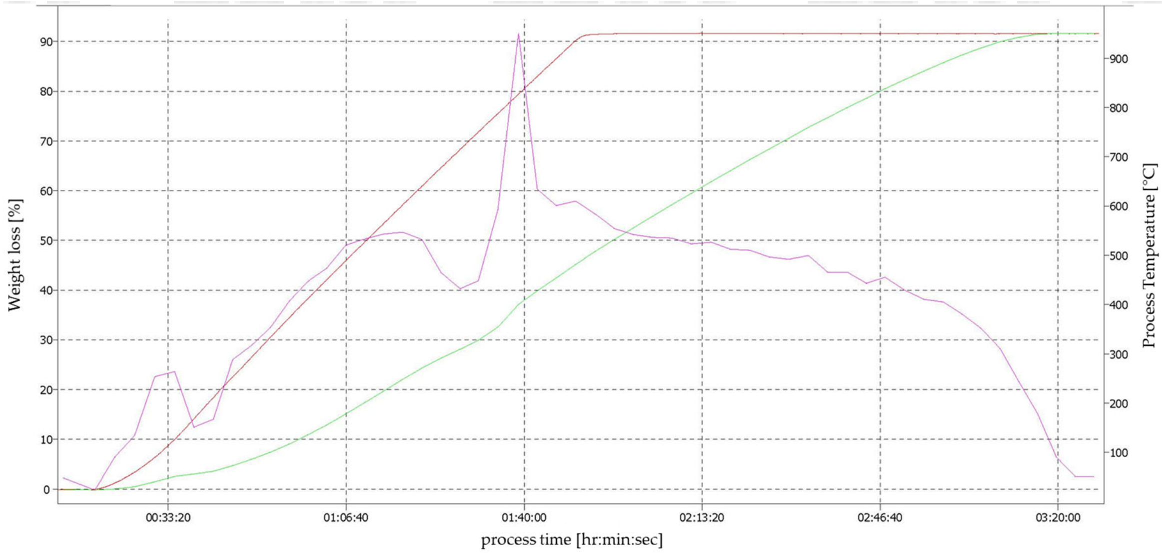

pink curve: weight loss per minute [%]; green curve: accumulated weight loss over pyrolysis time [%]; red curve: process temperature [°C]

**Figure 26.** Thermogravimetric analysis chart of **biochar No. 2** with 1.06 g sample.




pink curve: weight loss per minute [%]; green curve: accumulated weight loss over pyrolysis time [%]; red curve: process temperature [°C]

**Figure 27.** Thermogravimetric analysis chart of **biochar No. 3** with 1.03 g sample.




pink curve: weight loss per minute [%]; green curve: accumulated weight loss over pyrolysis time [%]; red curve: process temperature [°C]

**Figure 28.** Thermogravimetric analysis chart of **biochar No. 4** with 0.93 g sample.

## 8.3 Biochar influence on Soil Water Capacity

Besides the certification requirements, all four biochar types produced, showed remarkable WHC values, which allow presumption that if biochar is added to the soil, the maximum water capacity ($WC_{max.}$) will be improvement. The test runs confirmed this hypothesis. The observed values for the different soil types and biochar blends are shown in Table 19. All four biochars substantially increased the $WC_{max.}$, whereby based on the accumulated values (cp. Table 20, Table 21, Table 22) Biochar 4 provoked the highest, Biochar 3 the second highest, Biochar 2 the third highest and Biochar 1 the lowest increase in $WC_{max.}$ in all three soil types, with one outlier for cambisol, where Biochar 2 shows higher accumulated $WC_{max.}$ over Biochar 3. Based on the WHC values determined by the laboratory analysis, it was expected that Biochar 4 (with WHC 254 mass-%) unleashes the highest increase in WC, followed by Biochar 2 (with WHC 200 mass-%), followed by biochar 1 (with WHC 165 mass-%) and with the lowest WHC Biochar 3 (149 mass-%). But, the detected order of $WC_{max.}$ increase in all soils was tendentially Biochar 4 > Biochar 3 > Biochar 2 > Biochar 1. Hence, using biochar WHC as an indicator for soil water increase only, can be misleading.

Table 19 shows the mean $WC_{max.}$ values for all three soil types with all four biochars at six different admixtures and control. It is very valuable to recognize that even small amounts of biochar significantly increase water capacity. This applies to all four biochars and all three soils. The addition of just one percent by weight increases the water capacity by 1 to 10 percent. The fourteen-fold increase in biochar addition, however, only triggers a further increase of up to 25 %. It is also striking that increasing the amount of biochar in low concentration ranges (1 to 2 %) in some cases leads to a slight reduction in water capacity. However, from an addition of 3 % by weight, the water capacity increases statically with the addition.

**Table 19.** Mean Maximum Water Capacity values for Cambisol, Phaeozem and Calcisol with all four biochars at six different admixtures and control.

| | Biochar Soil Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|---|
| | Biochar Share in Soil [%] | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.4% |
| Soil Type | Biochar Share in soil [t/ha] | 0 | 12 | 17 | 25 | 42 | 83 | 166 |
| **Cambisol** $WC_{max.}$ [%] | Biochar 1 | 45.6% | 53.3% | 51.9% | 49.6% | 54.2% | 57.1% | 60.8% |
| | Biochar 2 | 45.6% | 52.2% | 52.2% | 52.5% | 54.7% | 56.7% | 64.8% |
| | Biochar 3 | 45.6% | 53.6% | 54.5% | 53.7% | 56.2% | 57.2% | 64.4% |
| | Biochar 4 | 45.6% | 53.6% | 53.6% | 53.6% | 56.0% | 56.9% | 66.4% |
| **Phaeozem** $WC_{max.}$ [%] | Biochar 1 | 47.7% | 49.1% | 48.1% | 50.8% | 52.8% | 54.7% | 60.3% |
| | Biochar 2 | 47.7% | 51.0% | 51.1% | 50.5% | 54.1% | 57.5% | 63.6% |
| | Biochar 3 | 47.7% | 50.0% | 49.2% | 50.8% | 52.8% | 55.3% | 64.4% |
| | Biochar 4 | 47.7% | 49.1% | 50.8% | 52.8% | 53.8% | 58.8% | 69.9% |
| **Calcisol** $WC_{max.}$ [%] | Biochar 1 | 50.7% | 50.9% | 50.4% | 51.9% | 56.0% | 57.4% | 63.2% |
| | Biochar 2 | 50.7% | 54.2% | 54.3% | 55.1% | 57.0% | 59.2% | 64.2% |
| | Biochar 3 | 50.7% | 55.1% | 55.1% | 56.2% | 57.1% | 60.6% | 68.2% |
| | Biochar 4 | 50.7% | 55.0% | 55.0% | 55.8% | 59.9% | 62.5% | 75.6% |

In order to facilitate an interpretation of the aforementioned results, diagrams for each soil were plotted. Figure 29 shows the results for Cambisol soil. What is striking for Cambisol is that a small addition of just 1 % by weight of biochar increases the water capacity of the soil compared to the control by 8 %, but further increases in the concentration up to an addition of 3.3 % by weight lead to a slight reduction in the water capacity of the soil. In particular, biochar 1 showed a significant reduction when increasing biochar concentration in soil up to 2 % by weight.

This phenomenon may be explained by the shift of interparticle pore space after soil and biochar are mixed. Biochar holds water in soil by adhesion and adsorption forces using inter- and intraparticle pores within the soil and biochar structure. When biochar is added to the soil, interparticle pores can rapidly be filled with soil particles that are smaller than the interparticle pores [34]. In the case of Cambisol, which was observed very `dusty´, this holds true. It seems comprehensive that the addition of biochar increases the water storage capacity due to the increased adhesion forces of the biochar. If the amount of biochar is increased and parts of the soil particles are smaller than the pores in the interparticle space and the biochar pores, the narrowing of the pore volume within the small particles initially leads to a reduction in the capillary force. As the biochar to soil ratio increases steadily, at a certain ratio all small soil particles are bound or replaced by biochar particles. At this point, the sum of inter- and intraparticle space increases, due to the elevated intraparticle biochar volume, which consequently increases the water holding capacity.

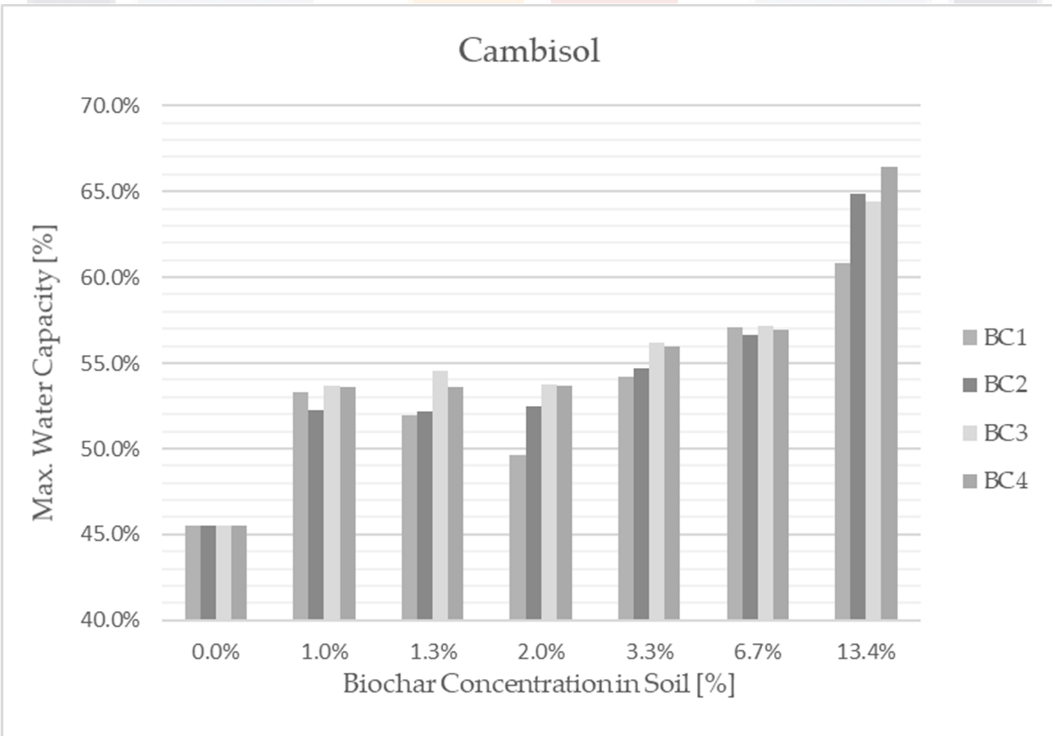



**Figure 29.** Histogram with mean $WC_{max.}$ values for Cambisol for all four biochars.

In contrast to cambisol, phaeozem and calcisol show a more straight-forward increase in water capacity with increasing addition of biochar. In these two soils, a reduction in water capacity with increasing biochar addition is only observed in the lower concentration range and with a significantly lower amplitude, whereas biochar 4 shows no volatility at all concentrations. Compared to calcisol, the phaeozem and the calcisol have a much lower dust content and therefore a lower potential for cavity sealing. The histograms in Figure 30 and Figure 31 illustrate the findings.

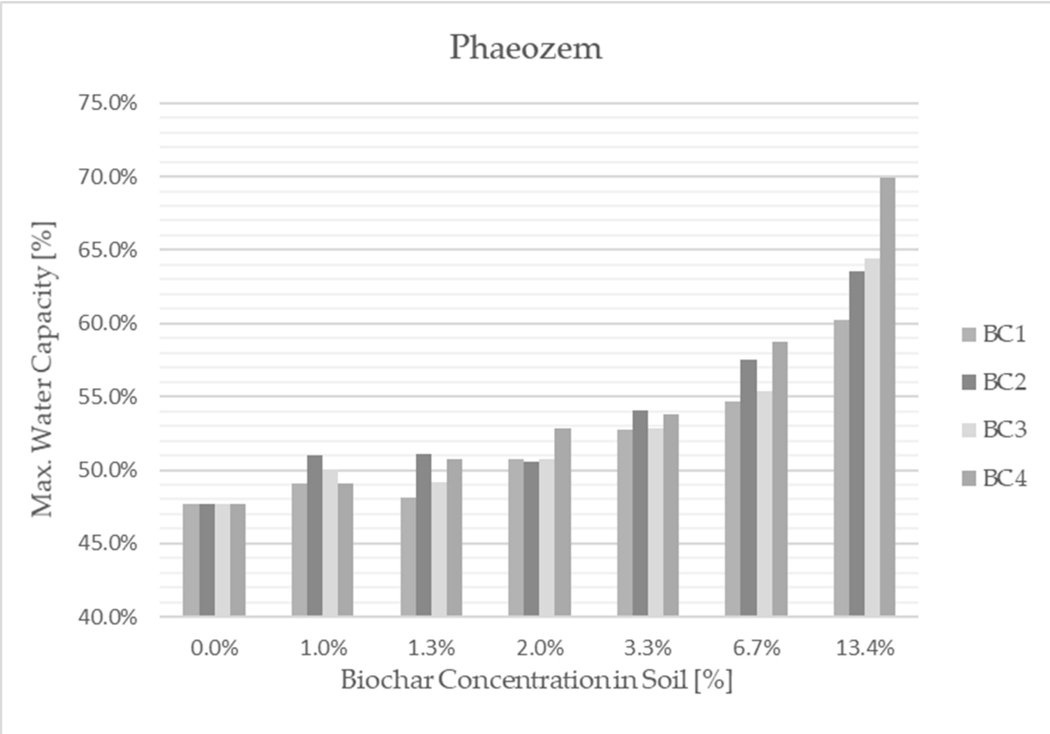



**Figure 30.** Histogram with mean $WC_{max.}$ values for Phaeozem for all four biochars.

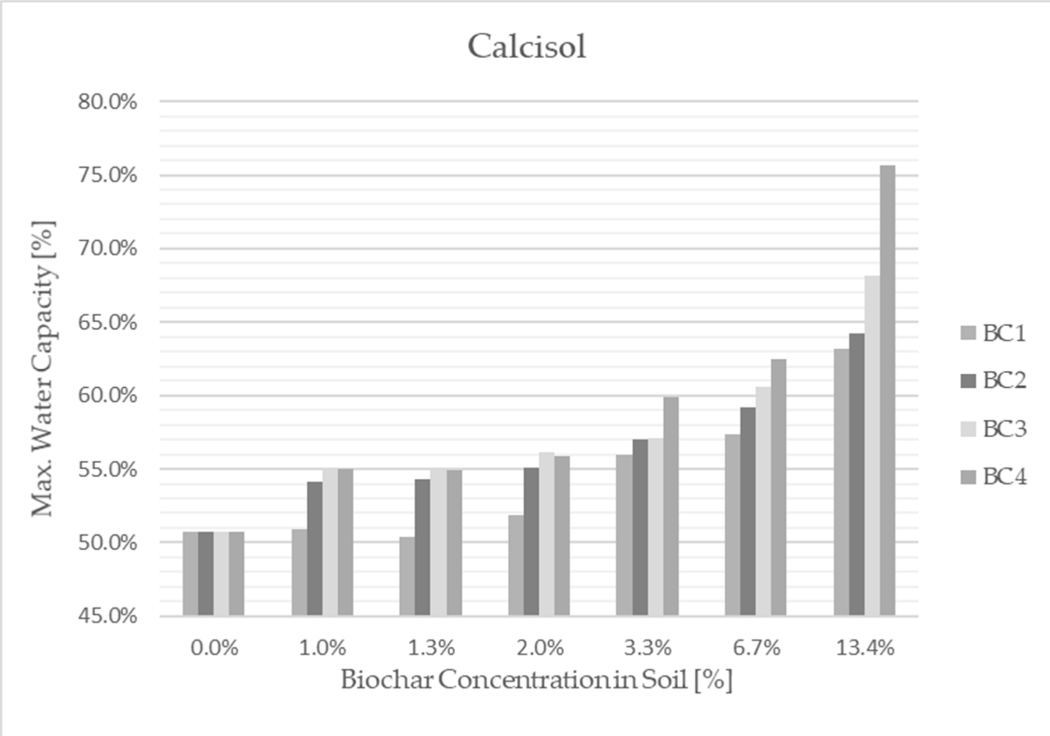



**Figure 31.** Histogram with mean $WC_{max.}$ values for Calcisol for all four biochars.

### 8.3.1 Direct comparison of biochars on three soil types

#### 8.3.1.1 Cambisol

All four biochars provoked an increase in the mean maximum water capacity when added to the cambisol soil. A clear tendency is visible that higher biochar ratios in soil consequently cause higher water capacity. In the first place, Biochar 4 showed highest effect on WC from 8.0 to 20.9 % (13 to 170 t $ha^{-1}$) with a mean increase of 3.5 %; followed by Biochar 2 with an increase from 6.7 to 19.3% (13 to 170 t $ha^{-1}$) with a mean increase of 3.2 %; followed by Biochar 3 with an increase from 8.1 to 18.9 % (13 to 170 t $ha^{-1}$) with a mean increase of 3.1 %, and the lowest increase gained with Biochar 1 from 7.7 to 15.3 % (13 to 170 t $ha^{-1}$) with a mean increase of 2.5 % (cp. Table 20).

With addition of 13 t $ha^{-1}$ of biochar to the cambisol, all biochars unleash a substantial increase in WC compared to control, but with a further increase in biochar additions in the range of 17 and 26 t $ha^{-1}$ a stagnation, even partial slight reduction of the WC in contrast to the predecessor is observed. Then, after a renewed increase in biochar additions in the range of 42, 85 and 170 t $ha^{-1}$ again a significant increase in the WC occurs. Measurement errors can be ruled out because all four repetitions confirm this "phenomenon". A simple and direct explanation seems difficult. It can be stated, however, that in comparison with the variant "no biochar in the soil" (control), with additions in the agro-economically feasible and toxicologically harmless range (cp. chapter 8.4.6.1) of 25 to 85 t biochar per hectare, water capacity increase between 4 and 12 % can be achieved.

**Table 20.** Cambisol: Calculation of $WC_{max.}$ delta (Δ) between each concentration and calculation of accumulated $WC_{max.}$ increase (Σ) from Control to highest concentration for all biochars.

| Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| BC Content [t/ha] | 0 | 13 | 17 | 26 | 42 | 85 | 170 |
| BC Concentr. | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.4% |
| WC - Biochar 1 | 45.6% | 53.3% | 51.9% | 49.6% | 54.2% | 57.1% | 60.8% |
| **Δ-predecessor** | | **7.7%** | **-1.4%** | **-2.3%** | **4.6%** | **2.9%** | **3.7%** |
| **Σ-cummulative** | | **7.7%** | **6.4%** | **4.0%** | **8.6%** | **11.6%** | **15.3%** |
| WC - BC2 | 45.6% | 52.2% | 52.2% | 52.5% | 54.7% | 56.7% | 64.8% |
| **Δ-predecessor** | | **6.7%** | **-0.1%** | **0.3%** | **2.2%** | **2.0%** | **8.2%** |
| **Σ-cummulative** | | **6.7%** | **6.6%** | **6.9%** | **9.1%** | **11.1%** | **19.3%** |
| WC - BC3 | 45.6% | 53.6% | 54.5% | 53.7% | 56.2% | 57.2% | 64.4% |
| **Δ-predecessor** | | **8.1%** | **0.9%** | **-0.8%** | **2.5%** | **1.0%** | **7.3%** |
| **Σ-cummulative** | | **8.1%** | **9.0%** | **8.2%** | **10.7%** | **11.6%** | **18.9%** |
| WC - BC4 | 45.6% | 53.6% | 53.6% | 53.6% | 56.0% | 56.9% | 66.4% |
| **Δ-predecessor** | | **8.0%** | **0.0%** | **0.1%** | **2.3%** | **0.9%** | **9.5%** |
| **Σ-cummulative** | | **8.0%** | **8.0%** | **8.1%** | **10.4%** | **11.4%** | **20.9%** |

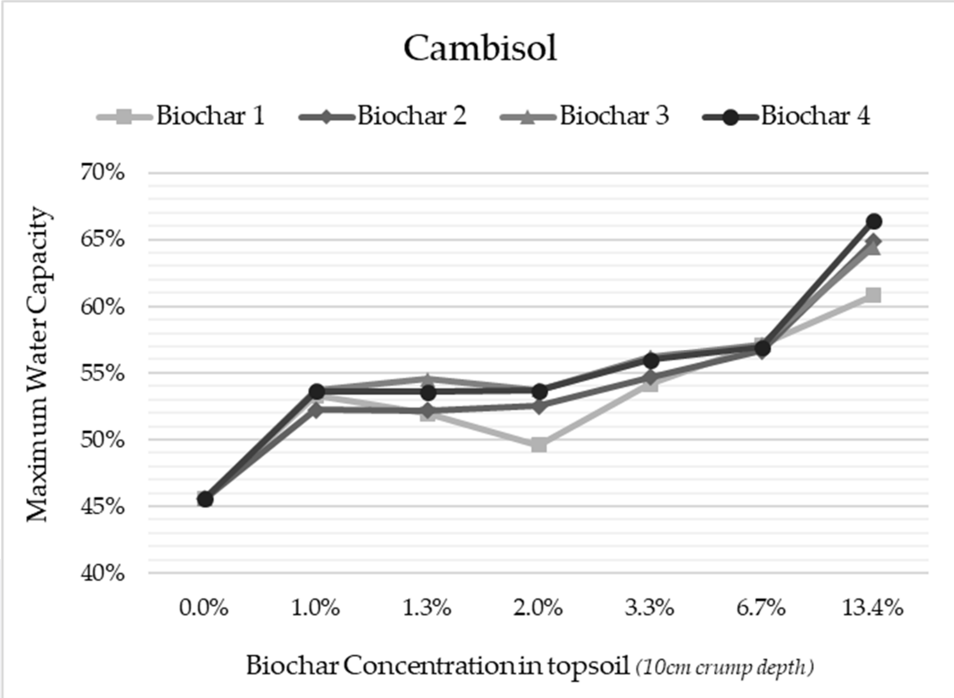



**Figure 32.** Graph comparison of water retention potential for all biochars in **Cambisol**.

**Table 21.** Mean Maximum Water Capacity values for all biochars with **Cambisol** at six different admixtures and control.

| Biochar Soil Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| Biochar Share in Soil [t/ha] | 0 | 12.5 | 17 | 25.5 | 42 | 85 | 170 |
| Biochar Share in Soil [%] | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.4% |
| Biochar 1 | 45.6% | 53.3% | 51.9% | 49.6% | 54.2% | 57.1% | 60.8% |
| Biochar 2 | 45.6% | 52.2% | 52.2% | 52.5% | 54.7% | 56.7% | 64.8% |
| Biochar 3 | 45.6% | 53.6% | 54.5% | 53.7% | 56.2% | 57.2% | 64.4% |
| Biochar 4 | 45.6% | 53.6% | 53.6% | 53.6% | 56.0% | 56.9% | 66.4% |

#### 8.3.1.2 Phaeozem

All four biochars provoked an increase in the mean maximum water capacity when added to the phaeozem soil. A clear tendency is visible that higher biochar ratios in soil consequently cause higher water capacity. In the first place, Biochar 4 showed highest effect on water capacity from 1.4 to 22.2 % (14 to 182 t $ha^{-1}$) with a mean increase of 3.7 %; followed by Biochar 3 with an increase from 2.4 to 16.7 % (14 to 182 t $ha^{-1}$) with a mean increase of 2.8%; followed by Biochar 2 with an increase from 3.3 to 15.9 %

(14 to 182 t ha$^{-1}$) with a mean increase of 2.6 %, and the lowest increase gained with Biochar 1 from 1.4 to 12.6 % (14 to 182 t ha$^{-1}$) with a mean increase of 2.1 % (see Table 22).

With addition of 14 t ha$^{-1}$ of biochar to the phaeozem, all biochars unleash a sub-stantial increase in water capacity compared to control, but with a further increase in biochar additions (different to cambisol) only for biochar 1 to 3 in the range of 18 and 28 t ha$^{-1}$ only very limited increase, even partial slight reduction of the water capacity in contrast to the predecessor is observed. Then, after a renewed increase in biochar additions in the range of 46, 91 and 182 t ha$^{-1}$ again a significant increase in the water capacity occurs. Measurement errors can be ruled out because all four repetitions confirm this "phenomenon".

A simple and direct explanation seems difficult. It can be stated, however, that in comparison with the variant "no biochar in the soil" (control), with additions in the agro-economically viable and toxicologically harmless range (cp. chapter 8.4.6.1) of 28 to 91 t biochar per hectare, water capacity increase between 2.8 and 11.1 % can be achieved, which is a bid less than in phaeozem.

**Table 22.** Phaeozem: Calculation of $WC_{max.}$ delta (Δ) between each concentration and calculation of accumulated $WC_{max.}$ increase (Σ) from Control to highest concentration for all biochars.

| Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| BC Content [t/ha] | 0 | 14 | 18 | 28 | 46 | 91 | 182 |
| BC Concentr. | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.3% |
| WC - BC1 | 47.7% | 49.1% | 48.1% | 50.8% | 52.8% | 54.7% | 60.3% |
| **Δ**-predecessor | | **1.4%** | **-0.9%** | **2.6%** | **2.0%** | **1.9%** | **5.6%** |
| **Σ**-cummulative | | **1.4%** | **0.4%** | **3.1%** | **5.1%** | **7.0%** | **12.6%** |
| WC - BC2 | 47.7% | 51.0% | 51.1% | 50.5% | 54.1% | 57.5% | 63.6% |
| **Δ**-predecessor | | **3.3%** | **0.1%** | **-0.5%** | **3.5%** | **3.5%** | **6.0%** |
| **Σ**-cummulative | | **3.3%** | **3.4%** | **2.8%** | **6.4%** | **9.8%** | **15.9%** |
| WC - BC3 | 47.7% | 50.0% | 49.2% | 50.8% | 52.8% | 55.3% | 64.4% |
| **Δ**-predecessor | | **2.4%** | **-0.9%** | **1.6%** | **2.0%** | **2.5%** | **9.1%** |
| **Σ**-cummulative | | **2.4%** | **1.5%** | **3.1%** | **5.1%** | **7.6%** | **16.7%** |
| WC - BC4 | 47.7% | 49.1% | 50.8% | 52.8% | 53.8% | 58.8% | 69.9% |
| **Δ**-predecessor | | **1.4%** | **1.6%** | **2.0%** | **1.0%** | **5.0%** | **11.1%** |
| **Σ**-cummulative | | **1.4%** | **3.1%** | **5.1%** | **6.1%** | **11.1%** | **22.2%** |

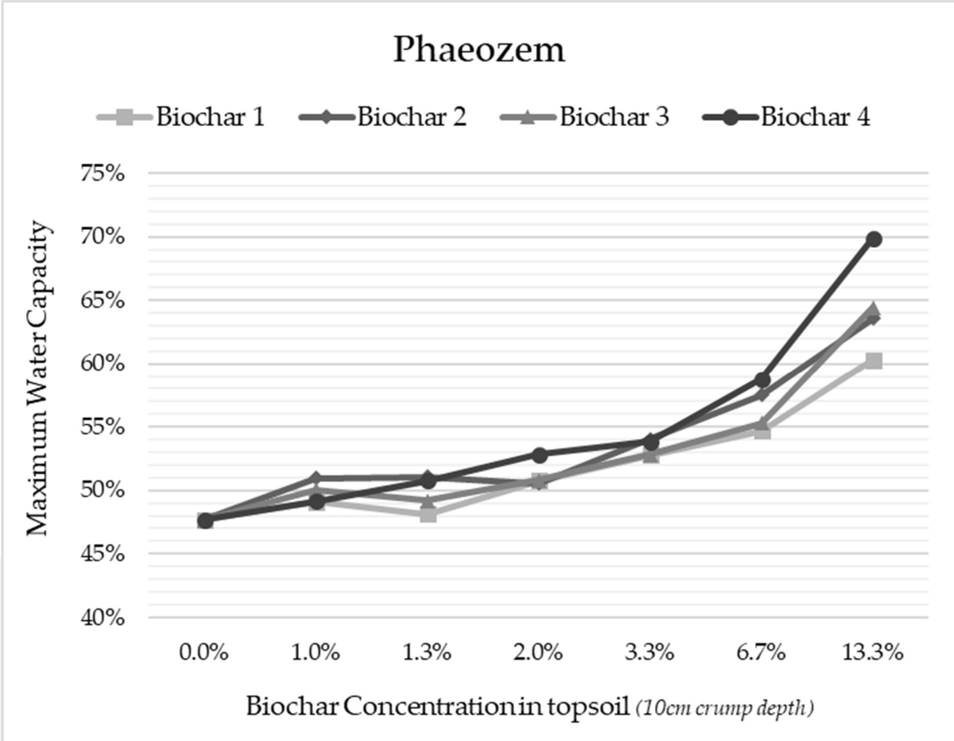



**Figure 33.** Graph comparison of water retention potential for all biochars in **Phaeozem**.

**Table 23.** Mean Maximum Water Capacity values for all biochars with **Phaeozem** at six different admixtures and control.

| Biochar Soil Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| Biochar Share in Soil [t/ha] | 0 | 13.5 | 18 | 27.5 | 45.5 | 91 | 182 |
| Biochar Share in Soil [%] | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.3% |
| Biochar 1 | 47.7% | 49.1% | 48.1% | 50.8% | 52.8% | 54.7% | 60.3% |
| Biochar 2 | 47.7% | 51.0% | 51.1% | 50.5% | 54.1% | 57.5% | 63.6% |
| Biochar 3 | 47.7% | 50.0% | 49.2% | 50.8% | 52.8% | 55.3% | 64.4% |
| Biochar 4 | 47.7% | 49.1% | 50.8% | 52.8% | 53.8% | 58.8% | 69.9% |

#### 8.3.1.3 Calcisol

All four biochars provoked an increase in the mean maximum water capacity when added to the calcisol soil. A clear tendency is visible that higher biochar ratios in soil consequently cause higher water capacity. In the first place, Biochar 4 showed highest effect on water capacity from 4.3 to 24.9 % (11 to 147 t $ha^{-1}$) with a mean increase of 4.2 %; followed by Biochar 3 with an increase from 4.4 to 17.5% (11 to 147 t $ha^{-1}$) with a mean increase of 2.9 %; followed by Biochar 2 with an increase from 3.4 to 13.5% (11 to 147 t ha$^{-1}$) with a mean increase of 2.3%, and the lowest increase gained with Biochar 1 from 0.2 to 12.5% (11 to 147 t ha$^{-1}$) with a mean increase of 2.1% (see Table 24).

All biochars unleash a substantial increase in WC compared to control when added to calcisol, and in contrast to phaeozem and cambisol only for biochar 1 at a ratio of 1:75 a decrease in the WC in contrast to the predecessor was observed. Biochars increase statically WC in calcisol, when biochar concentration is enlarged. It can be stated, however, that in comparison with the variant "no biochar in the soil" (control), with additions in the agro-economically viable and toxicologically harmless range (cp. chapter 8.4.6.1) of 22 to 73 t biochar per hectare, water capacity increase between 1.2 and 11.8 % can be achieved, which is a bid less than in calcisol soil.

**Table 24.** Calcisol: Calculation of $WC_{max.}$ delta (Δ) between each concentration and calculation of accumulated $WC_{max.}$ increase (Σ) from Control to highest concentration for all biochars.

| Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| BC Content [t/ha] | 0 | 11 | 15 | 22 | 37 | 73 | 147 |
| BC Concentr. | 0.0% | 1.0% | 1.3% | 2.0% | 3.4% | 6.6% | 13.4% |
| WC - BC1 | 50.7% | 50.9% | 50.4% | 51.9% | 56.0% | 57.4% | 63.2% |
| **Δ-predecessor** | | **0.2%** | **-0.5%** | **1.5%** | **4.1%** | **1.3%** | **5.8%** |
| **Σ-cummulative** | | **0.2%** | **-0.3%** | **1.2%** | **5.3%** | **6.6%** | **12.5%** |
| WC - BC2 | 50.7% | 54.2% | 54.3% | 55.1% | 57.0% | 59.2% | 64.2% |
| **Δ-predecessor** | | **3.4%** | **0.2%** | **0.7%** | **1.9%** | **2.2%** | **5.1%** |
| **Σ-cummulative** | | **3.4%** | **3.6%** | **4.4%** | **6.3%** | **8.4%** | **13.5%** |
| WC - BC3 | 50.7% | 55.1% | 55.1% | 56.2% | 57.1% | 60.6% | 68.2% |
| **Δ-predecessor** | | **4.4%** | **0.0%** | **1.1%** | **0.9%** | **3.5%** | **7.6%** |
| **Σ-cummulative** | | **4.4%** | **4.4%** | **5.5%** | **6.4%** | **9.9%** | **17.5%** |
| WC - BC4 | 50.7% | 55.0% | 55.0% | 55.8% | 59.9% | 62.5% | 75.6% |
| **Δ-predecessor** | | **4.3%** | **0.0%** | **0.9%** | **4.0%** | **2.6%** | **13.2%** |
| **Σ-cummulative** | | **4.3%** | **4.2%** | **5.1%** | **9.2%** | **11.8%** | **24.9%** |

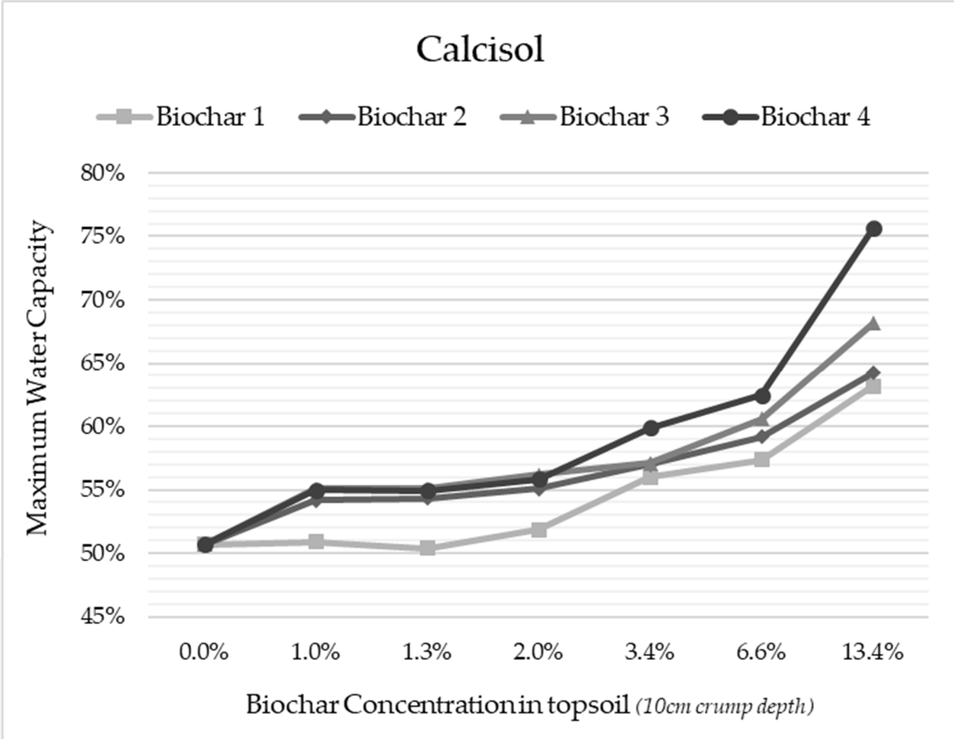



**Figure 34.** Graph comparison of water retention potential for all biochars in **Calcisol**.

**Table 25.** Mean Maximum Water Capacity values for all biochars with **Calcisol** at six different admixtures and control.

| Biochar Soil Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| Biochar Share in Soil [t/ha] | 0 | 11 | 14.5 | 22 | 37 | 73 | 147 |
| Biochar Share in Soil [%] | 0.0% | 1.0% | 1.3% | 2.0% | 3.4% | 6.6% | 13.4% |
| Biochar 1 | 50.7% | 50.9% | 50.4% | 51.9% | 56.0% | 57.4% | 63.2% |
| Biochar 2 | 50.7% | 54.2% | 54.3% | 55.1% | 57.0% | 59.2% | 64.2% |
| Biochar 3 | 50.7% | 55.1% | 55.1% | 56.2% | 57.1% | 60.6% | 68.2% |
| Biochar 4 | 50.7% | 55.0% | 55.0% | 55.8% | 59.9% | 62.5% | 75.6% |

### 8.3.2 Direct comparison of three soil types on each biochar

#### 8.3.2.1 Biochar 1

The data is summarized in Table 26 and Figure 35.

**Table 26.** Mean Maximum Water Capacity values for Cambisol, Phaeozem and Calcisol with **biochar No.1** at six different admixtures and control.

| Biochar Soil Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| Biochar Share in Soil [%] | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.4% |
| Cambisol | 45.6% | 53.3% | 51.9% | 49.6% | 54.2% | 57.1% | 60.8% |
| Phaeozem | 47.7% | 49.1% | 48.1% | 50.8% | 52.8% | 54.7% | 60.3% |
| Calcisol | 50.7% | 50.9% | 50.4% | 51.9% | 56.0% | 57.4% | 63.2% |

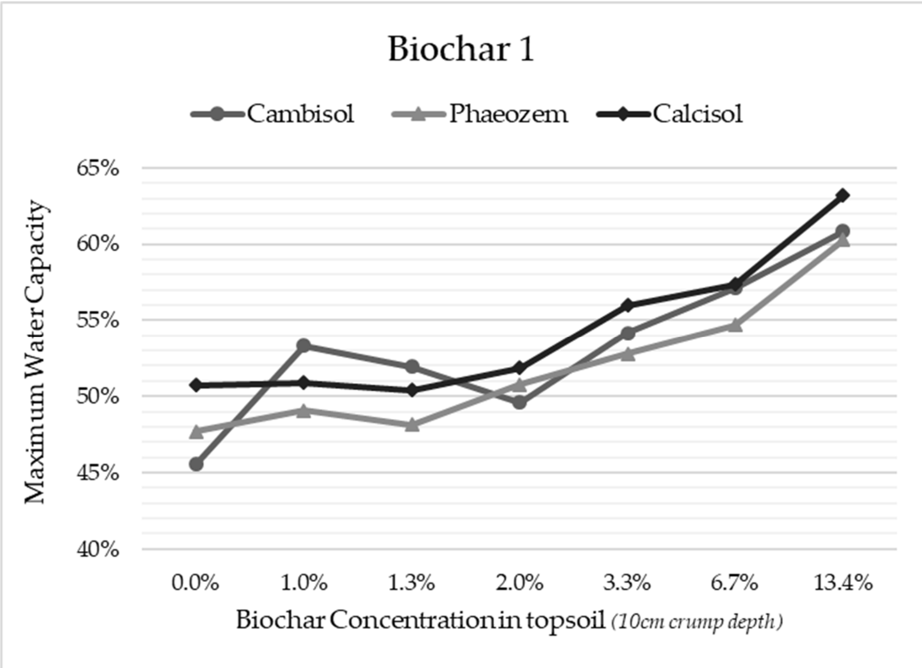



**Figure 35.** Graph comparison of water retention potential for selected soil types with Biochar **1**.

### 8.3.2.2 Biochar 2

The data is summarized in Table 27 and Figure 36.

**Table 27.** Mean Maximum Water Capacity values for Cambisol, Phaeozem and Calcisol with **biochar No. 2** at six different admixtures and control.

| Biochar Soil Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| Biochar Share in Soil [%] | 0.0% | 1.0% | 1.3% | 2.0% | 3.4% | 6.6% | 13.4% |
| Cambisol | 45.6% | 52.2% | 52.2% | 52.5% | 54.7% | 56.7% | 64.8% |
| Phaeozem | 47.7% | 51.0% | 51.1% | 50.5% | 54.1% | 57.5% | 63.6% |
| Calcisol | 50.7% | 54.2% | 54.3% | 55.1% | 57.0% | 59.2% | 64.2% |

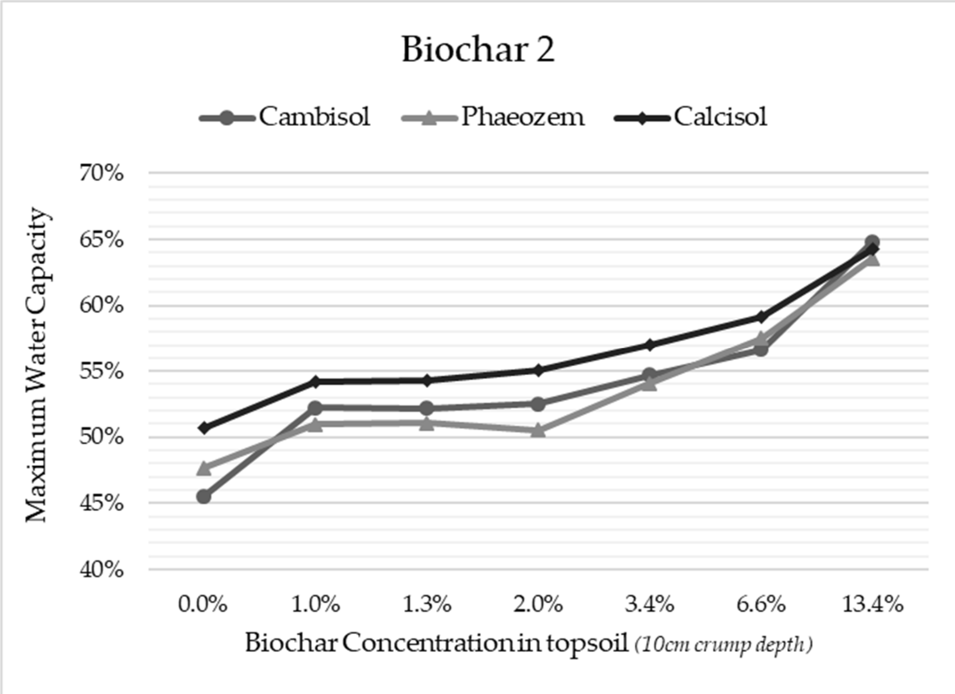



**Figure 36.** Graph comparison of water retention potential for selected soil types with Biochar **2**.

8.3.2.3 Biochar 3

The data is summarized in Table 28 and Figure 37.

**Table 28.** Mean Maximum Water Capacity values for Cambisol, Phaeozem and Calcisol with **Biochar No.3** at six different admixtures and control.

| Biochar Soil Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| Biochar Share in Soil [%] | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.4% |
| Cambisol | 45.6% | 53.6% | 54.5% | 53.7% | 56.2% | 57.2% | 64.4% |
| Phaeozem | 47.7% | 50.0% | 49.2% | 50.8% | 52.8% | 55.3% | 64.4% |
| Calcisol | 50.7% | 55.1% | 55.1% | 56.2% | 57.1% | 60.6% | 68.2% |

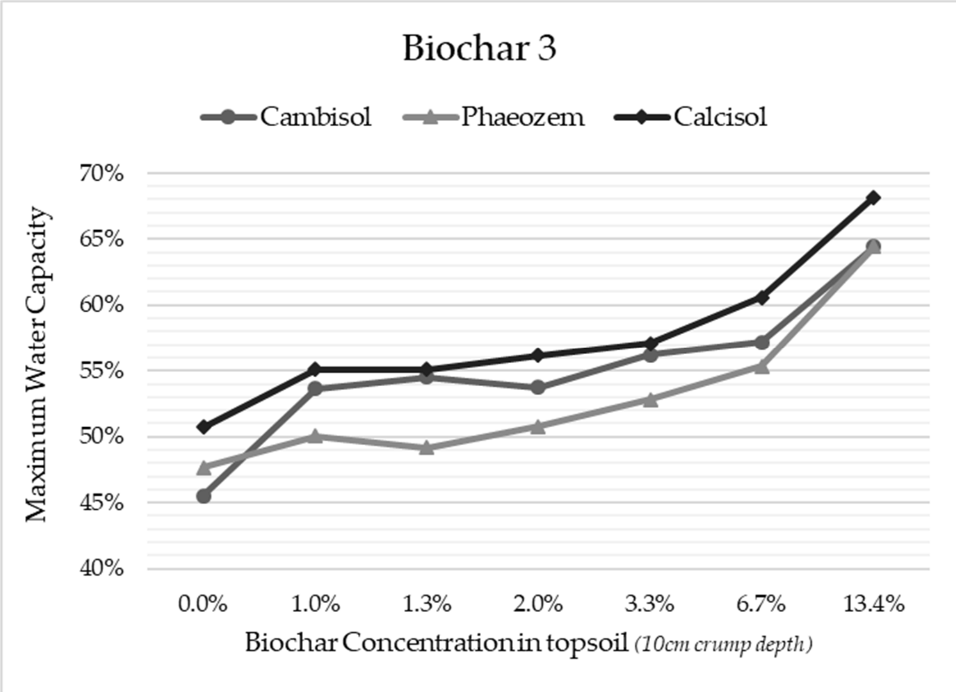



**Figure 37.** Graph comparison of water retention potential for selected soil types with Biochar **3**.

#### 8.3.2.4 Biochar 4

The data is summarized in Table 29Figure 38.

**Table 29.** Mean Maximum Water Capacity values for Cambisol, Phaeozem and Calcisol with **Biochar No.4** at six different admixtures and control.

| Biochar Soil Ratio [g/g] | Control | 1 : 100 | 1 : 75 | 1 : 50 | 1 : 30 | 1 : 15 | 1 : 7.5 |
|---|---|---|---|---|---|---|---|
| Biochar Share in Soil [%] | 0.0% | 1.0% | 1.3% | 2.0% | 3.3% | 6.7% | 13.4% |
| Cambisol | 45.6% | 53.6% | 53.6% | 53.6% | 56.0% | 56.9% | 66.4% |
| Phaeozem | 47.7% | 49.1% | 50.8% | 52.8% | 53.8% | 58.8% | 69.9% |
| Calcisol | 50.7% | 55.0% | 55.0% | 55.8% | 59.9% | 62.5% | 75.6% |

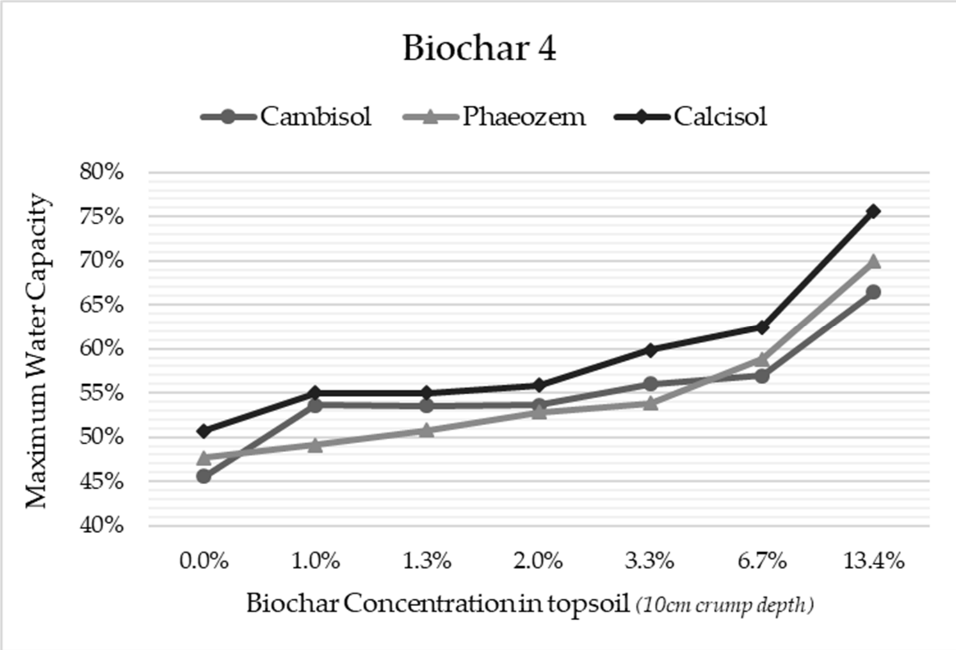



**Figure 38.** Graph comparison of water retention potential for selected soil types with Biochar **4**.

### 8.3.3 Chapter Discussion of the $WC_{max.}$ Test Results

The soil maximum water capacity (also known as water holding capacity, field capacity or water retention capacity) is defined as the difference between the water contents at field capacity and wilting point [154]. It is expressed in mm, cm or % of water for a given depth of certain soil; this characteristic is specific to the vegetation production, climate characteristics and other parameters [155]. Biochar potentially is able to alter soil hydrology whereby these changes provoke significant alterations in the water cycle and the soil-water-matrix based ecosystem. As soil water is essential for soil microbial processes, an increase in water availability will affect soil fauna positively [34]. Biochar has been found to decrease nutrient leaching on its own as well as after incorporation within soil. With greater nutrient retention by biochar additions to soil, timing of nutrient applications will become less critical [156,157]. Considering that plants to major extent receive nutrients solved with water uptake, an increased soil water capacity consequently increases nutrient provision.

Glaser et al. [17] reported that increased field capacity of 18% was observed in anthrosols rich in charcoal particles with surface areas three times higher than the surrounding soils. Already in the 1940s Tryon [112] presented similar results for sandy soil types, with charcoal addition 45 % (v/v). Liu et al. [48] reported a doubled plant-available WHC in a biochar : compost amended cambisol at 32.5 t $ha^{-1}$ compost mixed with 20 % biochar.

The physical characteristics of biochar suggest that it can change the pore-size distribution of the soil and possibly alter percolation patterns, residence times of soil solution and flow paths. Since the porosity of biochar largely consists of micro pores, the actual amount of additional plant available water will depend on the biochar feedstock and the texture of the soil it is applied to. The agronomic water-storage benefit of biochar application will thus dependent on the relative modification of the proportion of micro, meso and macro pores in the root zone. In sandy soils, the additional volume of water and soluble nutrients stored in the biochar micro pores may become available as the soil dries and the matric potential increases. This may lead to increased plant water availability during dry periods.

The present study results show that biochars produced from local woods have the ability to increase the water storage capacity of local soils. It is striking that even small addition from 1 percent by weight increase the storage capacity of the soils by up to 10 %. Further increases in biochar concentrations up to 13 percent by weight further increase the water capacity up to 25 %, but at a lower ratio to the input. The overall increase follows a degressive curve. Biochar 4 provokes the highest water increase in all soils, followed by Biochar 3, Biochar 2 and Biochar 1. In comparison to the WHC of the pure biochars, other results were expected, however the study underlies that measurements of pure biochar WHC are not necessarily relevant to the final $WC_{max.}$ of the final mixed biochar-soil substrate.

According to the law of Liebig, predominantly water constitutes the limiting factor in Aguascalientes agriculture. The present research work demonstrated that improving water storage capacity of soil by adding biochar constitutes a promising option. If Liebig's law applies, a farmer would strive for the maximum application of biochar contingent, to reach for the highest maximum water capacity increase possible, in order to minimize the limiting factor as far as possible. However, instead of applying the maximum amount of biochar possible to a specific terrain, an agronomist first would try to identify the input-output ratio where the optimum user performance ratio (OUPR) is achieved.

In simple words, which concentration of biochar in soil provokes the highest water capacity increase per ton of biochar applied? The respective unit is %WC/t, whereby %WC is percentage increase of water capacity and t is ton of biochar added.

Table 30 shows the results of this assignment. Cambisol for all four biochars has its optimum user performance ratio at 1 % (12.3 t $ha^{-1}$) biochar concentration, whereby %WC/t is highest for all four biochars. The ranking of biochars is BC3> BC4> BC1> BC2, from highest to lowest increase in water capacity. The optimum user performance ratio for the Phaeozem is found distributed over different concentrations for each biochar. For biochar 1 and 4 %WC/t is highest at a concentration of 2.0 % (27.5 t $ha^{-1}$). For biochar 2 and 3 %WC/t is highest at 1.0% (13.5 t $ha^{-1}$). The ranking of biochars is BC2> BC4> BC> BC1, from highest to lowest increase in water capacity. The optimum user performance ratio for the Calcisol lies at 1.0 % (11.0 t $ha^{-1}$) for biochar 2 to 4, whereby the highest %WC/t for biochar 1 is located at 2.0 % (37 t $ha^{-1}$). The ranking of biochars is BC4/3> BC2> BC1, from highest to lowest increase in water capacity.

Considering these numbers, an agronomist would closely stick to best %WC/t. However if Liebig's law applies it is still "agronomic" to apply higher quantities of biochar, in order to increase the water capacity and tackle water paucity.

**Table 30.** OUPR calculation of the four biochars in three soils. Numbers framed in black show the highest OUPR per soil and coal.

| Biochar Soil Ratio [g/g] | | Control | 1 : 100 | **0.0-1.0** | 1 : 75 | **1.0-1.3** | 1 : 50 | **1.3-2.0** | 1 : 30 | **2.0-3.3** | 1 : 15 | **3.3-6.7** | 1 : 7.5 | **6.7-13.4** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biochar Share in Soil [%] | | 0.0% | 1.0% | | 1.3% | | 2.0% | | 3.3% | | 6.7% | | 13.4% | |
| Biochar Share in soil [t/ha] | | 0 | 12.3 | **%WC/t** | 16.5 | **%WC/t** | 25.0 | **%WC/t** | 41.5 | **%WC/t** | 83.0 | **%WC/t** | 166.3 | **%WC/t** |
| | Biochar 1 | 45.6% | 53.3% | **0.63%** | 51.9% | **0.39%** | 49.6% | **0.16%** | 54.2% | **0.21%** | 57.1% | **0.14%** | 60.8% | **0.09%** |
| **Cambisol** | Biochar 2 | 45.6% | 52.2% | **0.54%** | 52.2% | **0.40%** | 52.5% | **0.28%** | 54.7% | **0.22%** | 56.7% | **0.13%** | 64.8% | **0.12%** |
| $WC_{max.}$ [%] | Biochar 3 | 45.6% | 53.6% | **0.66%** | 54.5% | **0.54%** | 53.7% | **0.33%** | 56.2% | **0.26%** | 57.2% | **0.14%** | 64.4% | **0.11%** |
| | Biochar 4 | 45.6% | 53.6% | **0.65%** | 53.6% | **0.49%** | 53.6% | **0.32%** | 56.0% | **0.25%** | 56.9% | **0.14%** | 66.4% | **0.13%** |
| Biochar Share in soil [t/ha] | | 0 | 13.5 | **%WC/t** | 18.0 | **%WC/t** | 27.5 | **%WC/t** | 45.5 | **%WC/t** | 91.0 | **%WC/t** | 182.0 | **%WC/t** |
| | Biochar 1 | 47.7% | 49.1% | **0.10%** | 48.1% | **0.02%** | 50.8% | **0.11%** | 52.8% | **0.11%** | 54.7% | **0.08%** | 60.3% | **0.07%** |
| **Phaeozem** | Biochar 2 | 47.7% | 51.0% | **0.24%** | 51.1% | **0.19%** | 50.5% | **0.10%** | 54.1% | **0.14%** | 57.5% | **0.11%** | 63.6% | **0.09%** |
| $WC_{max.}$ [%] | Biochar 3 | 47.7% | 50.0% | **0.17%** | 49.2% | **0.08%** | 50.8% | **0.11%** | 52.8% | **0.11%** | 55.3% | **0.08%** | 64.4% | **0.09%** |
| | Biochar 4 | 47.7% | 49.1% | **0.11%** | 50.8% | **0.17%** | 52.8% | **0.19%** | 53.8% | **0.14%** | 58.8% | **0.12%** | 69.9% | **0.12%** |
| Biochar Share in soil [t/ha] | | 0 | 11.0 | **%WC/t** | 14.5 | **%WC/t** | 22.0 | **%WC/t** | 37.0 | **%WC/t** | 73.0 | **%WC/t** | 147.0 | **%WC/t** |
| | Biochar 1 | 50.7% | 50.9% | **0.02%** | 50.4% | **-0.02%** | 51.9% | **0.05%** | 56.0% | **0.14%** | 57.4% | **0.09%** | 63.2% | **0.08%** |
| **Calcisol** | Biochar 2 | 50.7% | 54.2% | **0.31%** | 54.3% | **0.25%** | 55.1% | **0.20%** | 57.0% | **0.17%** | 59.2% | **0.12%** | 64.2% | **0.09%** |
| $WC_{max.}$ [%] | Biochar 3 | 50.7% | 55.1% | **0.40%** | 55.1% | **0.30%** | 56.2% | **0.25%** | 57.1% | **0.17%** | 60.6% | **0.14%** | 68.2% | **0.12%** |
| | Biochar 4 | 50.7% | 55.0% | **0.39%** | 55.0% | **0.29%** | 55.8% | **0.23%** | 59.9% | **0.25%** | 62.5% | **0.16%** | 75.6% | **0.17%** |

## 8.4 Toxicity Tests

### 8.4.1 *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 biochar elutriate. In the sublethal tests no growth inhibition was detected after 96 h in each biochar treatment with no dilution (elutriate at 100%) (Table 31).

**Table 31.** 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 |

In contrast to expected mortality rates with *Paramecium caudatum*, a growth in population was observed. After five days during cleaning of the microplates, some wells presented substantial growth in population. As no food was given to the organisms, it was not expected to find an increased population. Instead, organisms were observed that tapped biochar particles continuously with their mouth. Potentially some bacteria, which is the main food source for the ciliate, entered the microplates after the test run and used the biochar surface as growth medium.

### 8.4.2 *Lecane quadridentata* 48 h Acute and Sublethal Toxicity Tests

Lethal toxicity was detected when *Lecane quadridentata* was exposed to all four biochars (Table 32). Many particles of biochar in the stomach and digestive apparatus of *Lecane quadridentata* were observed (Figure 39).

**Table 32.** Acute toxicity values of *Lecane quadridentata* to four biochars.

| Parameter | Biochar 1 | Biochar 2 | Biochar 3 | Biochar 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 |

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.

$LC_{50}$ value in the range of 8.3 % to 25.1 % of effective concentration for *Lecane quadridentata* were obtained. 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 20 % as the maximum allowed threshold. Detected order of susceptibility: Biochar 3 > Biochar 4 > Biochar 1 > Biochar 2 (lowest to highest toxicity). No sublethal tests were conducted with *Lecane quadridentata* with the biochar elutriates since all biochars resulted in acute toxicity.

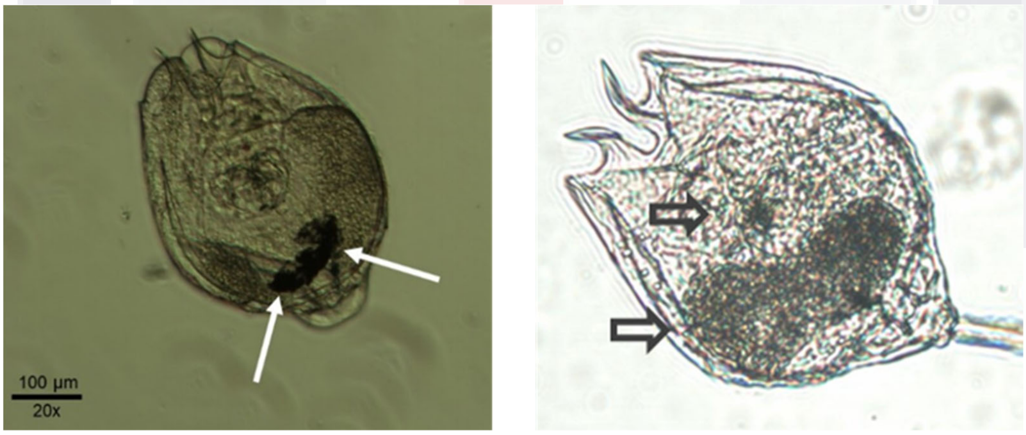



**Figure 39.** Photographs of dead *Lecane quadridentata*. Digestive tract filled with biochar particles as indicated by the arrows. (Resolution right: 40 amplifications; LEICA DLMS Moticam 2300 3.0 MPix). Universidad Autonoma de Aguascalientes, Flesch. F. Nov. 2017

The plotting of regressions helps to conduct a sound interpretation of the results. The coefficient of variation (CV) [also known as relative standard deviation (RSD)] values for all biochars are below 20 %. According to the Canadian norm for toxicity testing, CV values below that threshold prove, that the results are reliable and that the representative samples have been selected sound and that the analytical method performs well. Figure 40-43 show the regression plots for the four types of biochar.

Value $r^2$ is a measure of the strength of the linear regression. It indicates how well the independent variables are capable of explaining the variance of the dependent. The $r^2$ is always between 0% (or 0) [unusable model] and 100% (or 1) [perfect model adaptation] and is a measure of reliability for describing a linear relationship between the dependent and independent variable. Basically, $r^2$ values above 70 % [or 0.7] indicate that there is a confidential guarantee that the dependable variable relates to the independent variable, hence the model is sound (high model fit). In the present case using e.g. biochar 4, $r^2$ value is 0.811 (or 81.1%) (Figure 43), which means, that there is 81% reliability, that 50 % of the exposed animals die, because of the biochar. Unfortunately, $r^2$ values for Biochar 1 and 2 are below 70 %, indicating or a poor model fit (Figure 40 and Figure 41). As the present toxicity test does not work with identical clones, the $r^2$ values are anyhow confidential, even with the idea that twins still have differences.

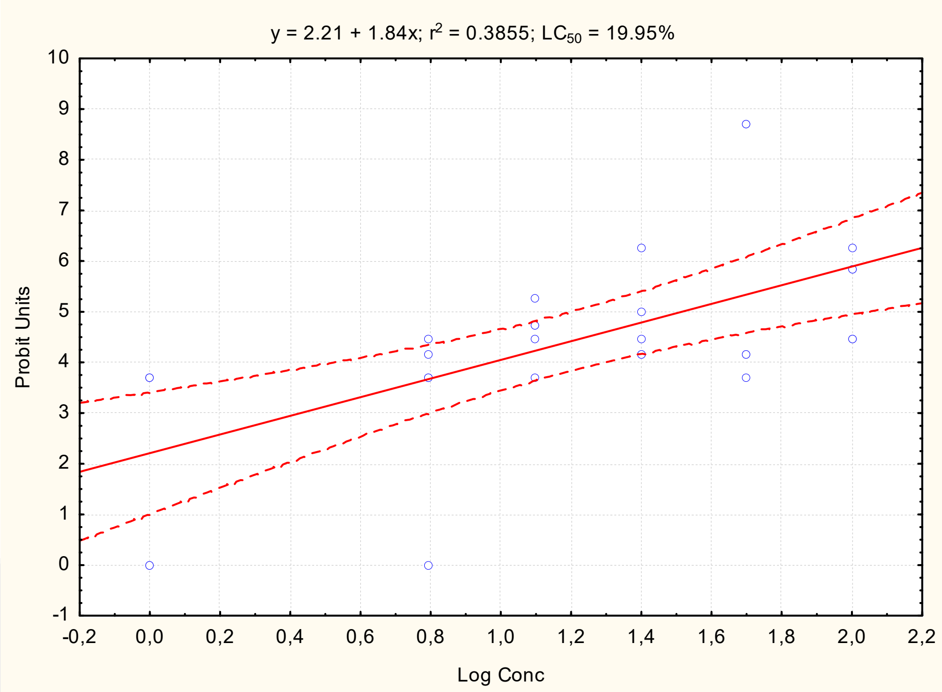



**Figure 40.** LC50 regression graph with *Lecane quadridentata* for Biochar **No. 1**. (Abscissa) Log Conc = decadic logarithm of elutriate concentration; (Ordinate) Probit Units = number of death animals.

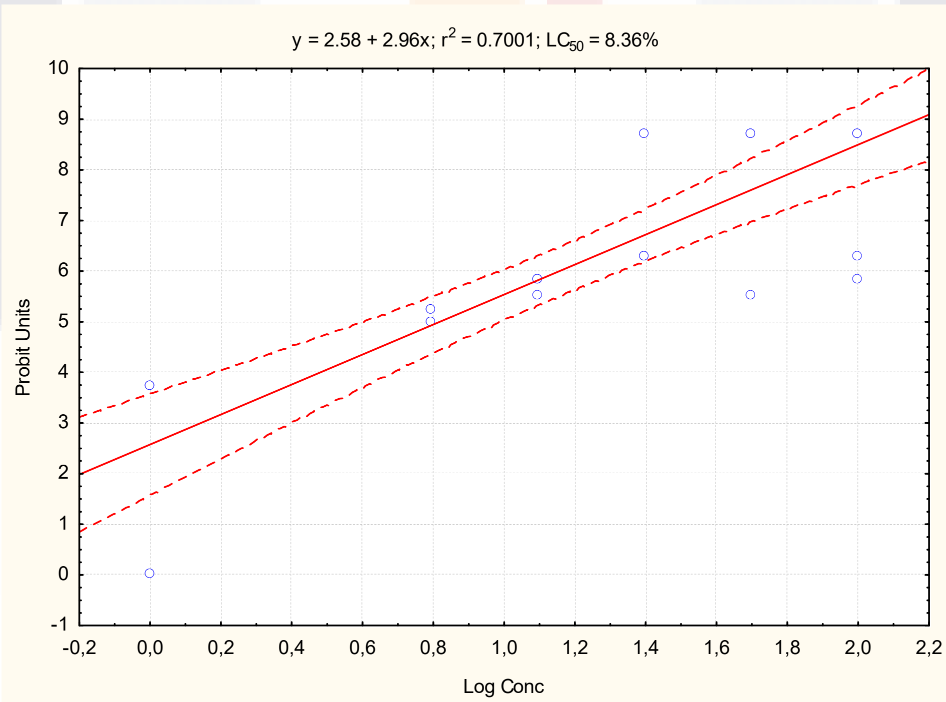



**Figure 41.** LC50 regression graph with *Lecane quadridentata* for Biochar **No. 2**. (Abscissa) Log Conc = decadic logarithm of elutriate concentration; (Ordinate) Probit Units = number of death animals.

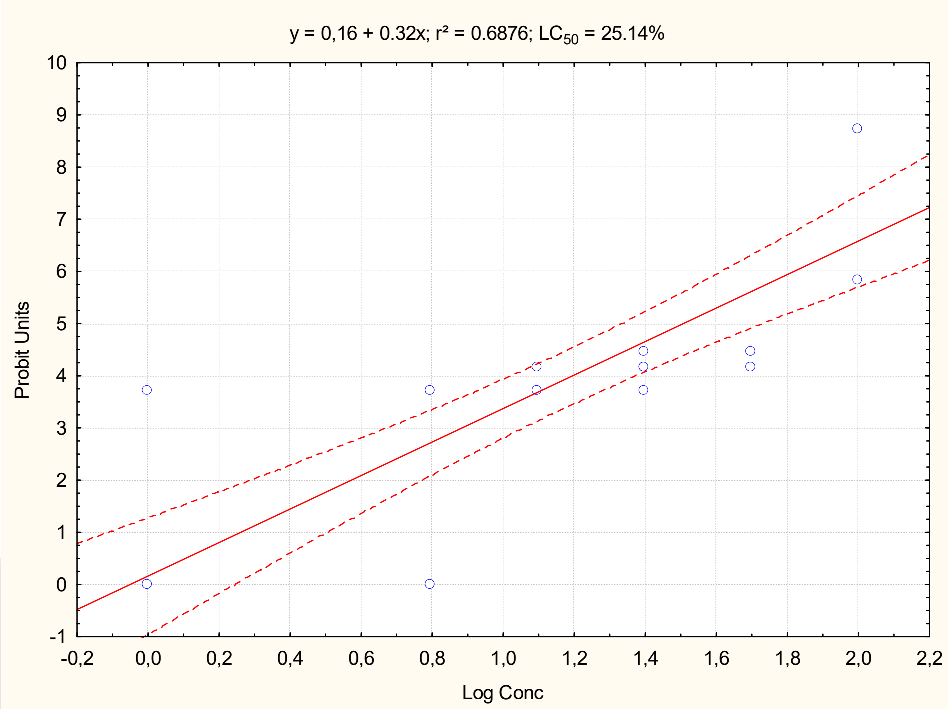



**Figure 42.** $LC_{50}$ regression graph with *Lecane quadridentata* for Biochar **No. 3**. (Abscissa) Log Conc = decadic logarithm of elutriate concentration; (Ordinate) Probit Units = number of death animals.

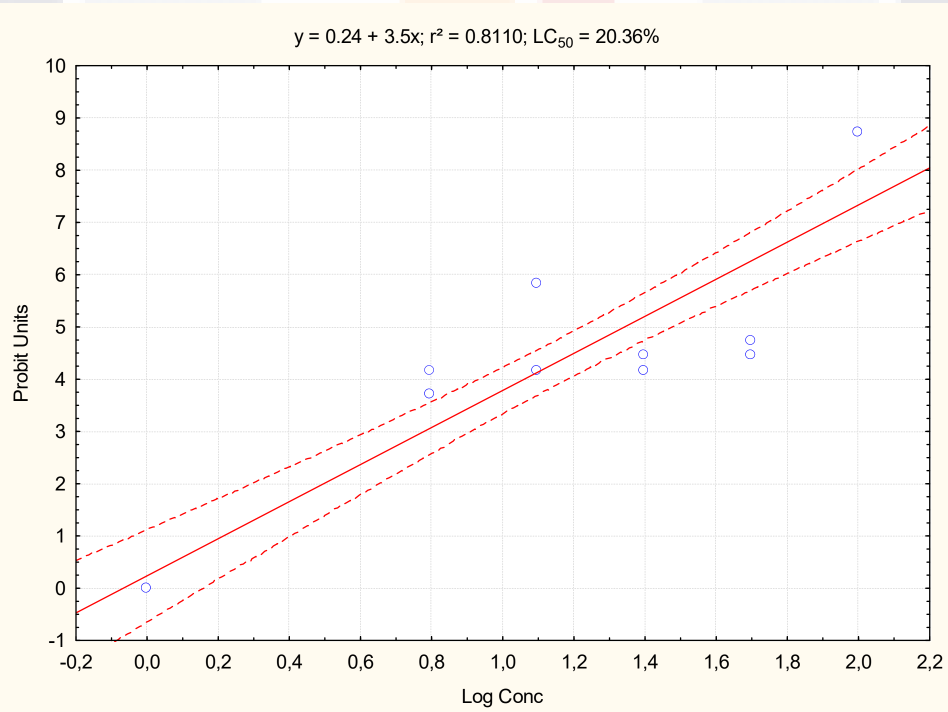



**Figure 43.** $LC_{50}$ regression graph with *Lecane quadridentata* for Biochar **No. 4**. (Abscissa) Log Conc = decadic logarithm of elutriate concentration; (Ordinate) Probit Units = number of death animals.

### 8.4.3 *Daphnia magna* 48 h Acute Toxicity Test

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

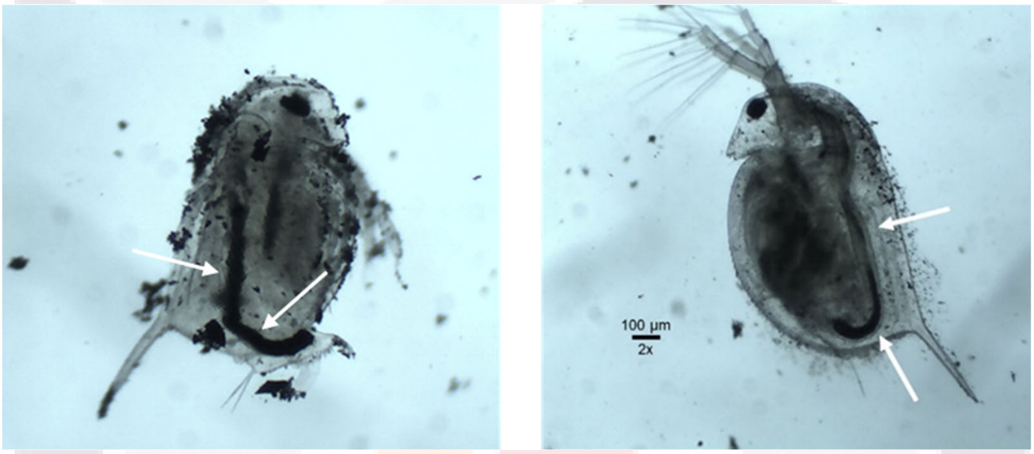



**Figure 44.** Photographs of dead *Daphnia magna*. Digestive tract filled with biochar particles as indicated by the white arrows. (Resolution left: 2 amplifications; LEICA DLMS Moticam 2,300 3.0 MPix). Universidad Autonoma de Aguascalientes, Flesch. F. Nov. 2017

### 8.4.4 *Moina macrocopa* 48 h Acute Toxicity Test

The digestive tract of *Moina macrocopa* was observed completely filled with biochar particles (Figure 45). Table 33 shows the results of the acute tests with *Moina macrocopa*. Low lethal toxicity levels were found with this cladocerans species which allow one to calculate $LC_{50}$ values only for two biochars. $LC_{50}$ value of effective concentration were obtained only for Biochar 2 and Biochar 3, with 134.87 % and 306.33 %, respectively.


**Figure 45.** Photograph of dead Moina Macrocopa. Digestive tract fully filled with biochar particles as indicated by the arrows. Universidad Autonoma de Aguascalientes, Flesch. F. Feb. 2019

**Table 33.** Acute toxicity values of *Moina Macrocopa* to four biochars.

| Parameter | Biochar 1 | Biochar 2 | Biochar 3 | Biochar 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 |

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 Biochar 1 and Biochar 4, it was decided to perform growth inhibition tests with this cladoceran species. Results of the sublethal growth inhibition tests are shown in Table 34. No sublethal toxicity was detected at the 100 % (no dilution) elutriate sample for Biochar 1 and Biochar 4 where no $EC_{50}$ values were calculated.

**Table 34.** Reproductive test with *Moina macrocopa* (7 days). *n* = 5.

| Treatment | Mean Value *r* |
|---|---|
| Control | 0.408 |
| Biochar 1 | 0.401 |
| Biochar 4 | 0.401 |
| Soil | 0.406 |

### 8.4.5 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 35 shows the growth inhibition sublethal tests with *L. quadridentata*, *M. macrocopa*, and *P. caudatum*.

**Table 35.** Results of the growth inhibition and reproductive tests with *Lecane quadridentata* (Lecane), *Moina macrocopa* (Moina), and *Paramecium caudatum* (Paramecium) exposed to the biochar:soil (1:8) elutriate. $n = 5$.

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

### 8.4.6 Chapter Discussion of the Toxicity Tests

#### 8.4.6.1 Toxicity of Biochar Elutriates to Aquatic Invertebrates

This experiment was conducted to investigate the toxicity on four aquatic invertebrate species exposed to four different biochars. We discovered that the respective biochars, 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 biochars.

Acute toxicity was only detected if the organisms were exposed to the pure biochar elutriate. When the organisms were exposed to elutriate obtained from a biochar–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 biochar 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 biochar does not guarantee 100% safety, particularly near sensitive habitats or with regard to biochar 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. [158] 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 biocharss 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) [79,113,142,158,159], the four biochars could be classified in their potential danger to each other in a direct comparison. Arranging the four biochars according to their toxicity potential, results in the following: Biochar 3 $<$ Biochar 2 $<$ Biochar 1 $<$ Biochar 4 (from low to high toxicity potential). Thus, biochar 4 is expected to generate the potentially lowest $LC_{50}$ values, causing the highest toxicity. Biochar 3 instead is expected to tend to result in the highest $LC_{50}$ values, causing the lowest toxicity to the organisms.

Interestingly the biochar with the highest concentrations of hazardous substances (e.g. PAHs), which undeniably was Biochar 4, did not provoke the highest toxicological impact on the organisms. Instead, Biochar 2, which is probably a “cleaner” and with regard to EBC thresholds (e.g., PAHs) an uncritical biochar, 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 Biochar 2, nor for the other biochars, which could be an explanation for the incongruous order of toxicity.

Cornelissen and Hale [115] state, that it is unlikely that the application of biochar poses any substantial PAH eco-toxicological risk to soil environments caused by the native PAHs in biochar. The study involving 60 biochars found that the release/leach ratio of PAHs with 0.2-2 ng $L^{-1}$ in comparison to polluted coastal waters is less than a 500 factor. Furthermore, Hale [152] states, that the dissolution/desorption process of contaminants from biochar into the soil biome is very limited due to the high physical bound and consequently bioavailability is limited to 1-10 % of the total content. The present investigation shows, that both insights may only be valid, if absorption of biochar micro-particles and associated digestion/metabolization processes of invertebrate organisms are neglected or foreclosed.

This result is an indication that there are possible synergistic responses among the entire mixture of hazardous substances and that digestion/metabolization processes are the trigger that causes 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 biochars, followed by *Moina macrocopa* and *Daphnia magna*.

#### 8.4.6.2 Toxic Mechanism, Actuator, and Relevance to the Environment

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

Except for ciliates, the results show that rotifers and cladocerans, which habitat in intercellular soil water, are capable of digesting micro-particles of biochar and thus release bounded and mobilize immobilized hazardous substances contained therein. Rotifers have an activity of phosphatases, ß-N-Acetylhexosaminidases, and lipases[160]. In the case of *D. magna*, many digestive enzymes have been found: galactosidases, esterases, trypsin, and cellulases [161]. 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 [162,163]. Volcanic eruptions, as well as forest and vegetation fires, will release PCDD/Fs and PAHs on a natural basis [164], which will most likely degrade in the soil. However, the man-made application of biochar 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 16) 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 Biochar 2 and Biochar 3, nevertheless this assumption was not confirmed by the present study, which is an indicator for the low heavy metal content of the soil where the mezquites grew.

Among the PAH-16 (Table 17) 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 biochar 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, Biochar 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. Biochar 4 additionally had an outlier in phenanthren content with 1.6 mg $kg^{-1}$. Basically, PAHs in biochar are very hydrophobic and hardly bio-accessible [151], 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 to 400 °C, whereas at a temperature level of 900°C, the chlorine-based pollutants are destroyed. Concentrations in all biochars were far below the permitted EBC thresholds, both for PCDD/Fs and PCBs (cp. Table 18); however, a there was a potential coherence between the toxicity potential of these POP substances and the detected mortality of rotifers and cladocerans.

## 8.5 Economic Evaluation of Biochar Use

In a first step, the unit costs (USD/t) for biochar production were calculated. Table 36 shows the underlying basic assumptions. The price for feedstock has been determined 22 USD/t including supply to the pyrolysis oven and pre-treatment and confection such as chopping, chipping, sieving, drying et cetera. The price is a mean value and is based on interviews hold with several biomass providers, the collection team of the municipality of Rincón de Romos, the Parque Mexico operators and landfill operators. The price is very volatile, as for instance, the disposal of biomass pruning at San Nicholas claims a disposal fee. The same could happen if the biomass material is used as feedstock for biochar production. In that case the feedstock could even have a negative price. However, that scenario becomes competitive and unlikely when huge volumes of feedstock are requested. In that case, even higher prices for feedstock can be expected, rising up to 35 USD/t, depending on quality of the wood.

**Table 36.** Basic assumptions for biochar production.

| Parameter | Value | Unit |
|---|---|---|
| Feedstock price | 22 | USD/t |
| ø carbon efficiency | 25% | |
| BC feedstock cost | 88 | USD/t |
| ø biochar density | 3.6 | |
| Production capacity | 460 | l/d |
| | 2.2 | d/1.000l |
| | 8 | d/t |
| | 30.5 | t/a |
| Working time | 8 | h/d |
| | 240 | d/a |
| Hourly rate | 1.2 | USD/h |
| Man power cost | 75.5 | USD/t |
| Maintenance | 1.50% | p.a. Inv. |
| | 39 | USD/a |
| | 312 | USD/LS |
| | 1.3 | USD/t |
| Grinding | 2.6 | USD/t |
| Packaging | 12.4 | USD/t |
| Application | 3.5 | USD/t |

The applied technology to produce biochar remains the Kon-Tiki flame curtain kiln. The carbon efficiency of the kiln has been determined to 25% (cp. Table 15) and the biochar density ratio determined to 3.6 (volumetric to gravimetric). The production capacity of the kiln by experience after several test runs amounts to 460 l/d (+/- 25%). Based on assumed work time (240 d/a; 8h/d) and labor cost of 1.2 USD/h, approx. 30t of biochar could be produced per day at 75 USD/t personnel cost. Furthermore, costs for maintenance, grinding, packaging and field application were determined (see Table 36). When all parameter values are totaled, OPEX of 183.3 USD/t are calculated (see Table 37).

**Table 37.** Financial assumptions for biochar production.

| Parameter | Value | Unit |
|---|---|---|
| Price Kon-Tiki | 2,600 | USD |
| Interest rate | 6.5% | p.a. |
| Loan period | 5 | a |
| Annuity | 625.6 | USD/a |
| Life span (LS) | 8 | a |
| BC cap. over LS | 244.0 | t |
| Financial cost | 20.5 | USD/t |
| OPEX | 183.3 | USD/t |
| CAPEX | 20.5 | USD/t |
| TOTEX | 203.8 | USD/t |

Based on the current market price (SEDACEI Automotive) the price for the Kon-Tiki is 2,600 USD. Assuming a 6.5% interest rate p.a. on a 100% credit for 5 years, financial costs (CAPEX) of 20.5 USD/t of biochar result. The total price for biochar expressed in TOTEX is 203.8 USD/t. In comparison to world market prices and other high-tech pyrolysis processes for EBC or IBI biochar, this price situates in a lower realm. Some biochar prices, especially in the feed and fodder application, range up to 1,500 USD/t [165]. However, biochar for soil amendment is retailed within a range of 200 to 850 USD/t.

### 8.5.1 Cost-Benefit-Analysis: Scenario Green Area at Universidad Panamericana

The identified optimal user performance ratio (OUPR) (see Table 30) for all three soil types tested and the associated water saving potentials and cost are contrasted with the costs for biochar (here TOTEX) based on the corresponding biochar share in soil of the OUPR. The division of TOTEX and cost saving results in payback period. Table 38 illustrates the results. In agronomic terms, the PBP are very short, reflecting that the biochar will last for more than a 100 years. Assuming that in contrary adequate credit times would not exceed more than 5 years, not all variants, e.g. `Phaeozem-Biochar 1´ or `Calcisol-Biochar 1´, may be implemented. The water price and the quantity applied at UP is comparably high. This leads to a very quick PBP with relatively low risk.

**Table 38.** Cost-Benefit-Analysis: Scenario Green Area at Universidad Panamericana.

| Soil type | Biochar | Increase $WC_{max.}$ | BC Share [t/ha] | Water Saving [m³/ha/a] | Cost Saving [USD/a] | TOTEX [USD] | Payback Period [a] |
|---|---|---|---|---|---|---|---|
| **Cambisol** | Biochar 1 | 7.7% | 12.3 | 1,232 | 1,785 | 2,507 | 1.4 |
| | Biochar 2 | 6.7% | 12.3 | 1,072 | 1,554 | 2,507 | 1.6 |
| | Biochar 3 | 8.1% | 12.3 | 1,296 | 1,878 | 2,507 | 1.3 |
| | Biochar 4 | 8.0% | 12.3 | 1,280 | 1,855 | 2,507 | 1.4 |
| **Phaeozem** | Biochar 1 | 3.1% | 27.5 | 496 | 719 | 5,606 | 7.8 |
| | Biochar 2 | 3.3% | 13.5 | 528 | 765 | 2,752 | 3.6 |
| | Biochar 3 | 2.4% | 13.5 | 384 | 556 | 2,752 | 4.9 |
| | Biochar 4 | 5.1% | 27.5 | 816 | 1,183 | 5,606 | 4.7 |
| **Calcisol** | Biochar 1 | 5.3% | 37.0 | 848 | 1,229 | 7,542 | 6.1 |
| | Biochar 2 | 3.4% | 11.0 | 544 | 788 | 2,242 | 2.8 |
| | Biochar 3 | 4.4% | 11.0 | 704 | 1,020 | 2,242 | 2.2 |
| | Biochar 4 | 4.3% | 11.0 | 688 | 997 | 2,242 | 2.2 |

Abbreviations: t, tons; ha, hectare; m³, cubic meter; USD, United States Dollar; a, years.

### 8.5.2 Cost-Benefit-Analysis: Scenario Green Areas Municipality of Aguascalientes

The PBPs in this scenario are longer in comparison to the Green Area at UP scenario. The price for application of treated effluent is higher (see Table 11), however the quantity applied is much lower, resulting in longer PBPs. Nevertheless, the PBPs indicate a beneficial, low risk investment. Table 39 shows the results of the respective scenario.

**Table 39.** Cost-Benefit-Analysis: Scenario Irrigated areas at Parque Ecológico Línea Verde

| Soil type | Biochar | Increase $WC_{max.}$ | BC Share [t/ha] | Water Saving [m³/ha/a] | Cost Saving [USD/a] | TOTEX [USD] | Payback Period [a] |
|---|---|---|---|---|---|---|---|
| **Cambisol** | Biochar 1 | 7.7% | 12.3 | 347 | 1,168 | 2,507 | 2.1 |
| | Biochar 2 | 6.7% | 12.3 | 302 | 1,016 | 2,507 | 2.5 |
| | Biochar 3 | 8.1% | 12.3 | 365 | 1,228 | 2,507 | 2.0 |
| | Biochar 4 | 8.0% | 12.3 | 360 | 1,213 | 2,507 | 2.1 |
| **Phaeozem** | Biochar 1 | 3.1% | 27.5 | 140 | 470 | 5,606 | 11.9 |
| | Biochar 2 | 3.3% | 13.5 | 149 | 500 | 2,752 | 5.5 |
| | Biochar 3 | 2.4% | 13.5 | 108 | 364 | 2,752 | 7.6 |
| | Biochar 4 | 5.1% | 27.5 | 230 | 773 | 5,606 | 7.2 |
| **Calcisol** | Biochar 1 | 5.3% | 37.0 | 239 | 804 | 7,542 | 9.4 |
| | Biochar 2 | 3.4% | 11.0 | 153 | 516 | 2,242 | 4.3 |
| | Biochar 3 | 4.4% | 11.0 | 198 | 667 | 2,242 | 3.4 |
| | Biochar 4 | 4.3% | 11.0 | 194 | 652 | 2,242 | 3.4 |

Abbreviations: t, tons; ha, hectare; m³, cubic meter; USD, United States Dollar; a, years.

### 8.5.3 Cost-Benefit-Analysis: Scenario Agrarian cultivation of corn in Aguascalientes

The PBPs in this scenario are far beyond any agronomic feasibility. The price for water in agriculture is very low, and even the quantities of water applied are not overdue (see Table 11). This leads to long PBPs. If a broad application of biochar should take place within the study area, further biochar benefits for agriculture, e.g. $CO_2$ sequestration credits, NPK savings, increase yield, etc. need to be monetized and included in the cost-benefit-analysis [44,166–168]. Table 40 shows the results.

**Table 40.** Cost-Benefit-Analysis: Scenario Agrarian cultivation of corn.

| Soil type | Biochar | Increase $WC_{max.}$ | BC Share [t/ha] | Water Saving [m³/ha/a] | Cost Saving [USD/a] | TOTEX [USD] | Payback Period [a] |
|---|---|---|---|---|---|---|---|
| **Cambisol** | Biochar 1 | 7.7% | 12.3 | 518 | 68 | 2,507 | 37 |
| | Biochar 2 | 6.7% | 12.3 | 451 | 59 | 2,507 | 42 |
| | Biochar 3 | 8.1% | 12.3 | 545 | 72 | 2,507 | 35 |
| | Biochar 4 | 8.0% | 12.3 | 538 | 71 | 2,507 | 35 |
| **Phaeozem** | Biochar 1 | 3.1% | 27.5 | 209 | 27 | 5,606 | 205 |
| | Biochar 2 | 3.3% | 13.5 | 222 | 29 | 2,752 | 94 |
| | Biochar 3 | 2.4% | 13.5 | 162 | 21 | 2,752 | 130 |
| | Biochar 4 | 5.1% | 27.5 | 343 | 45 | 5,606 | 124 |
| **Calcisol** | Biochar 1 | 5.3% | 37.0 | 357 | 47 | 7,542 | 161 |
| | Biochar 2 | 3.4% | 11.0 | 229 | 30 | 2,242 | 75 |
| | Biochar 3 | 4.4% | 11.0 | 296 | 39 | 2,242 | 58 |
| | Biochar 4 | 4.3% | 11.0 | 289 | 38 | 2,242 | 59 |

Abbreviations: t, tons; ha, hectare; m³, cubic meter; USD, United States Dollar; a, years.

### 8.5.4 Financial Statement

The structure of the entire calculation tool, is based on the initial tableau (see Table 12). The following Financial Statement corresponds to a dynamic business plan model which is developed to examine the economic performance of the project were all monetized effects are bundled for a final profitability check. As already mentioned, does the business plan (Financial Statement) consists of a standardized balance sheet, a profit and loss account, a cash flow statement and a conclusive calculation of key performance indicators (KPI's) (see Table 42) in order to judge the economic pre-feasibility of the designated project. Table 41 illustrates the result of the FS calculation.

Table 41. Financial Statement for a large-scale biochar production plant.

| Year | 2020<br>1 | 2021<br>2 | 2022<br>3 | 2023<br>4 | 2024<br>5 | 2025<br>6 | 2026<br>7 | 2027<br>8 | 2028<br>9 | 2029<br>10 | 2030<br>11 | 2031<br>12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Standardized Balance Sheet** | | | | | | | | | | | | |
| Net Working Capital | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Assets | -447,012 | -397,344 | -347,676 | -298,008 | -248,340 | -198,672 | -149,004 | -99,336 | -49,668 | 0 | 0 | 0 |
| Capital Employed | -447,012 | -397,344 | -347,676 | -298,008 | -248,340 | -198,672 | -149,004 | -99,336 | -49,668 | 0 | 0 | 0 |
| Equity | 82,089 | 69,681 | 61,319 | 57,064 | 57,141 | 61,788 | 71,256 | 85,812 | 105,735 | 131,322 | 197,654 | 266,910 |
| Loan | 367,899 | 336,540 | 303,143 | 267,575 | 229,694 | 189,352 | 146,388 | 100,630 | 51,899 | 0 | 0 | 0 |
| Cash | 2,975 | 8,877 | 16,786 | 26,631 | 38,496 | 52,468 | 68,640 | 87,106 | 107,966 | 131,322 | 197,654 | 266,910 |
| Capital Employed | 447,012 | 397,344 | 347,676 | 298,008 | 248,340 | 198,672 | 149,004 | 99,336 | 49,668 | 0 | 0 | 0 |
| **Income Statement** | | | | | | | | | | | | |
| Income | 211,200 | 221,549 | 232,405 | 243,793 | 255,738 | 268,270 | 281,415 | 295,204 | 309,669 | 324,843 | 340,760 | 357,457 |
| Raw material | -66,000 | -69,234 | -72,626 | -76,185 | -79,918 | -83,834 | -87,942 | -92,251 | -96,772 | -101,513 | -106,488 | -111,705 |
| Raw margin | 145,200 | 152,315 | 159,778 | 167,607 | 175,820 | 184,435 | 193,473 | 202,953 | 212,897 | 223,329 | 234,273 | 245,752 |
| Salary Manager | -16,400 | -17,204 | -18,047 | -18,931 | -19,858 | -20,832 | -21,852 | -22,923 | -24,046 | -25,225 | -26,461 | -27,757 |
| Salary Foremen | -13,600 | -14,266 | -14,965 | -15,699 | -16,468 | -17,275 | -18,121 | -19,009 | -19,941 | -20,918 | -21,943 | -23,018 |
| Salary Operators | -27,648 | -29,003 | -30,424 | -31,915 | -33,478 | -35,119 | -36,840 | -38,645 | -40,538 | -42,525 | -44,609 | -46,794 |
| Insurance | -9,934 | -10,420 | -10,931 | -11,467 | -12,028 | -12,618 | -13,236 | -13,885 | -14,565 | -15,279 | -16,027 | -16,813 |
| Accrued Liabilities | 0 | -513 | -586 | -674 | -766 | -863 | -965 | -1,072 | -1,183 | -1,301 | -1,424 | -2,795 |
| Maintenance | -7,450 | -7,815 | -8,198 | -8,600 | -9,021 | -9,463 | -9,927 | -10,413 | -10,924 | -11,459 | -12,021 | -12,610 |
| OPEX | -75,032 | -79,221 | -83,151 | -87,285 | -91,621 | -96,170 | -100,942 | -105,947 | -111,198 | -116,706 | -122,484 | -129,787 |
| EBITDA | 70,168 | 73,094 | 76,628 | 80,323 | 84,199 | 88,265 | 92,531 | 97,006 | 101,700 | 106,624 | 111,789 | 115,965 |
| Depreciation | -49,668 | -49,668 | -49,668 | -49,668 | -49,668 | -49,668 | -49,668 | -49,668 | -49,668 | -49,668 | 0 | 0 |
| EBIT | 20,500 | 23,426 | 26,960 | 30,655 | 34,531 | 38,597 | 42,863 | 47,338 | 52,032 | 56,956 | 111,789 | 115,965 |
| Interest | -25,827 | -23,913 | -21,875 | -19,704 | -17,392 | -14,930 | -12,308 | -9,515 | -6,541 | -3,373 | 0 | 0 |
| EBT | -5,327 | -487 | 5,084 | 10,950 | 17,139 | 23,667 | 30,555 | 37,823 | 45,491 | 53,582 | 111,789 | 115,965 |
| Tax | 0 | 0 | -1,525 | -3,285 | -5,142 | -7,100 | -9,167 | -11,347 | -13,647 | -16,075 | -33,537 | -34,790 |
| EAT | -5,327 | -487 | 3,559 | 7,665 | 11,997 | 16,567 | 21,389 | 26,476 | 31,844 | 37,508 | 78,252 | 81,176 |
| **Cashflow Statement** | | | | | | | | | | | | |
| EBITDA | 70,168 | 73,094 | 76,628 | 80,323 | 84,199 | 88,265 | 92,531 | 97,006 | 101,700 | 106,624 | 111,789 | 115,965 |
| Tax | 0 | 0 | -1,525 | -3,285 | -5,142 | -7,100 | -9,167 | -11,347 | -13,647 | -16,075 | -33,537 | -34,790 |
| Δ Net Working Capital | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cashflow of operation | 70,168 | 73,094 | 75,102 | 77,038 | 79,057 | 81,165 | 83,365 | 85,659 | 88,052 | 90,549 | 78,252 | 81,176 |
| Cashflow of investments | -496,680 | | | | | | | | | | | |
| Free Cashflow | -426,512 | 73,094 | 75,102 | 77,038 | 79,057 | 81,165 | 83,365 | 85,659 | 88,052 | 90,549 | 78,252 | 81,176 |
| Equity | 99,336 | | | | | | | | | | | |
| Dividend | -11,920 | -11,920 | -11,920 | -11,920 | -11,920 | -11,920 | -11,920 | -11,920 | -11,920 | -11,920 | -11,920 | -11,920 |
| Loan | 397,344 | | | | | | | | | | | |
| Interest | -25,827 | -23,913 | -21,875 | -19,704 | -17,392 | -14,930 | -12,308 | -9,515 | -6,541 | -3,373 | 0 | 0 |
| Redemption | -29,445 | -31,359 | -33,397 | -35,568 | -37,880 | -40,342 | -42,965 | -45,757 | -48,731 | -51,899 | 0 | 0 |
| Cashflow of financing | 429,487 | -67,193 | -67,193 | -67,193 | -67,193 | -67,193 | -67,193 | -67,193 | -67,193 | -67,193 | -11,920 | -11,920 |
| Total Cashflow | 2,975 | 5,901 | 7,909 | 9,845 | 11,865 | 13,973 | 16,172 | 18,466 | 20,860 | 23,356 | 66,332 | 69,255 |

This project is based on the technology used by Alfa Charcoal® to produce very good quality of biochar. It provides much larger quantities of biochar allowing to implement a biochar based soil management strategy in Aguascalientes. The Cashflow model modelling shows a positive Total Cashflow all through the business years, meaning that liquidity is given all over the project time. The operative performance, expressed by the EAT, is bid low, especially in the first two years, however increases positively over period, due to the inflation rate. The payback period of the present project inherits a moderate risk, as the expected life span of the plant is limited to 15 years. In contrast the net present value (NPV) is positive, hence the project shall be implemented. Additionally the IRR is above WACC (even 52 %), which means that the project is capable to produce money. Shareholders could count on a 12 % ROI margin, which is a promising option.

**Table 42.** Key Performance Indicator (KPI) of the Financial Statement.

| KPI | Value | Unit |
|---|---|---|
| IRR | 14.41% | |
| NPV | 146,946 | USD |
| PBP | 10 | a |
| ROI | 12% | |
| WACC | 7.60% | |

### 8.5.5 Chapter Discussion

#### 8.5.5.1 Economic evaluation up-shot

The economic assessment of the use of biochar to increase the water capacity in the soil and the associated cost savings through water savings in three different scenarios shows that the water price and the amount of water used for irrigation are crucial, whether an application is economically sensible or rather uneconomical. The price of biochar is also crucial. The price of the raw material plays a particular role. Using a simple kiln, under the given conditions, leads to a low biochar price in an international comparison. The study shows that agricultural applications in Aguascalientes are unprofitable, given the current low water price. On the other hand, applications in culturally and prestigiously motivated irrigation seems to be economically viable, with payback periods between 1-10 years. Based on the findings of the cost-benefit analysis, the economic viability of a commercial biochar production plant shows an optimistic result. Based on the positive total cash flows, liquidity problems can be factually excluded, which indicates a low risk. The relatively long payback period, on the other hand, indicates a moderate project scenario. On the other hand, there are clearly positive rates of return and beneficial net present values.

#### 8.5.5.2 Monetizing biochar based agrarian value chains

The application of biochar or biochar-based substrates in agriculture to improve soil properties is gaining popularity associated with positive agronomic and ecological outcomes. Besides plant cultivation and soil science related trials, further fields of application for biochar are discussed and investigated all around the globe. Some of these include the application of biochar in organic waste management, effluent purification, silage preparation, animal feeding, and in biogas plants as aggregates or within the treatment of natural fertilizer. Even up-stream effects, especially the renewable energy gaining feature of biochar and holistic effects such as the generation of regional added value throughout the utilisation of local untapped potentials for biochar production are going to be considered crucially. The tenor of this investigation is that biochar use offers a promising base to sustainably increase the efficiency in numerous fields of its application. Biochar applications not only lead to qualitative, substantial microbiological and physical soil improvements but also aid in closing the natural material cycles [6,47].

However, unfortunately, the gained insights from these laboratory and greenhouse trials are only conditionally transferable to practice and do not provide reliable economic or profitability assessments under market conditions. Nevertheless, it seems that at the current state of science and technology, the application of biochar as soil enhancer in large-scale application is profitable only in very few instances in industrialized countries with high price structures [30,78,168]. The setup of regional value chains, where multiple fields of biochar application are available, is the foundation of this doctoral research. Investment in the product (biochar) results in multiple benefits from different value-added stages generating higher revenues (see Figure 46).

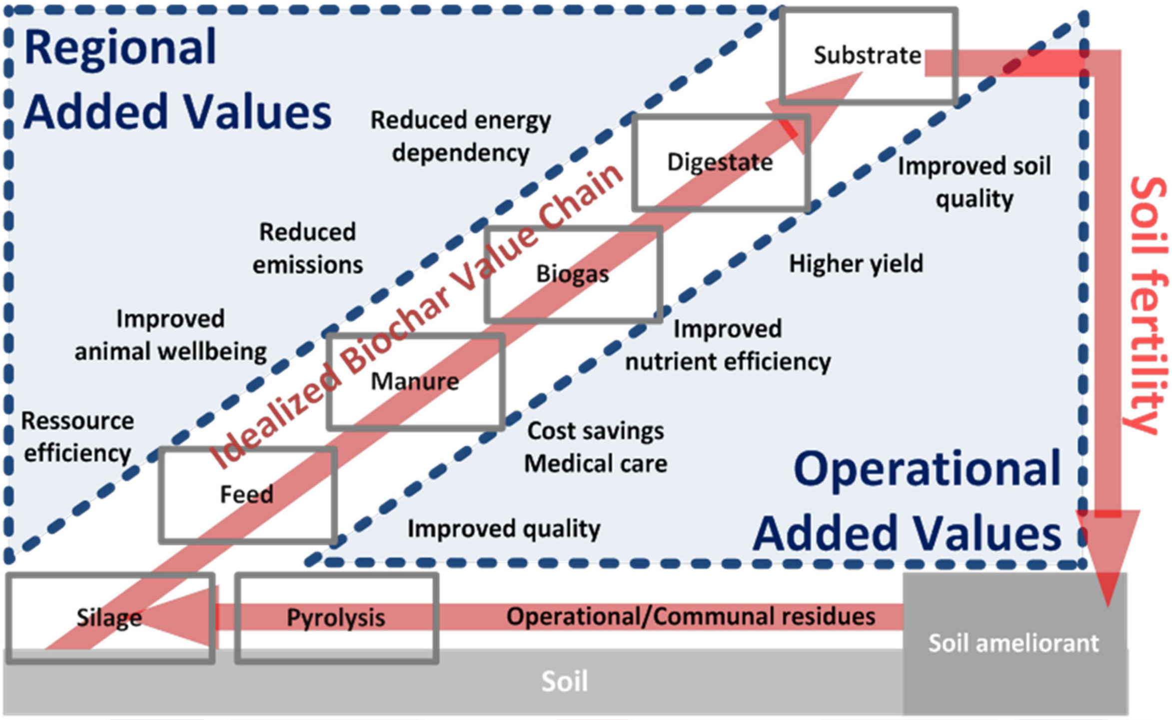



**Figure 46.** Idealized value chain for cascaded biochar use. University of applied Sciences Trier, Institute for applied Material Flow Management (IfaS), Flesch. F. Jan 2017

Hence, it is inferred that in contrast to the limited single utilization of biochar, multiple cascade utilization with its final use as a soil enhancer can be profitable in terms of agro economic payback periods. Placing the focus on cropping systems with high added value per unit area (e.g. wine, vegetables and fruits) could play a central role within the investigation. Apart from purely considering the financial costs and benefits to an individual farmer, it is also necessary to be mindful about the social costs and benefits, risks and uncertainties that a new technology may impose on people and the environment. Besides the direct microeconomic effects, positive environmental effects do play an important role as well. Biochar's ability to reduce greenhouse gases, its nitrate buffer function and carbon sequestration ability are examples of its versatility as an environmental technology, which are not yet been monetized or internalized [10,55,75,157]. These characteristics have an inevitable relevance to successfully implement a biochar based circular economy (see Figure 46).

# 9 Conclusion

The practice oriented design of the doctoral research covers relevant aspects with regard to biochar science, ranging from selection, collection and pre-treatment of feedstock, to the production technology applied, to physicochemical analysis of the biochar and associated soil water capacity trials, to toxicological risk evaluation with living organisms up to economic evaluation of biochar application.

## 9.1 Toxicological evaluation

The purpose of this research thesis was to use acute and chronic toxicity tests [142] 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 biochars that have been generated using local feedstock in Aguascalientes, Mexico. The present work shows that the application of a toxicological test using aquatic invertebrates could substantially increase biochar application safety in and close to sensitive habitats. The original hypothesis was proved by the experimental test results. Biochar, 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 *Lecane quadridentata* showed substantial toxicological response to the four tested biochars, with $LC_{50}$ values in the range of 8.3 % to 25.1 % of effective concentration. *Daphnia magna* and *Moina macrocopa* showed reduced lethal toxicity when compared with the rotifer. Only *Paramecium caudatum* did not show any negative response to the exposed biochars. Interestingly the biochar with the highest values in hazardous substances, which is clearly Biochar 4, does not show the highest toxicological impact on the organisms. Far from it, Biochar 3 which is obviously the "cleanest" and uncritical biochar, has the highest $LC_{50}$ values both for *Lecane*, *Daphnia* and *Moina*. This may is an indication that not the mixture of hazardous substances causes the toxicity rather a single contaminant is the trigger, such as Naphtaline or TCDD.

If biochar is applied soundly, adhering to the recommended mass thresholds, no serious adverse effects are to be expected. The present study provides a potential toxicity mechanism to benthic invertebrates when biochar is applied to soil. As a result of this study, it is recommended to not adhere only to the international certification thresholds, but also consider country-specific rules of application meticulously, especially with regard to application quantities.

The particular challenge in the scholarly comprehension of adverse effects provoked by convergent mechanisms of pernicious substances is the fact that they are promiscuous and non-static. However, these mechanisms are important, because in the living environment the toxicants are mixed, blended and, in many cases occur at low concentrations that may be regarded as non-adverse. Any toxicant, even at no observed effective concentrations (NOEC), can cause an increase of the toxicity of the other. With regard to the present results, it remains unclear, if a single hazardous substance or the blend of hazardous substances is responsible for the detected toxicities in rotifer *Lecane quadridentata* and cladoceran *Moina macrocopa*. Future research may focus on the explanation of the witnessed digestion processes and the assessment of susceptibility of each single toxicant and the hazardous mixtures of pernicious substances.

### 9.2 Application-oriented evaluation

Besides the fulfillment of EBC certification requirements, all four biochar types provoked remarkable soil water capacity increases when mixed with soil, whereby the initial hypothesis has been confirmed. Interestingly even small amounts of biochar significantly increase soils water capacity. This applies to all four biochars and all three soils. The addition of just 1 % by weight increases the water capacity by 1 % to 10 %. The fourteen-fold increase in biochar addition, however, only triggers a further increase of up to 25 %, whereas in some cases even small reductions of water capacity with increasing biochar addition have been observed.

The overall increase follows a degressive curve. The optimum cost-benefit addition ratio of biochar tends to be between 1 % and 2 %, however depending strongly on the type of biochar and soil. Cost-Benefit-Analysis demonstrate, that the use of biochar application to tackle chronic water paucity in Aguascalientes is beneficial for private and public green space scenarios with payback periods beneath 2 years, but is not yet economically viable for traditional agriculture, as water prices are comparably low. This is exactly where an expanded, holistic view is required, where all positive effects along the agrarian value chain of a cascading and multiple use of biochar are monetized. With regard to the identified quantity of untapped biomass potential in the study area of about 58,000 t/a, a commercial-scale business for biochar generation to initiate a comprehensive utilization shall be envisaged. First economic feasibility analysis indicate promising results, admittedly with moderate business risk but with sophisticating return rates and cash values.

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# Appendices

## Electron micrographs of the biochars: 15 kV zoom x 1,000

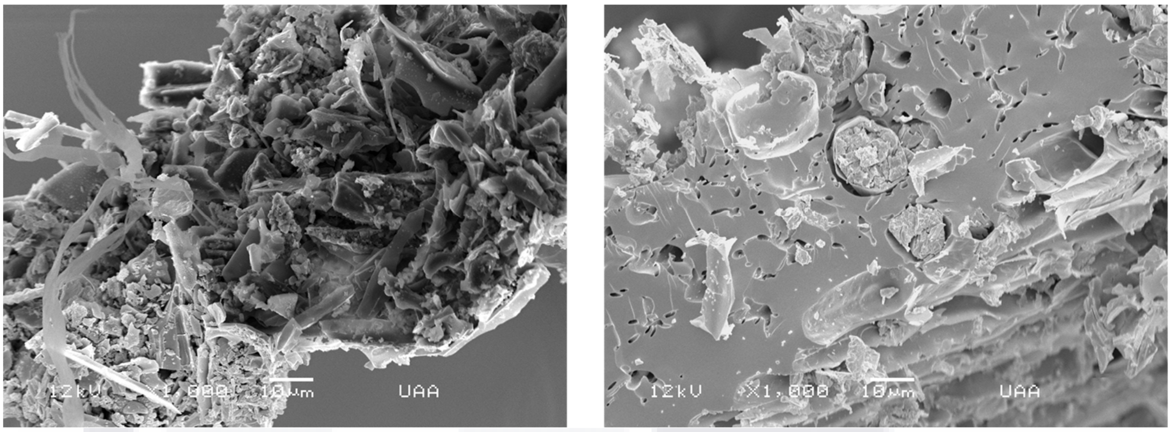


**Figure 47.** Structure of Biochar **No. 1** particle from different visual angel. Electron micrograph: 15 kV zoom x **1,000**, Universidad Autonoma de Aguascalientes, Flesch. F. November 2017

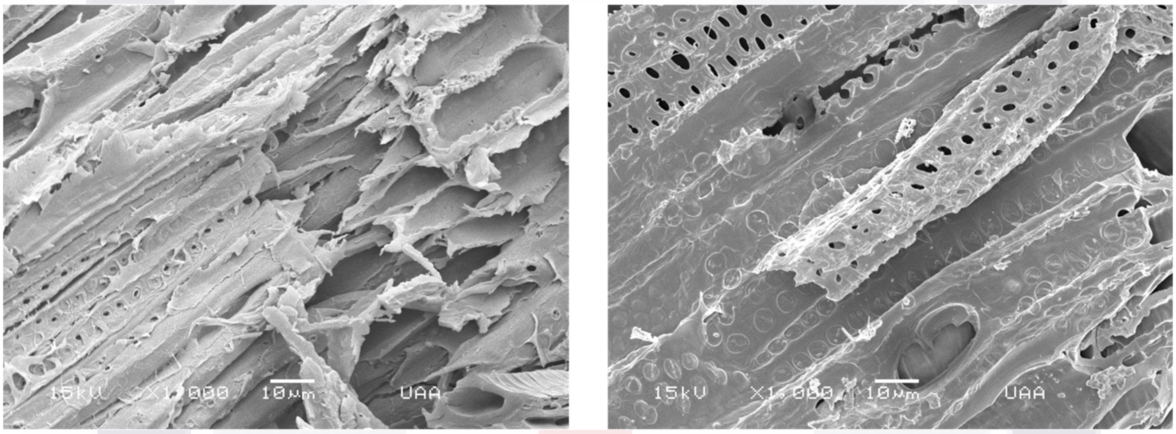


**Figure 48.** Structure of Biochar **No. 2** particle from different visual angel. Electron micrograph: 15 kV zoom x **1,000**, Universidad Autonoma de Aguascalientes, Flesch. F. November 2017

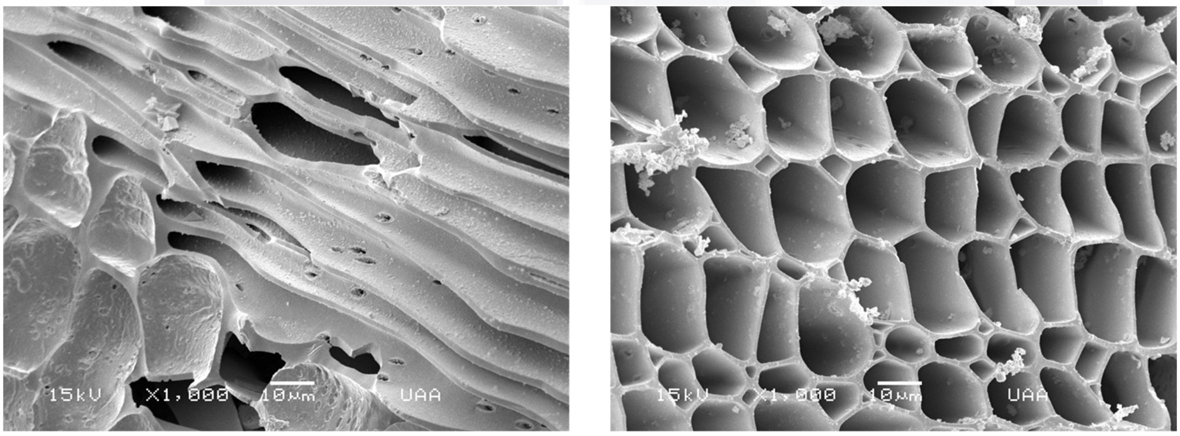


**Figure 49.** Structure of Biochar **No. 3** particle from different visual angel. Electron micrograph: 15 kV zoom x **1,000**, Universidad Autonoma de Aguascalientes, Flesch. F. November 2017

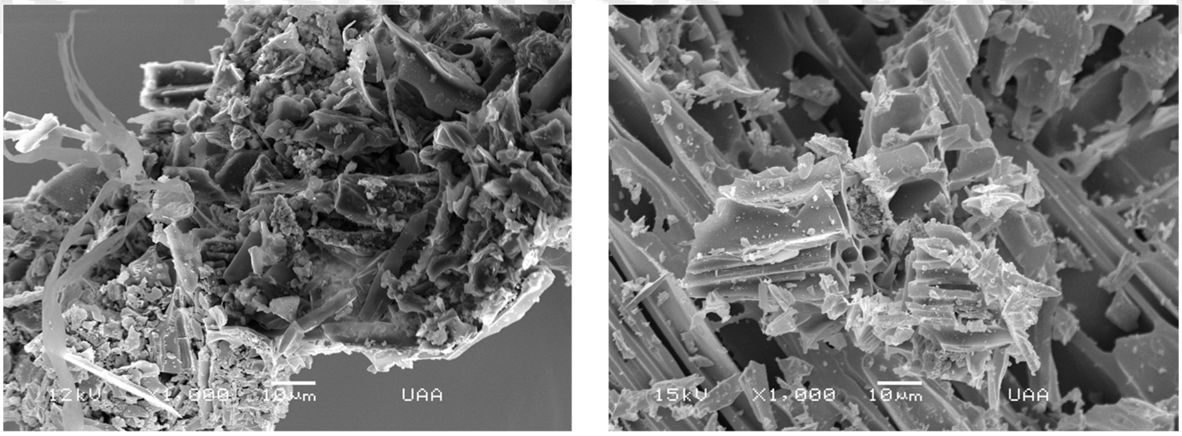


**Figure 50.** Structure of Biochar **No. 4** particle from different visual angel. Electron micrograph: 15 kV zoom x **1,000**, Universidad Autonoma de Aguascalientes, Flesch. F. November 2017