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ANALYTICAL METHODS FOR THE CHARACTERISATION OF VOLATILE AND WATER-SOLUBLE ORGANIC COMPOUNDS IN BIOCHAR. RELATIONSHIPS WITH THERMAL STABILITY AND SEED GERMINATION

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1) INTRODUCTION

The current global carbon footprint raises important concerns about the sustainability of human activities, largely dominated by the consumption of fossil fuels. Natural gas and oil will continue to be a bedrock of the global energy system for many decades to come.[1] Regulatory targets are required to reform the energy sector, responsible for at least two-thirds of greenhouse-gas emissions, and should promote the renewables. The Paris Agreement, which entered into force on 4 November 2016, is centered on the energy sector, and represents a major step forward in the fight against global warming. The target of limiting future temperature increase to "well below 2°C", as well as pursuing efforts towards 1.5°C is globally agreed.[2] Biomass feedstocks, particularly those derived from waste streams, are depicted as valuable options for the abatement of greenhouse gas emissions.[3] However, current values of atmospheric carbon dioxide are considered inacceptable, therefore strategies for its capture and storage become relevant to contain the increase of global average temperature. According to the scenarios developed by the International Energy Agency, implementing current international pledges will only slow down the projected rise in energy-related carbon emissions.[1] Carbon capture and storage (CCS) is the only solution for deep emissions reductions from industrial processes and from fossil fuel use in the power sector.[2] Pyrolysis and gasification are thermochemical processes that decompose organic materials in an oxygen-limited environment. Three fractions, variable in yields depending on the technology and process parameters are generated: a mixture of non-condensable gases, namely syngas, mainly composed of carbon monoxide and hydrogen; a liquid product with complex chemical composition, bio-oil and a solid carbonaceous material.[4,5] The syngas produced can be used as a fuel, while the upgraded bio-oil can generate biofuels or valuable chemicals. The remaining solid material can be burned to fulfil the energy requirements of the process. Alternatively, the valorization of this carbonaceous product by its application to soil was proposed as a novel strategy for the long-term storage of carbon dioxide into a stable form, allowing to reverse the climate change.[6,7] Biochar is therefore the solid product of pyrolysis, designed to be used for environmental management [8].The official definition of

biochar is given by the International Biochar Initiative "Biochar is a solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment. Biochar can be used as a product itself or as an ingredient within a blended product, with a range of applications as an agent for soil improvement, improved resource use efficiency, remediation and/or protection against particular environmental pollution, and as an avenue for greenhouse gas (GHG) mitigation".[9] Apart from the carbon sequestration potential, biochar was found to enhance the fertility of tropical soils named "Terra Preta". This was explained by the unique physicochemical properties of biochar. As low density highly porous material, biochar can reduce the soil bulk density and improving soil aeration and water holding capacity. Moreover, the mineral content and the cation exchange capacity can provide the soil with nutrients, preventing their leaching by increasing the nutrient retention capacity of the soil and increase soil pH.[10–12] Furthermore, the affinity of biochar for organic and inorganic compounds raised its potential use as sorbent for contaminants and reclamation of polluted areas.[13,14] Therefore, the production of biochar integrated with bioenergy production to maximize the efficiency of the process and increase carbon efficiency, can be a strategy for carbon sequestration and soil amelioration. However, biochar chemical and physical properties were found to be highly heterogeneous and strongly dependent on the variety of production conditions and biomass feedstocks.[15,16] These parameters can be therefore optimized to produce engineered biochars with unique properties for specific applications. Given the importance of the biochar characteristics on its quality for environmental applications, a thorough identification of the most important properties needs to be performed. The characterization should be aimed at the determination of threshold values and optimal range of the most important parameters. Given the complexity of biochar structure, a wide range of analytical techniques are employed for the determination of quality parameters. Moreover, standardization of analytical procedures is required as well as the definition of reference biochar materials.[17] Guidelines for the sustainable production of biochar, standardized testing and measurement methods, and parameters for quality and safety assessment are proposed, and periodically updated by the European Biochar Certificate (EBC) and

the International biochar Initiative (IBI).[9,18] In the following sections the most relevant biochar physicochemical properties to assure their environmental applications are presented, with particular attention to the analytical methods commonly used for their determination. In the last section, a series of non-conventional techniques are presented. These could represent a perspective to characterize non-regulated trace organic compounds, that could be trapped into the biochar matrix during pyrolysis (e.g. bio-oil residue), and may affect biochar performance (e.g. stability) and environmental behavior (e.g. plant growth), therefore enhancing the knowledge of biochar chemistry.

Figure 1: The benefits of biochar applied as a tool for soil fertility management[19]

1.1) Biochar bulk physicochemical properties

Biochar physical structure can directly influence soil mechanical properties and soil conditions like aeration, water holding capacity, in turn affecting soil biota. Moreover, biochar density and porosity will ultimately affect its transport in the environment (Figure 2).[20] Zhao et al. characterized a wide range of biochar physicochemical properties as effect of feedstock and process conditions: pore volume, average pore size and surface area are some examples of physical properties.[21] Specific surface area is an indication of biochar porous structure, in turn associated to the affinity for the adsorption of organic compounds and can influence contaminant mobility in soil.[22] Many studies

assessed the surface area with the Brunauer-Emmet-Teller (BET) method, which measures the nitrogen gas sorption at 77 K of biochar samples.[23–26] This method allows to characterize pores in the range of 2-50nm (micro and meso-pores), while for the analysis of sub-micropores (<2nm) the most common methods involve carbon dioxide adsorption.[20] Along with these techniques, biochar structure and surface topography, are often analyzed by scanning electron microscopy (SEM). With the SEM a visual indication of biochar macro-porosity (pores >50nm) and particle size distribution can be achieved. If SEM is coupled with Energy Dispersive X-ray spectroscopy (EDX) also compositional information can be investigated.[27] Brewer et al. proposed a combined skeletal- and envelope-density analysis as method for quantifying biochar porosity characteristics at micro- to macro-pore scales. Both measure the volume of a known mass by a displacement technique. In the case of skeletal density, the displaced material is helium gas, while for envelope density, a micro-granular suspension is used. Skeletal density and micropore volume were found to be primarily controlled by pyrolysis temperature, whereas envelope density, porosity, and macropore volume were feedstock dependent.[20]

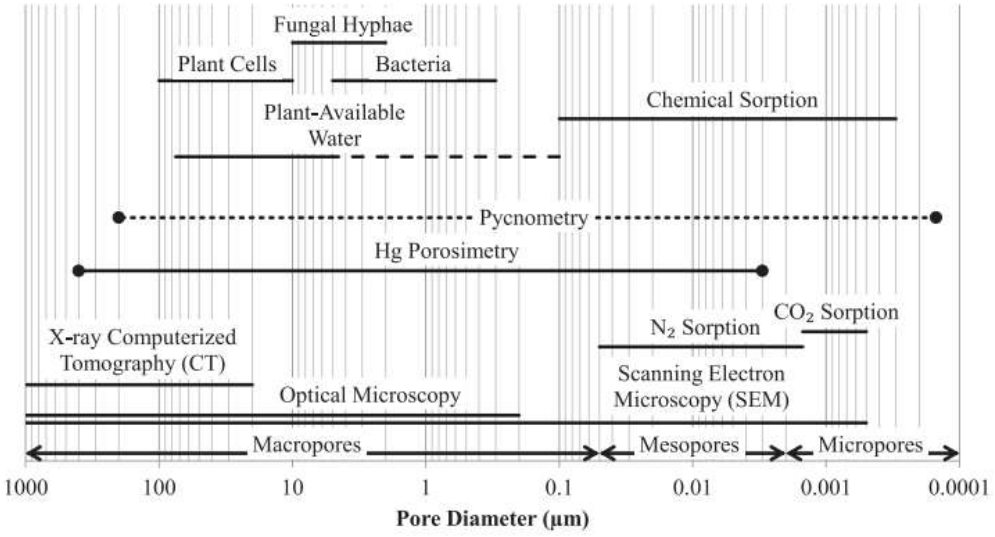

Figure 2: pore size ranges by classification, analytical characterization techniques available, and relevant physiochemical phenomena for biochar interactions with the environment. Solid lines indicate methods that produce pore size distributions. Ranges framed by dots indicate methods that give cumulative pore volumes in that range. Plant-available water pore size range based on soil pore size classifications (solid line) extended to pore sizes accessible if only capillary pressure is considered (dashed line).[20]

The electrical conductivity (EC) is determined in a given volume of deionized water (e.g. ASTM-D1125A: Electrical Conductivity of Non-Flowing Water Samples, or DIN 11265). However, the different conditions (e.g. dilution factors) resulted in different values. Cation exchange capacity (CEC) is a useful indicator to measure the potential capability of biochar to provide soil with its sorbed positively charged ions. Kloss et.al calculated CEC expressed in mmol/kg as the sum of Na, K, Mg, Ca, Al, Fe, and Mn cations in the extract of a sequential water-$BaCl_2$ extraction, by means of inductively coupled plasma mass spectrometry (ICP-MS).[24] Wu et al. measured extractable cations (K^+, Ca^{2+}, Na^+, Mg^{2+}) and the cation exchange capacity (CEC) of untreated biochar samples using 1 M ammonium acetate (pH 7) methods. pH is usually carried out by preparing a mixture of a certain quantity of biochar in a fixed volume of $CaCl_2$ solution.[28] However, dilutions with deionized water were also reported. Different dilution factors and the presence or absence of $CaCl_2$ will provide different pH values.[17] Kloss et al. made use of dispersive X-ray fluorescence to evaluate also the K, P and S content, while Brewer et.al employed this technique to gain information about the mineral content of biochar.[23,24] Wu et al. determined the atomic structure of biochar with traditional powder X-ray diffraction.[28] Proximate analysis, gives the content (as %) of moisture, ash, volatile matter (VM), fixed carbon (FC) and is a typical characterization method as official procedures have been developed, such as DIN51719, 51720. VM and FC contents give relative measures of the more labile and more stable components of chars, respectively. Usually VM decreased and FC increased with pyrolysis temperature.[29] For instance, Brewer et.al and Wu et al. determined the moisture, and proximate analysis with ASTM D1762-84. The Higher Heating Value (HHV) of chars was also calculated by oxygen bomb calorimeter.[23,28] The ASTM D1762-84 method was applied to a vast array of biochar samples by Enders et al.[30] Pereira et al. and Wang et al. calculated the ash content of biochar samples by thermal analysis with a thermogravimetric analyzer, while Kloss et al. coupled this analysis with the Differential scanning calorimetry (DSC).[24,31,32] The elemental analysis of carbon, hydrogen and nitrogen is usually performed by combustion,[30,31,33,34] while the oxygen content is generally determined by difference (all expressed

as mass %). Elemental analysis is useful to determine some important parameters like O/C, H/C and C/N atomic ratios as described in the next section. Different methodologies have been applied in the analysis of trace metals. Fellet et al. analyzed Al, Cd, Cr, Cu, Fe, Ni, Pb, Ti and Zn by means of Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).[27] Biochar samples were previously treated with a microwave assisted acid digestion (USEPA-EPA method 3052). Enders et al. performed digestion with HNO_3 and H_2O_2, then ICP-OES. Zhao et al. applied the USEPA method 3050B for the digestion of biochar in the analysis of trace metals.[21,30] According to an inter-laboratory comparison of analytical methods for biochar characterization, the digestion method significantly affect the concentration of the main biochar elements (P, K, Na, Mg, Mn, Ca, Fe). The digestion with aqua regia in a microwave system is proposed as the most practical and reliable.[17]

1.2) Biochar chemical structure

Biochar is usually produced under controlled conditions. However, the variability of the process parameters, such as highest treatment temperature (HTT), residence time of the biomass inside the reactor, and the wide range of feedstocks available, result in heterogeneous chemical structures and elemental composition of the biochar materials.[12,15,21] The combustion continuum concept is used to describe the several forms of pyrogenic carbon that occur in the environment and are produced during natural events, like lava flows, prairie and forest fires and geologic diagenesis, encompassing slightly charred plant matter, charcoal as well as soot and graphite.[12,15,29] The heat-induced transformation of plant biomass generates transient chemical properties, with gradual increase in aromaticity. The increasing charring temperature, produces the formation of aromatic ring structures, followed by a progressive condensation of smaller aromatic units into larger conjugated sheets.[29,35] Moreover, with increasing degree of thermal modification, biomass loses functional groups and C, H, O, N, and other elements, progressively aromatizes, via dehydration, decarboxylation, dehydrogenation, demethylation and cyclization reactions.[31] Keiluweit et al. studied phase transitions experienced by lignin rich (*Pinus ponderosa*) and lignin-poor (*Festuca arundinacea*) biomass as result of thermal alteration in the range of 100-700°C. Four distinct

categories of chemical phases and physical states were observed (Figure 3): transition chars, with preserved crystalline character of the precursor materials; amorphous chars, in which the heat-altered molecules and incipient aromatic polycondensates are randomly mixed; composite chars consisting of poorly ordered graphene stacks embedded in amorphous phases; and turbostratic chars, dominated by disordered graphitic crystallites (Figure 3).[29] The aromaticity is therefore a defining property of carbonized materials and can be generalized with a model with two phases: an amorphous phase, comprising randomly organized aromatic rings, and a crystalline phase, comprising condensed polyaromatic sheets that are turbostratically aligned.[36] The varying extent of these two features is assumed to largely determine the relatively high persistence of charred material in the environment.

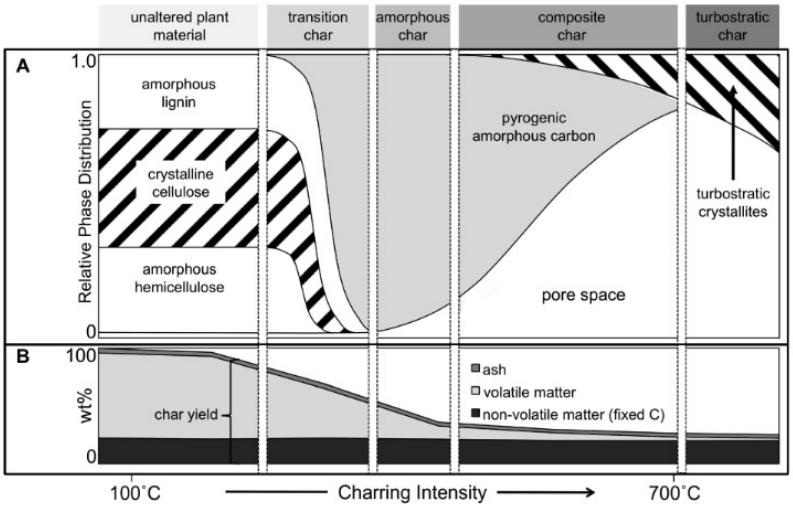

Figure 3: Dynamic molecular structure of plant biomass-derived black carbon (biochar) across a charring gradient and schematic representation of the four proposed char categories and their individual phases.[29]

Given that biochar is produced with the purpose of soil application, its stability is fundamental, as determines how long biochar C can be sequestered in soil, but also how long biochars can benefit the soil environment. The H/C and O/C molar ratios were used in coal research to describe maturity, decomposition rate, and thereby combustion behavior of fossil chars and coal.[12] Similarly, these ratios were used as indicators for the degree of condensation of biochar, with high values suggesting a large proportion of non-carbonized material. Spokas evidenced that the O:C ratio is a function of

biochar production temperature, but also accounts for other impacts (e.g., parent material and post-production conditioning/oxidation) that are not captured solely with production temperature. Therefore, the O:C ratio could provide a more robust indicator of biochar stability than production parameters like pyrolysis temperature and biomass type.[15] Schimmelpfennig et al. analyzed 66 biochars from animal and plant-based materials produced from pyrolysis, gasification and hydrothermal carbonization platforms under different temperature conditions. The study indicates that desirable ratios of biochar for soil application with the effect of sequestering carbon are H/C $\leq$0.6 and O/C $\leq$0.4.[12] Wang et al. examined models originating from coal chemistry to estimate the C aromaticity of biochar, based on its elemental composition (H/C) and fixed carbon (FC) by proximate analysis. All models were found able to successfully fit the literature data of biochar samples when atomic H/C ratio was below 1.[31] Solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy methods were applied in several studies to measure carbon functionality and aromaticity of chars.[23,34,37,38] In the analysis of a thermosequence of biochar samples in the range 250-550°C, Wang et al observed significant changes in peak intensity and chemical shifts of NMR spectra. As pyrolysis temperature increased, the resonances associated with hemicellulose, cellulose, lignin and proteins decreased. For 450°C and 550°C biochars no distinctive peaks for these compounds were observed in the spectra. This result was consistent with findings that hemicellulose degradation mainly occurred at 220–315°C, cellulose decomposition at 315–400°C, lignin within 250–450°C (with a small fraction of lignin that can be stable at higher temperature), and protein at 300–400°C.[31] A thermosequence of plant and animal-based biochars (250-650°C) revealed that aromatic C was the main C-containing functional group in all biochars and therefore, its formation was mainly controlled by the production temperature.[21] Wiedemeier et al. employed 7 methods (elemental analysis, MIR spectroscopy, NEXAFS spectroscopy, ^{13}C NMR spectroscopy, benzene polycarboxylic acids analysis, lipid analysis and helium pycnometry) to investigate the aromaticity and degree of aromatic condensation of two thermosequences of wood and grass biochars in the temperature range 100-1000°C. Aromaticity increased sharply from 200°C on,

reaching maximum values at 500–600°C, and remained constant at the maximum with higher HTT. Aromatic condensation measurements, on the other hand, increased smoothly from 300°C on, reaching highest values at 1000°C. The study concludes that some indirect, relatively inexpensive and simple methods like elemental analysis appropriately measured the aromaticity of the biochar like the more sophisticated spectroscopies.[36] VM was proposed as a parameter to assess the labile carbon prone to be biodegraded for applications related to soil remediation, amelioration and carbon sequestration.[39] Harvey et al. proposed a thermal oxidation method for assessing the biochar quality relative to its resistance to a/biotic degradation. The "recalcitrance index" R50 is based on the relative thermal stability of a given biochar to that of graphite and was determined by TGA under air from 30 °C at 10 °C /min until weight loss finished. R50 was described by the authors as an inexpensive and fast approach to evaluate the environmental recalcitrance and carbon sequestration potential of biochar.[40] The index was recently revised to take into account the ash content.[41] Analytical pyrolysis (Py-GC-MS) is another technique that can be used to infer biochar structure and stability through molecular indicators. Conti et al. characterized 35 biochars produced from switchgrass under different pyrolysis temperatures and residence times with Py-GC-MS.[42,43] The thermally labile fraction of biochar was classified into weakly, moderately and highly charred, according to the percent contribution of molecular markers. For example, dimethylfuran is a typical pyrolysis product of holocellulose, while naphthalene represents more charred aromatic structure. The study found that dimethylfuran/naphthalene ratio linearly correlated with H/C values indicating the progressive deoxygenation and polycondensation with the increasing pyrolysis temperature.[42] Further study revealed that toluene/naphthalene ratio was indicative of the carbonization degree of biochar from lignocellulosic biomass, but this molecular ratio along with the indole/naphthalene could be also indicative of the presence of proteinaceous biomass in the original feedstock. The 1-methylnaphthalene/naphthalene ratio was therefore proposed as a more reliable proxy of thermal stability of biochars from different feedstock and fitted the H/C trend.[43] Xiao et al. proposed the H/C ratio of biochar as the parameter that links biochar production temperature with its aromatic

clusters and sorption properties (Figure 4), and established quantitative relationships between these properties and H/C values.[44] In conclusion, all the aforementioned methods, based on elemental analysis, TGA, NMR, Py-GC-MS, and the corresponding parameters (e.g. H/C, O/C, VM, FC) correlate with the aromaticity and thermal stability of biochar. Moreover, biochar thermal stability reflects its recalcitrance against biological/abiotic oxidation, ultimately representing biochar environmental stability in soil.

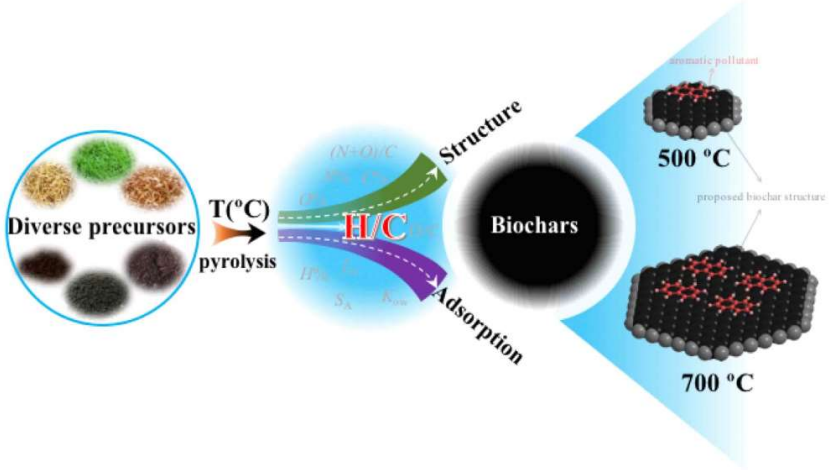

Figure 4: The relationships among pyrolytic temperature, structural characteristics and adsorption properties of biochars derived from diverse precursors via H/C ratio as a universal linkage. The H/C ratio acts as a linkage between all these parameters. The adsorption behavior of aromatic pollutant onto 700 °C derived biochar is higher than that produced at 500 °C.[44]

1.3) Guidelines for biochar quality and safety

Currently two international organizations published protocols for the determination of biochar quality and safety. IBI guidelines establish standardized testing and measurement methods for selected physicochemical properties of biochar materials. Production and handling parameters for biochar are not prescribed, but management practices for safe production and handling are recommended. Two categories of tests for biochar are required for all biochars, and include the most basic properties required to assess the utility of a biochar material for use in soil (Test Category A), and toxicant assessment (Test Category B). An additional test (Test Category C) for advanced analysis and enhancement properties is suggested as optional.[9] EBC guidelines prescribe precise indications of process conditions for biochar production and analytical methods for proper characterization. Two different grades of biochar quality are indicated according to a series of threshold values, “basic” and “premium” respectively.[18] The list of the parameters for the evaluation of biochar quality according to IBI and EBC is reported in Table 1, along with the analytical methods for their determination.

Table 1: Analytical methods for the determination of biochar quality/safety parameters according to the EBC and IBI guidelines

	EBC			IBI	
	Values		Methods	Values	Methods
Parameters	**Required parameters**			**Test Category A (basic utility properties)**	
Carbon content	> 50% (d.w.)		DIN 51732	Class 1: > 60%, Class 2: 30-60 %, Class 3: 10-30%	ASTM D4373
CHN	declaration		DIN 51732	-	ASTM D4373
S	declaration		DIN 51724-3	-	ASTM D4373
O	declaration		DIN 51733	-	ASTM D4373
H/C	< 0.7		DIN 51732	< 0.7	ASTM D4373
O/C	<0.4		DIN 51733 ISO 17247	-	-
VOC	declaration		TGA	-	-
N, P, K, Mg, Ca	declaration		DIN EN ISO 17294 – 2 (E29) DIN 51729 DIN EN ISO 11885 DIN EN ISO 17294-2	-	-
pH	declaration		DIN ISO 10390	declaration	TMECC (2001)
EC			DIN ISO 11265	declaration	TMECC (2001)
ash content			DIN 51718 TGA 701 D4C	declaration	ASTM D1762-84
moisture			DIN 51718 TGA 701 D4C	declaration	ASTM D1762-84
BET surface area			ISO 9277	-	-
bulk density			VDLUFA-Method A 13.2.1	-	-
carbonate	declaration		DIN 51726	declaration	AOAC 955.01
particle sieze distribution	-		-	declaration	Progressive dry sieving with 50, 25, 16, 8, 4, 2, 1 and 0.5 mm sieves.
	Basic	**Premium**		**Test Category B: Toxicant Assessment**	
As	-	-	-	13-100 mg/kg (d.w.)	TMECC (2001), US EPA 7000 (2007), US EPA 6010 (2007)
Co	-	-	-	34-100 mg/kg (d.w.)	
Mo	-	-	-	5-75 mg/kg (d.w.)	
Se	-	-	-	2-200 mg/kg (d.w.)	
B	-	-	-	declaration	
Cl	-	-	-	declaration	
Na	-	-	-	declaration	
Pb	< 150 g/t (d.w.)	< 120 g/t (d.w.)	DIN EN ISO17294-2 (E29), DIN 22022-2, DIN 22022-7, DIN EN ISO 17294-2 / DIN EN 1483	121-300 mg/kg (d.w.)	
Cd	< 1.5 g/t	< 1 g/t (d.w.)		1.4-39 mg/kg (d.w.)	
Cu	< 100 g/t (d.w.)	< 100 g/t (d.w.)		143-6000 mg/kg (d.w.)	
Ni	< 50 g/t (d.w.)	< 30 g/t (d.w.)		47-420 mg/kg (d.w.)	
Zn	< 400 g/t (d.w.)	< 400 g/t (d.w.)		416-7400 mg/kg (d.w.)	
Cr	< 90 g/t (d.w.)	< 80 g/t (d.w.)		93-1200 mg/kg (d.w.)	
Hg	< 1g/t (d.w.)	< 1g/t (d.w.)	DIN EN1483 (E12)	1-17 mg/kg (d.w.)	US EPA 7471 (2007)
PAHs	< 12 mg/kg (d.w.)	< 4 mg/kg (d.w.)	DIN EN 15527: 2008-09, DIN ISO 13877: 1995-06, DIN CEN/TS 16181	6 – 300 mg/kg (d.w.)	US EPA 8270 (2007)
PCB	< 0.2 mg/kg (d.w.)		AIR DF 100, HRMS	0.2-1 mg/kg (d.w.)	US EPA 8082 (2007), US EPA 8275 (1996)
dioxins/furans (PCDD/Fs)	< 0.02 mg/kg (d.w.)		AIR DF 100, HRMS	17 ng/kg WHO-TEQ	US EPA 8290 (2007)
Germination inhibition assay	n.d	n.d	n.d	pass/fail	OECD(1984), Van Zwieten et al. 2010
Additional parameters				**Test Category C: optional parameters**	
calorific value	declaration		DIN 51900	-	-
ash content (815°C)	declaration		DIN 51719	-	-
VM	declaration		DIN 51720	declaration	ASTM D1762-84
WHC	declaration		DIN ISO 14238-2011	-	-
total surface area/ external surface area	-		-	declaration	ASTM D6556
NH4 and NO3	-		-	declaration	Rayment and Higginson 1992
Total P and K	-		-	declaration	Enders and Lehmann 2012
Available P	-		-	declaration	Wang et al. 2012
Total Mg and S	-		-	declaration	Enders and Lehmann 2012
Available Ca, Mg and Sulfate-S	-		-	declaration	Camps Arbestain et al. 2015

1.4) Biochar priority contaminants

Biochar environmental applications can positively impact soil functions and mitigate climate change, however, thorough evaluation on the presence of potentially toxic elements must be monitored prior to large scale application. IBI and EBC guidelines define polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzodioxins (PCDDs) and heavy metals as biochar priority contaminants. The proposed threshold levels together with the methods for their determination were reported in the previous section (Table 1). However, the analysis of these priority contaminants is challenging due to the effect of the biochar complex matrix. In the case of trace metals, the recalcitrance of the carbonaceous matrix to degradation and acid dissolution is the main hurdle. As for the extraction of the main biochar elements (P, K, Na, Mg, Mn, Ca, Fe) described in section 1.1, the combination of acid and microwave digestion are considered appropriate procedures for the analysis of metals and metalloids in biochar.[17] On the other hand, biochar aromaticity hinders the extractability of PAHs with the traditional extraction under reflux conditions.[17] To this purpose, Soxhlet extraction was proved to be the best performing method for the extraction of PAHs from biochar. Hilber et al. compared the use of Soxhlet and Accelerated Solvent Extraction (ASE) under different conditions but concluded that ASE was consistently inferior.[45] Therefore, different methods based on Soxhlet extraction were developed, that made use of different solvents, like toluene,[45,46] dichloromethane,[47] acetone/cyclohexane.[48] However, ASE was used for method development by some authors.[49] Schimmelpfennig et al. evidenced that biochars from different technological processes can be distinguished by their PAH composition. Especially the naphthalene/phenanthrene ratio and the total PAH content appeared to be useful parameters to distinguish biochars from different production processes.[12] In fact, biochar production process could intrinsically generate potentially harmful organic compounds such as PAHs, that are carcinogenic and persistent pollutants ubiquitous in the environment. Madej et al. evidenced that among the process parameters for biochar production, neither the feedstock nor the oxygen content had a significant influence on the PAH formation, but the temperature range of 500–

700° C was considered suitable to obtain minimal PAH concentrations.[50] Buss et al. evidenced that residence time at peak temperature did not influence the PAH concentration in biochar, but the feedstock selection played an important role.[51] However, both studies stressed that complete removal of gas-phase pyrosynthesized PAHs from the reactor due to high carrier gas flow led to biochars with low PAH concentrations. Fabbri et al. developed a method for the determination of the most relevant PAHs defined by the US Environmental Protection Agency (16 US EPA PAHs) in biochar. The extraction procedure with Soxhlet and acetone/cyclohexane (1:1) solvent mixture is followed by GC-MS analysis. Biochars produced by slow pyrolysis from woody biomass presented the lowest concentrations (<10 μg/g).[48] Naphthalene was the most abundant PAH, while higher molecular weight homologues were present in trace concentrations, but their presence pose the highest health and environmental hazards due to the established carcinogenic potential of this class of compounds.[48] The method was further applied to quantify the concentration of PAHs in a vineyard soil after amendment with 33 t/ha of biochar over a period of two years.[52] The content of PAHs increased remarkably (five times on average) after biochar treatment and remained significantly higher than that in the control soil without biochar addition. However, the levels in the amended soils remained within the range reported for background soils and decreased over time. The nature of the feedstock also affects the formation of noxious organic compounds that could be adsorbed onto biochar structure during its production. For example, the presence of chlorine can lead to the formation of PCDFs and PCDDs.[46] Hale et al. quantified total and bioavailable PAHs and dioxins in a set of 59 biochars produced from slow pyrolysis, fast pyrolysis and gasification. Passive samplers were by exposed to a mixture of biochar and water, followed by solvent extraction of the cartridges and GC-MS analysis. Concentrations of bioavailable PAHs in slow pyrolysis biochars ranged from 0.17 ng/L to 10.0 ng/L which is lower than concentrations reported for relatively clean urban sediments. The gasification produced biochar sample had the highest bioavailable concentration. Total dioxin concentrations were low (up to 92 pg/g) and bioavailable concentrations were below the analytical limit of detection.[46] On the other hand, in ash-rich

biomass, the enrichment of metal and metalloids within biochar can occur during pyrolysis, even if some elements can be volatilized during the process, such as Cd, Pb, Hg and As.[17,49] Biochar contamination by Zn and Ni could also occur due to the contact with steel and tin materials during its production and storage.[17] Freddo et al. analyzed metal and metalloids in lignocellulosic biochars produced at 300 and 600°C, with ICP-MS after acid digestion of the samples. The concentrations of Cd, Cr, Cu, Ni, Pb, Zn and As were variable, however, the study concluded that biochar application to soil (up to an application rate of 100 t/ha) is unlikely to make any real difference to metal and metalloid concentrations in the receiving soil.[47] Oleszczuk et al. estimated the content of contaminants in four lignocellulosic biochar and correlated the results obtained with ecotoxicological estimation on bacteria, plants, algae, invertebrates. The content of trace metals (Cd, Cu, Cr, Ni, Pb, Zn) was comparable to uncontaminated soils. Therefore, the study concluded that no significant negative effect on the environment after the introduction of biochars to soils should be expected.[49] Luo et al. 2014 determined total, bioavailable and leachable trace metals in a thermosequence of sewage sludge biochar (200-700°C). The concentrations in most of the sample were below the control standards of sludge for agricultural use in China, USA, and Europe. On the other hand, the leachable Mn concentrations in sludge biochars produced at below 500 °C exceeded the groundwater or drinking water standards of these countries.[53]

1.5) Biochar volatile organic compounds (VOCs) and water-soluble organic compounds (WSOCs)

Chemical characterization of biochar is necessary prior to large scale application in order to prevent possible detrimental environmental effects or avoid exposure to toxicants during its handling and storage. As above mentioned, biochar quality and safety were primarily focused on its physicochemical bulk properties, chemical structure and priority contaminants. However, other parameters could affect biochar performance in soils. There are indications that organic compounds trapped onto biochar can be released in the environment potentially causing adverse as well as beneficial effects on soil biota, such as phytotoxicity.[54,55] or growth promotion.[56,57] Their release

could occur in the gaseous phase (air), as volatile organic compounds (VOCs) or in the water phase (e.g. pore water) as water-soluble organic compounds (WSOCs). Studies on the chemical nature, concentration, mobility and bioavailability of these organic compounds are required and certainly represent a new interesting field of research. Condensation of pyrolysis vapors with subsequent adsorption on the porous biochar structure during its synthesis could be the source of these chemical species.[58,59] Other possible hypotheses of their presence onto the biochar structure could be the formation during pyrolysis by *in-situ* reactions or sorption during the storage of biochar.[58,60] VOCs and WSOCs have not been inserted in present guidelines, thus they are considered here as non-conventional parameters of biochar quality, given their potential influence on its environmental performance. Accordingly, as no methods for their characterization have been standardized, the analytical techniques devoted to their detection are considered non-conventional. This section describes the analytical techniques that could potentially be suitable for the study of biochar mobile organic compounds.

Volatile organic compounds (VOCs)

During pyrolysis, the breakdown and rearrangement of the original biomass chemical structure can generate variable amounts of VOCs that can eventually be adsorbed onto biochar surface. Spokas et al. employed headspace instrumentation (headspace thermal desorption) coupled to GC-MS to qualitatively identify VOCs sorbed on biochar. This technique was selected because is common for the analysis of sorbed compounds on charcoal sample tubes. Over 70 biochars encompassing a variety of parent feedstocks and manufacturing processes (fast pyrolysis, slow pyrolysis, traditional kilns, gasification, wood fire boilers, activated chars, hydrothermal biochars and microwave assisted biochars) were evaluated and were observed to possess diverse sorbed VOC composition. Biochar samples (0.5 grams) were placed into a 10mL headspace vial and VOCs were thermally desorbed from the biochars using a head space sampler. For headspace methods, vial temperature and equilibration time are the most important parameters. A fixed thermal desorption temperature of 150°C was selected similarly to other studies using headspace methods for charcoal desorption.

Furthermore, a desorption time of 10 min was used. Over 140 individual chemical compounds were qualitatively detected. Feedstock type seemed not to be the primary determining factor for VOCs, while the presence of oxygen appeared to be a controlling factor, decreasing the amount and number of VOCs. Lower pyrolytic temperatures (< 350°C) produced biochars with VOCs consisting of short carbon chain aldehydes, furans and ketones; elevated temperature biochars (>350°C) were typically dominated by aromatic species and longer carbon chain hydrocarbons. The top ten most frequently observed compounds were: acetone, benzene, methylethyl ketone, toluene, methyl acetate, propanal, octanal, 2,3-butadiene, pentanal, and 3-methylbutanal. Carbon dioxide, methane, ethylene/acetylene, and ethanol were detected in all of the sampled biochars.[61] Bernardo et al. used the same technique to determine the concentration of 15 VOCs in the eluates of a leaching test in which biochar from the pyrolysis of pine biomass was extracted with a calcium chloride solution (0.001mol/l). The headspace sampling was performed using an equilibration time of 30min and an extraction temperature of 60°C. Organic compounds monitored and quantified were: benzene, toluene, ethylbenzene, o/m/p/-xylenes, cumene, propylbenzene, 4-ethyltoluene, tert-butylbenzene, 1,2,4-trimethylbenzene, 1-methylpropylbenzene, butylbenzene, 1,4-diethylbenzene and 1,2,4,5-tetramethylbenzene.[62] These studies opened the way for the characterization of a wide range of compounds that could be mobilised from biochar. However, further investigation on the quantity of these species and the relationships between VOCs and biochar bulk properties could better explain their role on biochar quality. Becker et al. used head space chromatography with mass spectrometry and flame ionization detector to separate, identify and quantify VOCs thermally desorbed from a series of carbonized materials produced by hydrothermal carbonization at 190-270°C. A variety of potentially harmful benzenic, phenolic and furanic volatiles along with various aldehydes and ketones were identified in feedstock- and temperature-specific patterns. These species were indicative of the degradation and partial carbonization of the parent biomass material under high pressure and relatively low temperature compared to those usually maintained with pyrolysis.[63] Solid-Phase Microextraction (SPME) is a simple and efficient, solventless sample

preparation technique [64]. Since its first applications SPME has been widely used in different fields of analytical chemistry, like environmental, food and bioanalytical analysis and is ideally suited for coupling with mass spectrometry (MS).[65–67] SPME proved to be powerful for the analysis of trace compounds in complex matrices, due to the integration of multi-staged procedures (extraction, concentration, purification) into a single step, considerably simplifying the sample preparation and reducing the risk of analytes loss. The technique is based on the use of a fused-silica fibre coated with a polymeric stationary phase. In combination with GC-MS, the analytes in the sample are directly extracted onto the fibre coating compounds and transferred into the injector of a gas chromatograph for thermal desorption and analysis. The versatility of fibre SPME allows the possibility of sampling the analytes in the head-space (HS-SPME), for the detection of volatile compounds or by direct insertion (DI-SPME) into the sample matrix for the analysis of less volatile components.[64,68] Piri-Moghadam et al. compared SPME methods for the detection of environmental pollutants in water samples (VOCs, PAHs) with officially standardized procedures (US EPA, ASTM). The accuracy of SPME was in good agreement with the traditional ones based on liquid-liquid extraction (LLE) and solid phase extraction (SPE), but SPME can overcome some concerns regarding the greenness of LLE technique and provides broader range of applications.[68] Soria et al reported HS-SPME as an advanced method for the analysis of pyrolysis products in bio-oil. Its application was seldom reported for biochar analysis while detailed performance studies have not been conducted yet.[69] Clough et al.2010 applied the SPME to qualitatively detect VOCs in a biochar sample. Divinyl benzene fibre was selected for the analysis in the head space mode, with the fibre exposed at 40°C for 40 minutes. Only α,β-pinene and acetaldehyde were identified among the VOCs evolved from the biochar.[70] Higashikawa et al.2013 selected DVB fibre (20°C and 20 minutes fibre exposure) to quantitatively detect model VOCs in complex environmental matrices and demonstrated that poultry manure and oak biochars presented high affinity for the spiked compounds.[71] Studies specifically aimed at the determination of VOCs in biochar in relation to its bulk chemical properties have not been published. Therefore, SPME has the potential for the

evaluation of trace amounts adsorbed onto biochar as consequence of re-condensation of pyrolysis vapours.

Water-soluble organic compounds

In comparison to VOCs, more studies are available on compounds that can be released in water from biochar. Lin et al. investigated the composition of water-extractable fraction of four lignocellulosic slow pyrolysis biochars produced at increasing temperatures (380-600°C) by means of liquid chromatography organic carbon detection (LC-OCD), that allowed to compare the separated substances with classes usually employed to characterize natural organic matter.[72] The detected species were attributed to: biopolymers consisting of polysaccharides and nitrogen containing materials, humic acids, fulvic acids and their degradation products, low molecular weight acids and neutral species, and hydrophobic components. Organic acids were important species even in biochars produced at higher temperatures, while humic-like acids and low molecular weight neutral species were the principal components of the compounds extracted from the low temperature chars (<450°C). Similar results were observed by Tahesimoosavy et al 2015 in which dissolved organic carbon (DOC) decreased in biochar produced from 450 to 650°C. The pyrolysis temperature greatly affected the composition of DOC as led to a degradation of the high molecular weight species into low molecular weight acids.[73] Lievens et al. investigated the nature of the leached organic compounds from biochar produced from mallee leaf and bark, by a fluidized-bed pyrolysis reactor at 400 and 580°C. The biochar solvent-extractable organic compounds (chloroform/methanol) were analyzed by GC-MS, and a total of 9 compounds were detected, including phenolic species (phenol, 4-methoxy phenol, 3,4-dimethoxy phenol), organic acids (acetic acid, propionic acid, methyl butanoic acid), levoglucosan, 1,2,4-trimethoxybenzene and 4-hydroxy-3-methoxybenzaldehyde. The structure of water-leached aromatic compounds was suggested to be composed of structures with 2–3 and 3–5 fused aromatic rings, according with maxima of synchronous fluorescence spectra centred around 340 and 390 nm, respectively.[74] Qu et al. characterized the dissolved black carbon released into water by two slow pyrolysis biochars

(400°C) with different analytical techniques (elemental analysis (EA), X-ray photoelectron spectroscopy (XPS), UV-vis, Raman, X-ray diffraction (XRD), FTIR, and solid-state ^{13}C NMR). The characteristics of biochar dissolved black carbon were similar to that of the humic acids, but with a higher aromatic and carboxylic structures.[75] Fluorescence excitation-emission spectrophotometry is a rapid, sensitive and non-destructive technique often used for tracing the dynamics of dissolved organic matter (DOM) in marine and freshwaters as well as soils and sediments.[76–80] The specific excitation and emission wavelengths are characteristic of the molecular nature of the fluorophores.[81] The concentration and chemical composition of DOM influence the intensity and shape of the fluorescence spectra.[82] At low concentrations, the peak intensity is directly proportional to the concentration of a given fluorophore in solution. This relationship is not valid at high sample concentrations as a result of absorption of excitation and emission light by the sample matrix.[81] The results of the fluorescence analysis can be assembled in Excitation-Emission Matrices (EEMs), obtained by combining fluorescence emission spectra measured from a series of different excitation wavelengths and subsequently arranging the composite scan in a grid (excitation X emission X intensity). Interpretation of fluorescence EEMs can be performed by means of multivariate analysis and multiway techniques, like the Principal Component Analysis, Partial Least Squares Regression, Principal Filter Analysis. Parallel factor analysis (PARAFAC) which can decompose the fluorescence signal into underlying individual fluorescent phenomena has been widely used.[81,82] Uchimiya et al. used the PARAFAC analysis to resolve the overlapping spectra obtained from the EEM to investigate the structural changes in biochar-derived DOC as a function of feedstock and pyrolysis temperature. In that experiment, five feedstocks (almond shell, broiler litter, lignin, cottonseed hull and pecan shell) were pyrolysed at 4 incremental temperatures between 350 and 800°C and each kind of biochar was sequentially extracted using hot water (80°C) and a cold NaOH solution (0.05M) for 16h.[83] Zhang et al. investigated the properties of water-extractable fractions of four biochar samples with the fluorescence excitation-emission matrix (EEM) analysis and adopted the fluorescence regional integration (FRI) technique for the interpretation of the

spectra. Wood, bamboo and rice based biochars were extracted by shaking with room temperature deionized water at 200r/min for 4h. EEM spectra were normalized to the TOC concentration of the samples. According to FRI, EEM spectra were divided into five excitation-emission regions (tyrosine-like organic compounds, tryptophan-like organic compounds, fulvic acid-like materials, soluble microbial byproduct-like materials, humic acid-like materials). The cumulative Ex/Em areas of region indicative of fulvic acid-like materials and humic acid-like materials were the highest for the wood biochar sample, whereas bamboo biochar had the lowest levels of humification materials.[84] Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) is a technique that effectively converts ionic mass to charge ratio to an experimentally measurable ion cyclotron orbital frequency. Because frequency can be measured more accurately than any other physical property, FT-ICR-MS offers highest resolving power and mass accuracy among all types of mass spectrometers. Its unique analytical characteristics made FT-ICR-MS important tool for proteomics, metabolomics, petroleomics, and investigation of complex mixtures.[85–88] Electrospray ionization (ESI) is a soft ionization method designed to produce charged species that could be manipulated within the gaseous or high-vacuum phase of mass spectrometers. ESI takes place at atmospheric pressure, ionizes a wide range of polar, hydrophilic molecules with both acidic and basic functional groups. The production of positive or negative ions depends on the ionization efficiency. Samples with easily ionizable and/or numerous acidic groups, such as carboxylic acids, will readily lose a proton and be negatively ionized very efficiently. In contrast, samples with many basic groups, such as amines, will easily pick up a proton and be positively ionized.[89] ESI-FT-ICR-MS has been used to unravel the molecular complexity of natural organic matter.[90–93] Given the similarity between some of the structures of biochar WSOCs and those of NOM evidenced by spectroscopy studies, ESI-FT-ICR-MS analysis could be extended to the detailed characterization of biochar WSOCs. Smith et al. studied the compounds released in water by three different biochar samples produced from peanut shell, chicken litter, and pinewood at 450°C with negative ESI-FT-ICR-MS. The molecular composition of pinewood-derived biochar water extracts showed unique

carbohydrate ligneous components and sulfur containing condensed ligneous components that are both absent from the peanut shell water extracts.[94] In a following study, the same authors investigated the chemical composition of lignocellulosic biochar produced at 300, 400 and 500°C together with the residues of lignin and cellulose produced at the same temperatures. The amount of WSOCs extracted from biochar, irrespective of biomass starting material, decreased significantly as a function of pyrolysis temperature.[59] Riedel et al. conducted batch and soil column experiments to investigate the composition of DOM leached from an arable topsoil amended with biochar. Negative ESI-FT-ICR-MS revealed a marked change in the composition of the OM mobilized. The most saturated and reduced compounds were removed in the solutions leached from the biochar amended soil. Newly-appeared ‘‘lignin-type’’ compounds were detected, indicating that non-black carbon was also leached from the amended soil in the form of highly oxygenated DOM.[95]

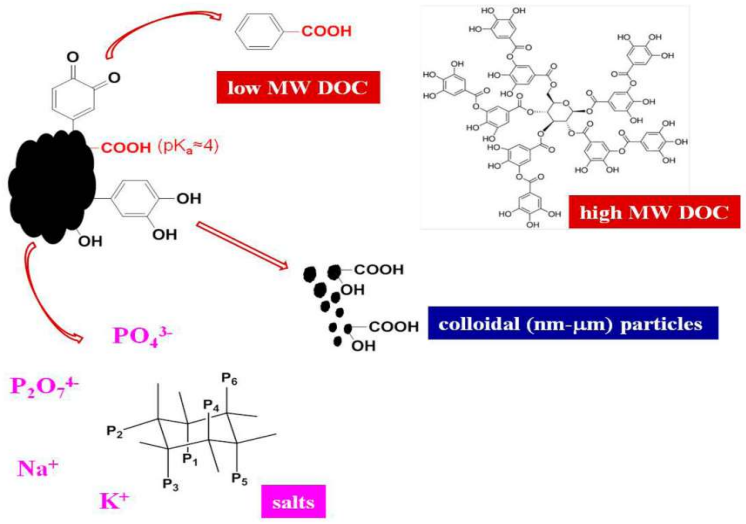

Figure 5. Proposed constituents of biochar-borne pyrogenic dissolved organic carbon.[96]

Environmental importance of biochar VOCs and WSOCs

As previously discussed, the biochar heterogenous complex structure can adsorb and retain several organic and inorganic compounds during its production, and these fractions can be subsequently mobilized in the environment after biochar application to soil. It is therefore fundamental to screen the potential effects of biochar mobile species prior to large scale application. IBI guidelines for biochar quality recognizes the importance of the evaluation of potential toxicity arising from the biochar in soil applications and proposes biochar toxicity assessment by following the requirements commonly used for soil amendments, composts and fertilizers. In particular, the germination inhibition assay is indicated as compulsory test for the determination of suitable biochar quality.[9] Seed germination and root elongation tests are among the simplest short-term bioassays for estimating the potential impacts of contaminants in the soil.[97] Many procedures used for testing biochar phytotoxicity were followed by different authors. One of the reference method is the OECD Guideline for testing of chemicals "Terrestrial plants, growth test", in which the test substance has to be incorporated at various concentrations into soil where the seeds are sown, and the number of seedlings that emerge is recorded.[98] Van Zwieten et al. applied this procedure to test the toxicity of two biochar produced by slow pyrolysis of papermill waste at 550°C with two agricultural soils, using three plant species, radish (*Raphanus sativus*), wheat (*Triticum aestivum*) and soybean (*Sorghum bicolor*). The study concluded that no negative effects on plant germination in the presence of biochar were observed suggesting the absence of detrimental components. Contrarily, germination of wheat in the ferrosol was significantly improved in the presence of either biochar.[99] Free et al. tested the effects of the incorporation of biosolids and corn stover biochar produced at 550°C at different rates (0-10 t/ha) into soil. Germination tests were performed with 50 seeds of maize (*Zea mays*) and showed no effects of biochar feedstock or rate of biochar application neither on germination of maize or dry weight of coleoptiles, roots and coleoptile length.[100] Alburquerque et al. tested biochars produced from five feedstocks (olive stone, almond shell, wheat straw, pine woodchips, and olive-tree pruning) at different temperatures from 368 to 507°C with sunflower

(*Helianthus annuus*). The percentage of germinated seed with respect to control soil increased with increasing biochar-application rates. Statistically significant correlations between the number of germinated sunflower seeds and soil variables such as pH, EC, field capacity, bulk density, and DOC were proposed to explain the positive effect.[101] The bioactivity of the compounds that can be released into water by biochar was highlighted by Rogovska et al.[54] Six biochars produced from hardwood, corn and switchgrass at different pyrolysis temperatures ranging from 450 to 850°C were extracted with deionized water for 24 hours and twelve corn seeds were germinated with the aqueous extracts. Biochars produced at high conversion temperatures caused a significant reduction in the shoot and root lengths of the seedlings, while the germination rate was not affected. The comparison with the results of nutrient solutions prepared to mimic the nutrient composition and pH of the biochar extracts suggested that the nature of biochar phytotoxicity was due to water-soluble organic compounds. Polycyclic aromatic hydrocarbons were detected in the aqueous extracts and attributed responsible for the reduction in seedling growth.[54] Busch et al. developed germination and growth tests for the determination of phytotoxic substances and salt stress of biochars and hydrochars. The first one with chars mixed into unfertilized peat substrate and barley, as plant not sensitive to salt stress. The second test was carried out on salad (*Lactuca sativa*) a salt-sensitive plant. Negative effects on salad germination, but not barley germination and growth, may indicate high ash contents but no harmful substances, whereas effects on barley germination would indicate potential toxic effects. Moreover, a germination test with cress (*Lepidium sativum*) designed for compost testing, was modified to evaluate the effect of gaseous substances emitted by the chars. While the biochar did not induce negative effects hydrochar showed negative effects in all tests. A sequential test with the same hydrochar showed positive results and allowed to hypothesize that the harmful substances must have been degraded or they were water soluble and leached. It was proposed that the negative effect observed with fresh hydrochar was most likely associated with the emission of phytotoxic volatile substances, such as formic or acetic acid.[102] Bargmann et al developed tests with spring barley (*Hordeum vulgare*) to assess phytotoxic effects of biochar,

hydrochar and process-water from hydrothermal carbonization (HTC) as soil amendments.[103] The content of dissolved organic carbon (DOC) in the chars correlated with the formation of seedlings (Figure 6). While low DOC of the biochars did not show inhibiting effect, higher values associated to the extracts of hydrochars presented significant reduction of the germination rate. It was concluded that the inhibiting substances are partly gaseous and are released to the atmosphere after application, and partly water-soluble. An additional experiment was performed to test 11 selected potentially phytotoxic substances on cress. Total inhibition of the germination was observed for glycolic acid and levulinic acid, while guaiacol caused a 50 % impediment of germination. Acetic acid, glycolaldehyde dimer and catechol had a negative impact on growth. It was concluded that substances such as organic acids, phenols and aldehydes may be responsible for phytotoxic effects of the hydrochars.[103]

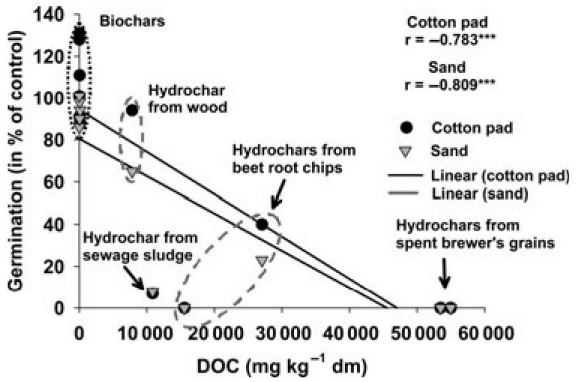

Figure 6: Correlation between germination of barley seeds and DOC content of hydrochars and biochars in substrates with 10 % char amendment.[103]

Zakaria et al. proposed Petri dish bioassays as simple and rapid ecotoxicological tests for preliminary assessment of biochars in soil-less conditions. The study compared the performance of five biochars in soil-less versus soil-based germination and growth tests. The soil-less procedure was finally proposed as a simpler preliminary screening method than a soil-based bioassay using a standard soil like OECD 1984.[104] Buss et al. studied softwood biochar produced at 550°C that were contaminated with pyrolysis gases during process operation (Figure 7). VOC contaminated biochar showed phytotoxicity on cress seeds (germination tests) while non-contaminated biochar produced

under the same conditions did not present any negative effect.[58] Even after four weeks of storage, the contaminated biochar released small quantity of vapours inhibiting germination. The nature of toxicity was only hypothesized but the chemical nature not determined. Further studies led Buss et al. to conclude that VOCs posed greater concern for plant growth than PAHs.[105] In summary, biochars produced from different feedstocks and conditions exhibited contrasting effects in germination tests. The summary of biological effects on a variety of plant species is reported in Table 2 along with the possible cause.

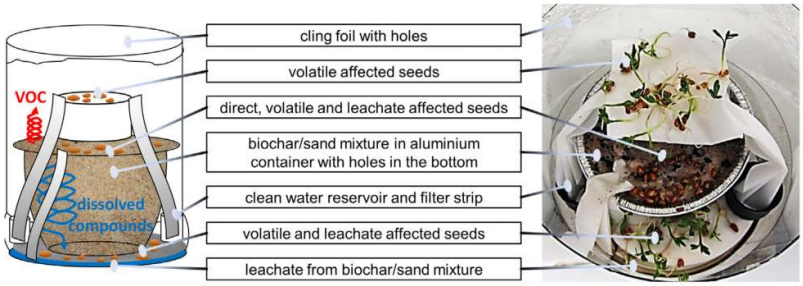

Figure 7: Germination test for assessing the effect of biochar mobile compounds on cress seeds.[58]

Table 2: Summary of the biological effects of biochar and hydrochar on seed germination and seedling growth.

Feedstock	Production temperature (°C)	Test	Effect	Proposed cause	Reference
papermill	550	*Raphanus sativus* (radish), *Triticum aetivum* (wheat), *Sorghum bicolor* (soybean)	Improved germination of wheat	liming effect, CEC,	Van Zwieten et al.[99]
biosolids, corn stover	550	*Zea mays* (maize)	no significant effect in the germination and early growth	-	Free et al.[100]
olive stone, almond shell, wheat straw, pine woodchips olive-tree pruining	368-507	*Helianthus annus* (sunflower)	increase in the germination rate with increasing biochar application rate	pH, EC, field capacity, bulk density, DOC	Albuquerque et al.[101]
hardwood, corn, switchgrass	450-850	*Zea mays* (maize)	No effects in the germination rate. Reduction in shoot and root lengths with high temperature biochar	PAHs in water extracts of the biochar	Rogovska et al.[54]
peanut hull residue, beet-root chip	203 (hydrochar), 498 (biochar)	*Hordeum vulgare* (barley), *Lactuca sativa* (salad), *Lepidium sativum* (cress)	Biochar did not inhibit germination, while hydrochar showed negative effects	phytotoxic VOCs in hydrochar (formic acid, acetic acid)	Busch et al.[102]
several lignocellulose, manure, sewage sludge	190 (hydrochar), 860 (biochar)	*Hordeum vulgare* (barley)	DOC content correlated with the formation of seedlings	VOCs and WSOCs inhibited seedlings growth (glycolyc acid, levulinic acid, guaiacol)	Bargmann et al.[103]
oil mallee, rice husks, jarrah, wheat chaff	550-700	*Triticum aetivum* (wheat)	biochar both increased and decreased seed germination rates	trace organic compounds, high alkalinity acidity can cause the negative effects	Zakaria et al.[104]
softwood pellets	550	*Lepidium sativum* (cress)	inhibition of germination	high VOCs emission from biochar	Buss et al.[58]

2) AIM OF THE STUDY

Biochar application to soil requires the careful consideration of organic compounds that can be adsorbed or generated onto biochar surface during its production. The mobility of these species in the environment can produce impacts on soil system and biota. The importance of priority contaminants like PAHs, PCDD/Fs and heavy metals, was established by the definition of threshold levels for biochar quality and safety. However, compounds that can be mobilized in the air as volatile organic compounds (VOCs), and in water as water-soluble organic compounds (WSOCs) were less investigated. A wide array of compounds could be present in VOCs and WSOCs, ranging from highly polar to less polar species. Due to its versatility for the analysis in the gas and water phases, solid-phase microextraction (SPME) could be eligible for the sampling of VOCs and WSOCs in biochar. The principal aim of this study was to evaluate the potential of SPME for the characterization of VOCs and WSOCs in biochar. Preliminary experiments showed that Carboxen-PDMS SPME fiber resulted adequate for the analysis of pyrolysis products of biomass. A second objective was the correlation of mobile organic compounds with biochar bulk properties and production conditions, to shed lights on the definition of quality parameters. Finally, the chemical information obtained by the developed analytical methods tailored for the determination of biochar organic compounds was used interpret the biological effects observed on plant growth. The present thesis is structured into the following sections:

- Volatile organic compounds: the first section is focused on the development of an analytical method based on SPME and gas chromatography-mass spectrometry (GC-MS) for the determination of VOCs released from biochar, in relation to its carbonization degree and thermal stability.
- Water-soluble organic compounds: the section is aimed at the characterization of organic compounds released from biochar into water (WSOCs) in relation to its carbonization degree and thermal stability. Due to the limitation of SPME-GC-MS to the determination of volatile

and semi-volatile species, fluorescence spectroscopy and ultrahigh resolution mass spectrometry techniques were employed to investigate the composition of higher molecular weight constituents of WSOCs.

- Characterization of biochar produced from a pilot plant pyrolysis process: the section presents the application of the methods developed on biochar from a bench scale reactor, to samples produced with the thermo-catalytic reforming process at Fraunhofer UMSICHT (Germany). Differences in the profiles of VOCs and WSOCs can be used to test the incidence of the process on biochar quality.
- Relationships between VOCs, WSOCs and seed germination: the section provides the correlation between the fingerprints of VOCs and WSOCs released by biochar and their effects on plant growth at different stages.

3) DETERMINATION OF VOLATILE ORGANIC COMPOUNDS (VOCs) IN BIOCHAR

3.1) INTRODUCTION

Biochar is the solid carbonaceous material produced by the pyrolysis of biomass for a variety of applications in the agro/environmental field.[7] The wide range of feedstocks that can be thermally converted, including animal (poultry litter, residues from anaerobic digestion) as well as vegetable based materials (agricultural residues, energy crops), and the different technologies currently under optimisation for biochar production highly influence its physical and chemical properties.[36,106] The increasing interest in finding suitable biochar applications (e.g soil amendment, greenhouse gas mitigation, feed additive, new materials, remediation of polluted areas)[14,107] led to a comprehensive investigation on its chemical characteristics to determine its quality prior to large scale use.[23,108] Standardisation of analytical methods for biochar testing is under investigation with conventional and non-conventional techniques and standard parameters for its quality/safety were proposed.[9,17,18] During pyrolysis condensable gases could be retained onto the biochar porous structure and these compounds could have a certain degree of mobility. Few studies dealt with the determination of trace compounds that can be released in air or water, however, these are important parameters for biochar quality because their mobility could exert positive or negative effect on plants, microorganisms or other organisms in natural environments as well as soil chemical properties.[52,58,109–111] Biochar labile substances can have a priming effect on soil biota[112] and the potential to influence the pyrogenic black carbon cycle.[113] Moreover, if large amounts of biochar are produced for soil application, the presence of harmful compounds such as monoaromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes: BTEX), polyaromatic hydrocarbons (PAHs) and heavy metals, has to be monitored for health and safety concerns during its handling and storage and to prevent impacts on plants,[52,58,109,111] or other organisms.[49] Spokas et al.[61] qualitatively investigated volatile organic compounds (VOCs) released by 77 biochars produced from different processes and different temperatures with traditional head space gas

chromatography-mass spectrometry (HS)-GC-MS instrumentation. Differences in the amounts of VOCs (total ion current) from similar feedstocks (oak hardwood), pyrolysis units and temperatures were found as well as VOCs profiles of corn stover biochar produced at similar conditions but from different units. The top ten most frequently observed compounds were acetone, benzene, methylethyl ketone, toluene, methyl acetate, propanal, octanal, 2,3-butadiene, pentanal, and 3-methyl butanal.[61] The overall trend associated with an increase in pyrolysis temperature within the same unit was a net decrease in total sorbed VOCs with an increasing proportion of aromatic compounds. Buss et al.[58] studied softwood biochar produced at 550°C that were contaminated with pyrolysis gases during process operation. VOCs contaminated biochar showed phytotoxicity on cress seeds (germination tests) while non-contaminated biochar produced under the same conditions did not present any negative effect. Even after 4 weeks storage, contaminated biochar released small quantity of vapours inhibiting germination, but the chemical nature of the contamination was only hypothesized. Further studies led Buss et al.[111] to conclude that VOCs posed greater concern for plant growth than PAHs. EBC guidelines for biochar quality indicates that the VOCs are very important for the determination of biochar quality but no quantitative information or thresholds were given.[18] The report cites the qualitative study conducted by Spokas et al.[61] and concluded proposing the assessment of total VOCs by thermogravimetric analysis (TGA). IBI proposed the same determination of VOCs as advanced analysis optional for biochar quality.[9] Solid phase microextraction (SPME) is a powerful solventless technique for the analysis of trace components in complex matrices by GC-MS, that integrates several analytical steps (sampling, extraction, pre-concentration and sample introduction for instrumental analysis).[65] The potential of SPME for the analysis of mobile organic compounds was described in the characterisation of poultry litter biochar where the technique enabled the detection of alkyl phenols, small hydrocarbons, alkyl aromatics, small PAHs and nitrogen containing compounds.[55] However, the effect of biochar characteristics on the qualitative and quantitative distribution of VOCs determined by HS-SPME has not been investigated yet. In this study, a method based on HS-SPME-GC-MS was developed in order to

understand the pattern of VOCs retained or formed in biochar during pyrolysis of biomass and how they relate to process conditions and biochar bulk properties. The method was tested on seven biochars obtained from the pyrolysis of a typical lignocellulosic biomass, corn stalk, at increasing pyrolysis temperatures (350-650 °C). The purpose of this study was the detailed characterisation of the mobile organic compounds that could be volatilised from biochar, potentially affecting its environmental performance, and the correlation of the VOCs species with biochar carbonisation degree. Secondly, the potential of HS-SPME data for the evaluation of biochar quality for sustainable applications was discussed.

3.2) MATERIALS AND METHODS

Chemicals

2-allyl-6-methoxy phenol (*o*-eugenol) and methanol were purchased from Sigma Aldrich. SPME fibre holder and Carboxen-polydimethylsiloxane (Car-PDMS) fibre were purchased by Supelco.

Biochars

Pellettised corn stalks were pyrolysed in a bench-scale horizontal quartz reactor[114] at different temperatures (350, 400, 450, 500, 550, 600 and 650 °C) with fixed residence time of 20 minutes and 1000 ml/min nitrogen flow. During pyrolysis actual temperature in the reactor was measured with a thermocouple. For each pyrolysis batch about 15 grams of biomass were processed. The char produced was let to cool in the reactor under nitrogen flow, collected and after homogenisation, stored in the freezer. The syntheses of biochar were performed in triplicate, with relative standard deviation (RSD) of the yields for each pyrolysis condition less than 2%. Biochar samples obtained at a given pyrolysis temperature *XXX* °C were grouped, homogenised and stored in closed vials at –25°C before analysis; the resulting sample was named as *CSXXX*. Spent mushroom substrate was pyrolyzed under the same conditions at 500°C and the resulting biochar was named SM500

Biochar characterisation

Elemental analysis of biochar samples was performed with a Thermo Scientific FLASH 2000 Series CHNS/O Elemental Analyser. TGA of thermosequence biochar samples was performed with a Mettler Toledo TGA/DSC. Between 5 and 10 mg of biochar were placed in alumina pans. TGA analyses were performed with nitrogen purge gas (50ml/min) and the following temperature program: 25°C for 2 min, 25-110°C at 25°C/min, 110°C for 10min (segment A); 110-900°C at 25°C/min, 900°C for 10min (segment B); 900°C for 20 min in air at 50ml/min (segment C). A blank curve of an empty pan was acquired for baseline correction during the evaluation of the biochar samples. Proximate analysis was conducted from the weight loss curve of each sample. Moisture content was calculated from the weight loss during segment A, while the volatile matter (VM) and fixed carbon (FC) from segments B and C, respectively. The ash content was calculated from the residual weight after fixed carbon oxidation (segment C). Analyses were performed in triplicate. Data were normalised by sample weight and expressed in % dry basis.

HS-SPME procedure

One gram of biochar was exactly weighed in 20 ml HS vials and spiked with 1 µl (weighed at ± 0.01 mg) of methanol containing *o*-eugenol as surrogate pyrolysis product (1.00 mg/ml). Sealed vials were placed on a heating plate at 150°C where only the bottom part containing biochar was in contact with the heated surface. The Car-PDMS fibre was placed in the HS where the temperature was about 40°C. Temperatures were constantly measured by means of a PSC-MS Plus Portable Handheld infrared thermometer. The exposure time was fixed at 30 minutes according to Becker et al.[63] Spokas et al.[61] Preliminary experiments during method development showed that: Car-PDMS fibre gave better performance in terms of signal intensity compared to polyacrylate and polyethylene glycol SPME fibres; increasing exposure time more than 30 minutes did not significantly increase GC signal. The HS-SPME procedure was also applied to calibration solutions,

1 g of $CaCO_3$ spiked with 1µl of internal standards and CS biochar samples heated at different temperatures (25, 50, 100, 150°C) during sampling.

GC-MS conditions

After fibre exposure, the Car-PDMS was inserted into the split/splitless injector of an Agilent 5977 gas chromatograph equipped with a straight SPME liner. Analytes were thermally desorbed at 250°C for 10 minutes and separation performed with a DB-FFAP polar column (30m length, 0.25mm i.d, 0.25µm film thickness). Starting GC oven temperature was set to 36°C (5 minutes) and increased to 250°C (10°C/min). Detection was made with a quadrupole mass spectrometer Agilent 7820A operating under electron ionization at 70eV with acquisition at 1 scan/sec in the *m/z* 29 and 450 range. Mass spectra were acquired in full scan mode properly adjusting the electron multiplier voltage. Identification was based on library mass spectra matching (NIST) and literature data. Quantification was made from the peak area integrated by extracting characteristic ions from total ion current.

Quantification and statistical analysis

The quantity of each analyte released by biochar and captured by the SPME fibre in the HS of test vials (20 mL) was expressed in terms of normalised peak area *NA*. *NA* was calculated from the peak areas of the analyte ($A_{analyte}$) and the internal standard (A_{is}), the quantity of added internal standard (μg_{is} of methanol) and the analysed biochar using the following equation (Eq.1):

$$\text{Eq.1: } NA = \frac{A_{analyte}}{A_{is}} \frac{\mu g_{is}}{g_{biochar}}$$

This approach was considered adequate for the purposes of this study intended to compare the relative behaviour of different biochar samples produced from the same feedstock. The linearity of the HS-SPME method was tested on *o*-eugenol. Ordinary least squares regression computed on the peak area of *o*-eugenol in the calibration range of 108-0.108 ng (12 data points) resulted in the following linear model ($y = ax + b$): $y = 4.43E06x - 4.56E06$, where x and y are the amount of

internal standard and its peak area respectively. 95% confidence intervals for a and b were $[4.34E06; 5.55E06]$ and $[9.42E06; 8.38E05]$ respectively, while the standard errors were $5.43E04$ and $2.94E06$. R^2 and Pearson correlation coefficient (r) were both 0.999. Average S/N values for 0.1 ng level was 50 (n=3, 4% RSD) indicating that under the selected conditions VOCs at the sub-ng level could be detected in the HS of biochar. When the methanolic solution of *o*-eugenol was added to the biochar matrix, the GC signals of *o*-eugenol were strongly reduced in comparison to those observed from the HS-SPME of the neat calibration solution. The values were rather low (less than 5 % the value of the calibration solution), especially for the more carbonised biochars. This effect was attributed to the strong hydrophobic adsorption of *o*-eugenol onto the biochar surface. In fact, when the calibration solution was added to powdered $CaCO_3$ the HS-SPME produced a value more similar (73% ± 15) to that in the absence of a solid matrix (i.e. the calibration solution 100% ± 7). A different behaviour was exhibited by methanol that produced a signal lower than that in the absence of biochar, but similar in all the tested biochar samples (17.0 % ± 1.3) with a reasonable precision (RSD < 10%) for each biochar. For these reasons, methanol was selected as internal standard in the calculation of the quantity of analyte evolved from a given quantity of biochar under HS-SPME conditions. The RSD of methanol peak area (A_{is}) from triplicate analyses of each biochar was < 10%. All the analyses were conducted in triplicate; data were reported as mean values ± 1 standard deviation (s.d.) or % relative standard deviation (RSD). Blanks were performed to ascertain the absence of cross-contamination. Ordinary least squares regression was computed with the software PAST (Paleontological Statistic vers. 2.16) while Pearson product moment correlation coefficients (*r*, with *P* level of significance) were calculated with the software SigmaPlot vers.12.0. Kruskal-Wallis and Jonckheere-Terpstra statistics were performed with the NSM3 package of R according to Hollander et al.[115]

3.3) RESULTS AND DISCUSSION

Biochar characteristics

The elemental composition of biochar samples produced in this study was reported in Table 3. The hydrogen and oxygen content decreased with increasing pyrolysis temperature and accordingly the values of H/C and O/C atomic molar ratios decreased (from 0.80 to 0.32 and from 0.17 to 0.07 from 350 °C to 650 °C, respectively). The content of nitrogen also decreased with increasing pyrolysis temperature. Proximate analysis of biochar samples were reported in Table 4. The FC and ash content tended to increase with the pyrolysis temperature while the VM decreased. These trends were typical observed in thermosequence biochars due to the more severe thermal decomposition of biopolymers at higher temperature with enhanced elimination of volatile compounds and polycondensation, aromatisation and defunctionalisation of the carbonaceous matrix.[116–119] H/C and O/C atomic molar ratios were proposed as an index of biochar carbonisation degree or its degree of aromaticity.[42]

Table 3: Elemental composition (% d.w.) and atomic molar ratios of corn stalk (CS) biochar samples. Mean values and %RSD (n=3). Oxygen content by difference.

biochars	***N***	**RSD**	***C***	**RSD**	***H***	**RSD**	***O***	***H/C***	***O/C***	***C/N***
CS350	1.2	*2.1*	55	*0.43*	3.7	*1.2*	13	0.80	0.17	56
CS400	1.0	*2.9*	54	*1.1*	3.2	*1.2*	9.6	0.71	0.13	61
CS450	1.0	*2.2*	56	*1.3*	2.7	*0.2*	7.1	0.59	0.095	63
CS500	0.92	*3.6*	56	*1.7*	2.3	*5.4*	5.5	0.49	0.074	70
CS550	0.87	*5.3*	55	*5.5*	2.0	*6.3*	5.6	0.44	0.078	73
CS600	0.84	*5.0*	55	*2.2*	1.6	*1.6*	3.9	0.36	0.053	77
CS650	0.69	*12*	54	*7.0*	1.4	*9.0*	5.1	0.32	0.071	91

Table 4: Proximate analysis of corn stalk thermosequence. Data are reported as % on dry basis with %RSD (n=3)

Biochars	VM	*%RSD*	FC	*%RSD*	Ash	*%RSD*
CS350	33	*1.4*	43	*1.0*	24	*3.6*
CS400	26	*0.67*	45	*2.1*	28	*3.8*
CS450	21	*2.3*	49	*1.2*	30	*2.9*
CS500	20	*0.46*	49	*3.3*	31	*4.9*
CS550	18	*3.0*	50	*2.6*	32	*4.5*
CS600	16	*4.3*	50	*0.87*	34	*2.0*
CS650	15	*2.9*	51	*1.7*	35	*3.2*

H/C values were preferred in this study given that O/C values may not be adequate for biochars with high ash content.[36] The trend of H/C demonstrated to be statistically significant with the Jonckheere-Terpstra statistics ($P < 0.01$). FC increased with the decreasing H/C of the biochars, with correlation coefficients of 0.83 ($P < 0.05$) indicating a good correlation between different parameters representative of the carbonisation degree, while the volatile matter progressively decreased with the increasing H/C ($r = 0.97$, $P < 0.01$).

Qualitative analysis: biochar volatilome

Thermosequence biochars evolved a variety of organic compounds in the HS that were detected by SPME GC-MS. A typical chromatogram obtained from the HS-SPME of the biochar sample CS350 (H/C 0.80) is presented in Figure 8. In total 88 compounds were tentatively identified belonging to several compounds classes (Tables S1 and S2). The initial part of the chromatogram presented an intense and poorly resolved sequence of peaks formed by volatile compounds, principally C_3-C_4 oxygenated compounds, for example propanaldehyde, butanaldehyde, butan-2-one, methyl acetate. Some of these compounds were also detected in previous studies with different biochars.[55,61,70,71] Blank analyses confirmed that these compounds were not present in reagents and glassware utilised in the analytical procedure, however, it cannot be excluded that biochar might adsorb volatile compounds from the ambient air and release them under HS conditions even though precautions were made to limit contact with air. The attention in this study was focused to $C_{\geq 5}$ compounds that eluted later in the chromatogram and were structurally related to the original biopolymers composing the original plant biomass or indicative of their charring intensity (Figure 8). Typical thermal degradation products of lignin (2-methoxyphenol, i.e. guaiacol, and its alkylated derivatives) and holocellulose (e.g. 1,4:3,6-dianhydro-β-D-glucopyranose, C_1-C_3 furans and furanones, C_1-C_2 cyclopentenones and pyranones) could be identified in CS350 (Figure 8).[42,120,121] Aromatic (benzoic and benzeneacetic) and short chain (up to C_8) aliphatic acids

principally in the form of methyl esters were identified. Apparently, methylation of carboxylic groups could take place during sampling. Lipids could be the source of these acids. These compounds were detected in HS and aqueous extracts of biochar from poultry litter where they could play a role in the inhibition to plant seed germination.[55]

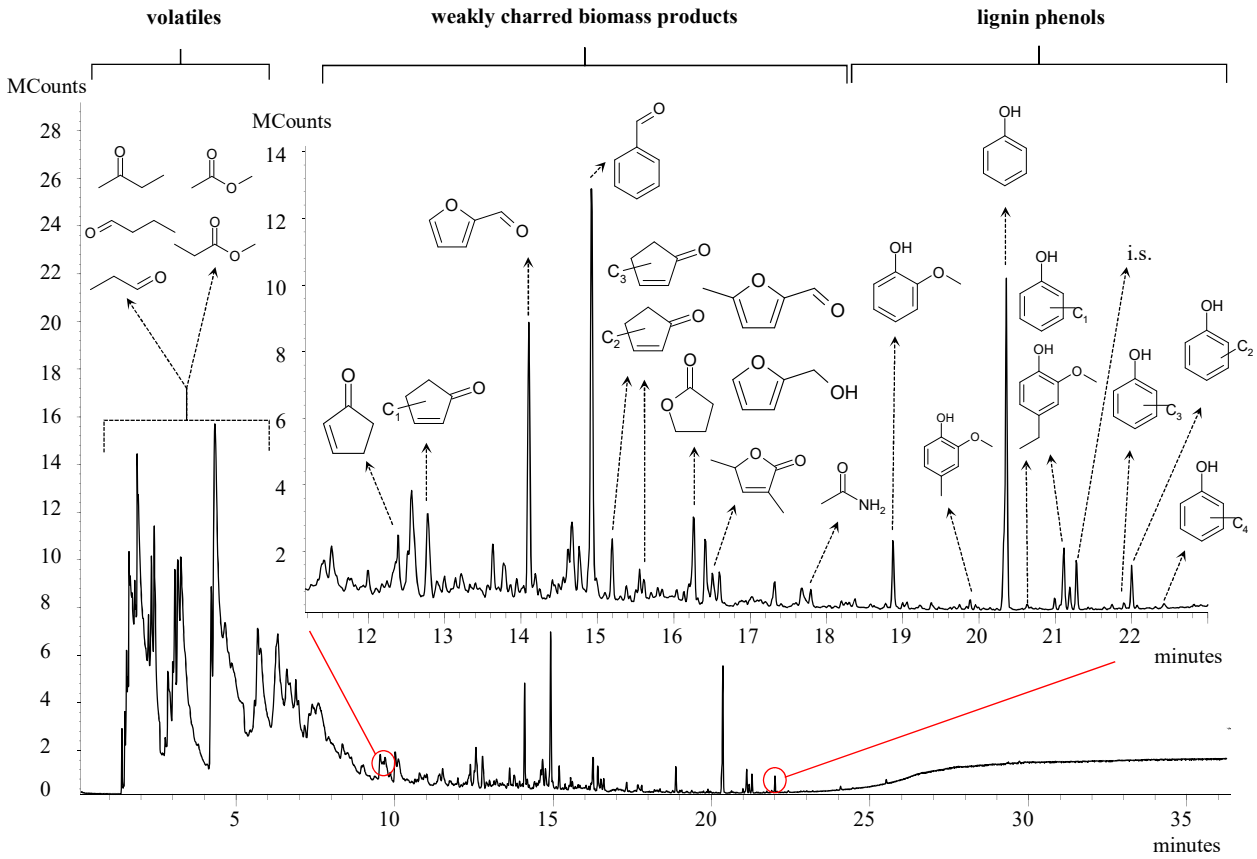

Figure 8: Total ion current chromatogram from HS-SPME-GC-MS analysis of corn stalk biochar produced at 350°C. The pattern of the most relevant VOCs was reported in the expanded chromatogram between 10 and 22 minutes.

Aromatic compounds not directly related to the structure of original biomolecules, but indicative of their carbonisation were detected in almost all biochar samples. These compounds included monoaromatic (benzenes, biphenyls, indanes) and polycyclic aromatic hydrocarbons (PAHs), benzonitrile and benzofurans.[42,120] Benzene, toluene, C_2-benzenes, naphthalene, benzonitrile, and benzofurans were released from all the samples including the highly carbonised biochars of the thermosequence (CS600 and CS650). Interestingly, these aromatics compounds were proposed as molecular indicators of charring in analytical pyrolysis studies.[42,120,121]The ratio of alkylated/non-

alkylated analogues (e.g. toluene/benzene, methylnaphthalene/ naphthalene) were found to decrease with decreasing H/C ratios. Similarly, the degree of alkylation of VOCs released by biochars tended to diminish with charring intensity. While C_1-C_2 benzenes were observed in all thermosequence biochars, C_3, C_4 and C_5 benzenes were not detected for biochar with H/C lower than 0.44, 0.49 and 0.7 respectively. Alkyl substituted benzenes are well known harmful compounds[122] and their presence in biochar could pose direct issues on its safety during handling and storage. The number of products detected by GC-MS after HS-SPME decreased with increasing charring degree expressed by the H/C ratio of biochars, from 88 in CS350, to 15 in CS650. For instance, the large group of phenols (phenol, methyl phenols and C_2-C_4 phenols) and guaiacols (guaiacol and its C_1-C_2 derivatives) released from CS350 was restricted to only phenol, 4-methylphenol, guaiacol and 4-methylguaiacol in biochar with higher carbonisation degree. To the purpose of investigating the relationship between the degree of charring and the presence of mobile compounds, a quantitative approach was applied and discussed in the next section.

Relationship with biochar properties

The quantity of VOCs, expressed as normalised areas (*NA*) were reported in Tables S1 and S2. These values were meant to estimate the magnitude of VOCs that could be released in air from different biochars under fixed conditions rather than absolute concentrations. The aim was to evaluate the dependence of emitted VOCs on the carbonisation degree of biochars produced with the same feedstock and pyrolysis apparatus. Pearson product-moment correlation coefficient (r) showed statistically significant ($P < 0.05$) correlation (r = 0.95) of VOCs vs. H/C trend. Similarly, total VOCs and VM were strongly correlated (Figure 9, r> 0.9, $P < 0.05$). The strong correlation with indexes of the degree of charring and aromaticity (H/C, VM) indicated that highly carbonised biochars should be produced in order to reduce the risk of VOCs emission. Notably, the molecular pattern of the volatilome of biochars produced at different temperatures was different. This was evidenced in Figure 10 showing the quantity of the different chemical families as a function of H/C values, for the most abundant (Figure 10 A) and less abundant (Figure 10 B) compounds.

Aldehydes and ketones (mostly benzaldehydes and furaldehydes) were the most abundant family in weakly charred biochars (around 30%), but their quantity decreased sharply with decreasing H/C becoming negligible in biochars with H/C < 0.59.

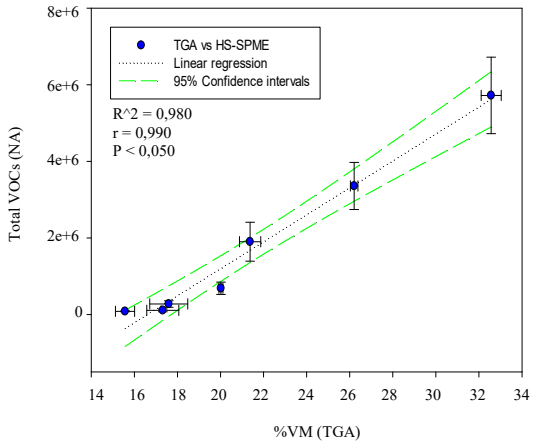

Figure 9: Correlation between the normalized area (NA) of VOCs (HS-SPME-GC-MS) and the VM measured by TGA of cornstalk biochars. Mean values and SD from three replicates.

Aromatics (principally benzene, toluene and C_2-bezenes) and phenols were the principal class of VOCs in all the biochars. Their contributions to total VOCs of slightly carbonised biochar were 20 and 14%, respectively. In all other corn stalk biochars (H/C 0.71-0.32) aromatics were the most abundant compounds. Interestingly, aliphatic as well as aromatic (benzoic acid) acids represented a significant class of compounds featuring biochar volatilomes (25% for CS350). These compounds were detected principally as methyl ester derivatives probably from biochar-catalysed methylation. Traces of cyclic ketones like cyclopentanone and cyclohexanone were detected only in biochars with H/C of 0.49 and 0.44. In this range of temperature there could be a cyclic rearrangement of their smaller linear precursors. Aromatic aldehydes like furaldehyde and benzaldehyde were identified in all thermosequence biochars. Cyclopentenones, lactones, alkyl and aryl furans are representatives of thermal degradation of holocellulose.[42,114] Their signal was strongly suppressed in biochars with H/C < 0.49. C_1-C_3 cyclopentenones were not found at H/C lower than 0.59. Volatile aldehydes and ketones could also originate from pyrolysis of holocellulose.[120] C_3-C_4 alkyl aldehydes and substituted derivatives of benzaldehyde were detected only in biochars with H/C >

0.59. Incomplete degradation of cellulose at low pyrolysis temperature (0.80 < H/C < 0.59) could be evidenced by the presence of 1,4:3,6-dianhydro-β-D-glucopyranose.

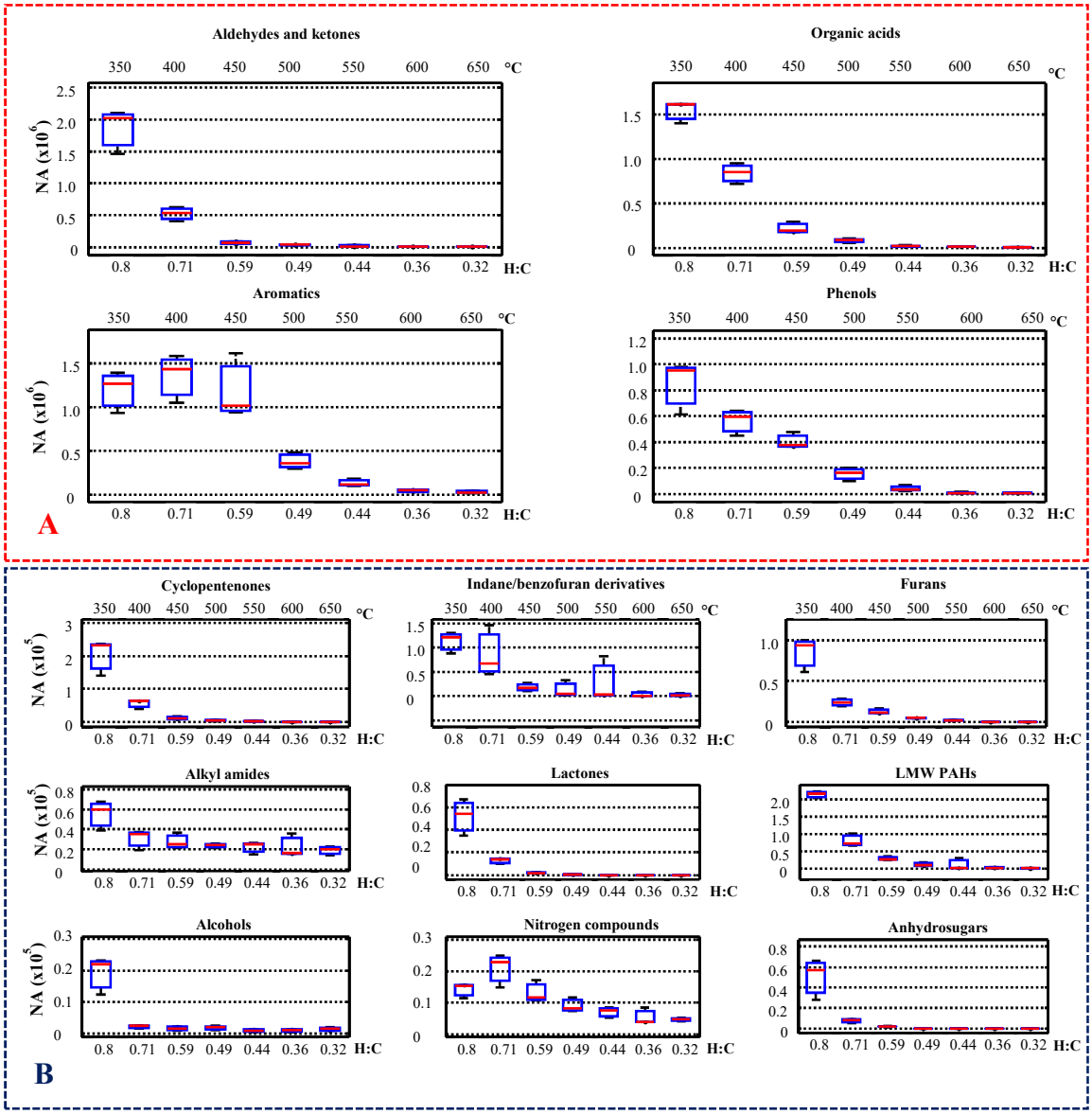

Figure 10: Box plots of the normalized areas (NA) of major (A) and minor (B) VOCs compound classes vs. H/C of corn stalk biochars and pyrolysis temperature. The tops and bottoms of each "box" reported the 25th and 75th percentiles of the samples, respectively, while the whiskers were drawn from the ends of the interquartile ranges to the furthest observations within the whisker length.

Kruskal-Wallis test was applied to determine differences of VOCs concentrations in biochars with increasing carbonization degree. Results evidenced statistically significant differences (1% level of significance) on the medians of all VOCs compound classes except alkyl amides. The level of significance of alcohols was 5%. Jonckheere-Terpstra statistic was also performed to test the

alternative hypothesis of an increasing trend in the medians of VOCs with the increasing H/C. The outcome evidenced statistically significant differences (1% level of significance) for all compound classes. These results were in accordance with those of Pearson product-moment correlation coefficients and confirmed the relevant effect of the carbonisation degree on the volatiles released by biochar. Quantitative analysis highlighted differences in VOCs relative abundance (Table S1 and S2), but all corn stalk biochars had toluene, acetic acid methyl ester and phenol among the most abundant compounds. The presence of aromatic compounds, volatile fatty acids and phenols in biochar could be therefore a crucial marker for its safety and quality for environmental applications. The %RSD calculated on total VOCs concentrations in triplicate analysis were 17, 18, 27, 23, 31, 34 and 14% for corn stalk biochars with H/C of 0.80, 0.71, 0.59, 0.49, 0.44, 0.36 and 0.32 respectively. Considering the complexity of the matrix and the number of compound identified, %RSD values were acceptable and demonstrated the suitability of the HS-SPME method for the analysis of VOCs in biochar. A rough estimation of the concentrations could be accomplished by utilising for all the compounds the response factor of the surrogate pyrolysis product *o*-eugenol. VOCs concentrations progressively decreased with the increasing carbonisation degree from about 6 to 0.09 $\mu g/g_{biochar}$. These values are within the range conservatively estimated in biochars (pg-$\mu g/g_{biochar}$) through direct HS-GC-MS analysis and could be sufficient to affect the soil microbiome.[61]

VOCs emissions at ambient temperatures

The effect of biochar temperature on the pattern of evolved compounds detectable by HS-SPME was investigated at 25, 50, 100 °C (the results at 150 °C were described in the previous section). The lowest value was chosen in order to verify if biochar could release volatile compounds at ambient temperature, while 50°C approximated a possible condition achieved in terrestrial soils during warm periods. The values of 100 °C was tested by Becker et al.[63] As expected, temperature greatly affected the pattern and intensity of GC traces. As an example, the mass chromatograms relative to the ions *m/z* 122 in the elution region of interest indicative of alkyl aromatics, alkyl

aldehydes and lignin phenols were shown in Figure 11 for different temperatures. GC peaks were barely detected at 25 and 50 °C, they could be revealed at 100 °C and became intense at 150°C. This latter temperature was selected in the procedure. Concerning the possibility of VOCs emission at ambient temperatures, quantitative results (Table S3) showed that VOCs could be detected only in CS350 and CS400 biochars with low carbonisation degree (H/C 0.80 and 0.71).

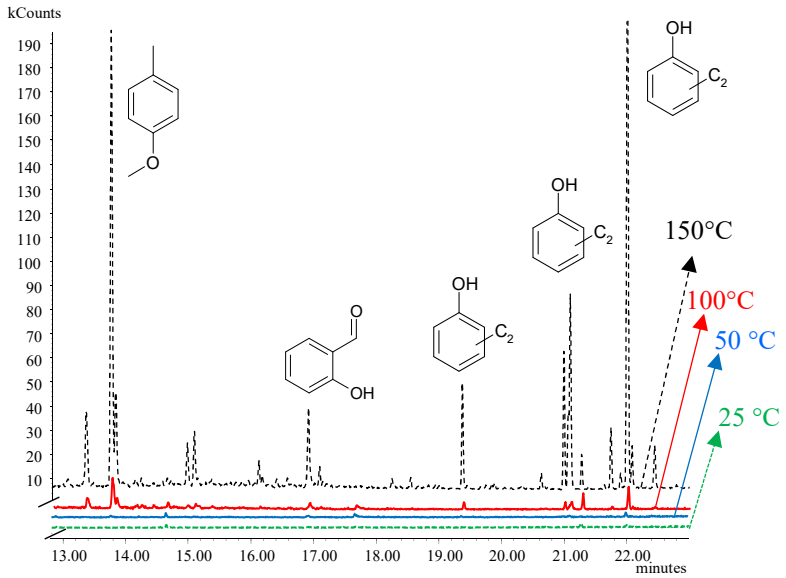

Figure 11: Mass chromatograms at m/z 122 from cornstalk biochar (CS350) heated at different temperatures (25, 50, 100 and 150 °C) during HS-SPME.

The most abundant compounds were aldehydes like propanal, furfural, benzaldehyde, formic and acetic acids. The possible correlation of such compound classes, especially organic acids and phenols with the environmental performance of biochar were highlighted[55,58,111] and their presence could be a useful proxy to discriminate biochar quality. Biochars with H/C < 0.71 did not exhibit detectable compounds in HS at 25 and 50 °C. The threshold value of 0.70 for H/C was considered as a quality parameter for biochar.[9,18] Similarly, the results of this study suggest that sufficiently carbonised biochars (H/C ≤ 0.70) obtainable at relatively high pyrolysis temperatures (≥ 450 °C) are not prone to evolve VOCs at normal conditions.

VOCs profiles evolved from biochar produced from different feedstocks

The HS-SPME-GC-MS method was tested on a biochar produced from spent mushroom substrate at 500°C (SM500) to compare its applicability to the detection of VOCs in biochars from different feedstocks. SM500 evolved 76 VOCs in the HS that were captured by the SPME fibre (Table S4) and its volatilome is presented in the chromatogram of Figure 12. Given the presence of lignocellulose in spent mushroom substrate, several compounds found in CS analysis were detected in the HS of the corresponding biochar obtained at 500 °C (SM500). However, a wider range of alkyl aldehydes and ketones was present (C_3-C_6), including unsaturated species (e.g methyl butenal, methyl pentenal, pentenone). SM500 evolved also a distinctive pattern of heterocyclic compounds, especially with nitrogen and sulfur that could be attributed to the protein content as well as specific biopolymers, like chitin.[123–125] The origin of nitrogen and sulphur containing organic compounds could be tentatively attributed to the thermal degradation of proteins (e.g. benzeneacetonitrile) and heat promoted reactions of proteins with carbohydrates (Maillard products such as pyrazines) and lipids (e.g. aliphatic alkylnitriles). Other characteristic compounds were homologues of thiophenes, pyridines, thiazoles and amides. The approximated average concentration of total VOCs on three replicates was 166 ± 12 ng cm^{-3}. The value was similar to corn stalk biochar with H/C 0.71, but in this case the H/C value was slightly lower (0.62). The average %RSD value of all VOCs quantified (n=76) was 7%, indicating that the proposed method could have a satisfactorily precision for the characterization of VOCs in biochar. Consistently with the results of corn stalk biochars with H/C lower than 0.71, the most abundant compound class in SM500 was the alkyl and methoxy monoaromatics (41% of total VOCs), with toluene as most abundant compound. Nitriles and phenol accounted for 19 and 18% respectively while the characteristic heterocyclic compounds represented the 2% of total VOCs.

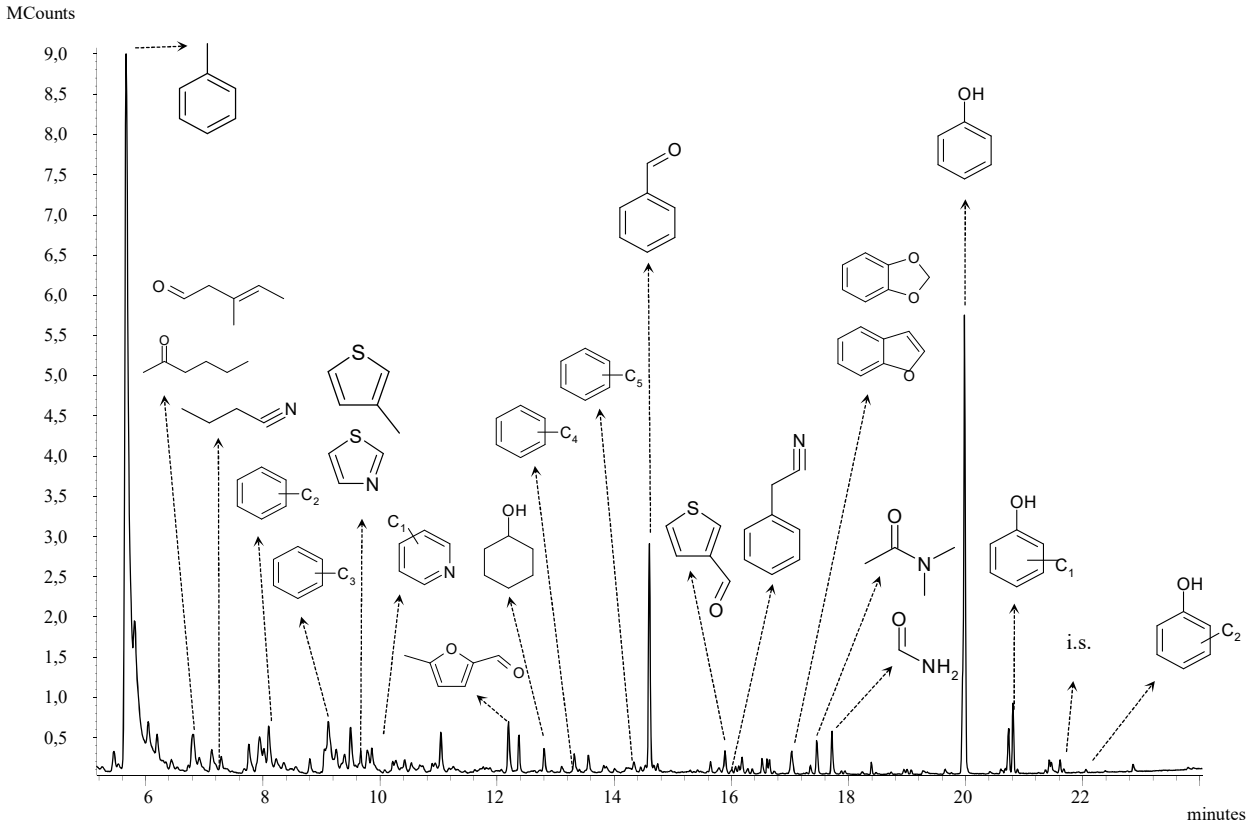

Figure 12: total ion current chromatogram of HS-SPME-GC-MS analysis of VOCs evolved from spent mushroom substrate biochar produced at 500°C.

Evaluation of the HS-SPME-GC-MS method for biochar quality assessment

The presence of trace VOCs in biochar was investigated with a green analytical method based on SPME. SPME application to biochar was seldom reported.[70,71] Clough et al. applied the technique using DVB/CAR/PDMS fibre exposed at 40°C for 40 minutes but only α,β-pinene and acetaldehyde were identified.[70] Higashikawa et al. selected DVB/CAR/PDMS fibre (20°C and 20 minutes fibre exposure) to quantitatively detect model VOCs in complex environmental matrices and showed that the biochar-matrix reduced method performance due to high affinity for the spiked compounds.[71] In the present study, several compound classes with different polarities and representative of biomass thermochemical degradation products were detected. Recently, it was shown that the composition of pyrolysis vapours provided by SPME with Car-PDMS fibre was similar to that of the bio-oil analysed by direct GC-MS.[114] This similarity confirms the suitability of the Car-PDMS fibre for the detection of biomass pyrolysis products. The ability of the methods to detect molecular markers of

feedstock and carbonisation could allow the control of the occurrence of biochar contamination from vapour re-condensation during pyrolysis.[59,126] The analytical performance of the method for quantitative analysis was found to be satisfactory when considering the high number of compounds identified, the complexity of the matrix and the low concentrations of analytes. In fact, the method resulted adequate to the scope of evidencing quantitative relationships between VOCs and biochar properties (degree of carboniastion). The composition and quantity of biochar VOCs was dependent on chemical characteristics, as VOCs decreased with increasing carbonisation degrees, while the molecular pattern shifted from the prevalence of oxygenated compounds towards that of aromatic hydrocarbons. The risk of VOCs emissions can be reduced by producing biochar with a high carbonisation degree, represented by low values of H/C and volatile matter. The emission of VOCs at ambient conditions resulted minimal for cornstalk biochars with H/C<0.70 and volatile matter < 20%. These limits that favour highly carbonised biochars should be taken into consideration for the selection of sustainable biochar. However, in soil applications the extent of carbonisation could affect in an opposite direction other biochar properties beneficial to plant growth, such as the cation exchange capacity (CEC).[127] Therefore, a careful consideration of several parameters should be taken into account in order to identify the suitable biochar for the intended use.

4) CHARACTERIZATION OF ORGANIC COMPOUNDS RELEASED FROM BIOCHAR INTO WATER

4.1) INTRODUCTION

Biochar (BC) research has made consistent progress since its first appearance, when it was proposed as a sustainable strategy for the abatement of greenhouse gases in terrestrial ecosystems.[6] However, the ameliorating effect of BC in soil applications is highly dependent on its physical and chemical properties, in turn affected by production technology and biomass feedstocks. The definition of BC quality is therefore fundamental, and different criteria were proposed for its classification, like carbon content, aromaticity, and the presence of harmful chemical species such as heavy metals or polyaromatic hydrocarbons (PAHs).[17] Apart from priority contaminants, the role of BC mobile organic compounds is being evaluated as potentially affecting its performance in soil. Volatile organic compounds (VOCs) and water soluble organic compounds (WSOCs) were deemed responsible for the positive [56] and negative[55,58] effects on plants, microorganisms[128] and aquatic organisms.[59,129] BC labile carbon structures could also affect the composition of soil derived dissolved organic matter, in turn influencing soil ecosystem processes. The presence of organic species leached from BC was confirmed in soil application. Riedel et al.[95] evidenced a compositional change in the molecular fingerprint of the organic matter released from a soil mixed with BC compared to untreated soil in column experiments. The amendment caused a marked reduction of the organic matter mobilization from the soil, but a net increase in the intensities of black carbon-type and lignin-type compounds was observed. Uchimiya et al.[130] demonstrated the existence of polyaromatic moieties in the dissolved organic carbon (DOC) extracted from a BC-amended soil, attributed to unique structures of pyrogenic DOC. Vapors re-condensation and pyrolysis temperature were found to be of primary importance for the production of BC suitable for soil application.[58–60] The contact of the pyrolysis vapors with the carbonized biomass inside the reactor and their removal is critical to prevent BC contamination.[58,59] Temperature and residence

time are crucial process parameters. At temperatures higher than 400°C a sharp decrease in VOCs adsorbed on BC was evidenced.[59,60] High nitrogen flow can reduce the content of PAHs.[50,51] The effect of process conditions on the composition of WSOCs has not been widely investigated, nonetheless WSOCs may play an important role in BC environmental impact due to their mobility in water. WSOCs were investigated by means of two dimensional GC[59] and liquid chromatography,[56,72,131] while ultrahigh resolution mass spectrometry (Fourier Transform Ion Cyclotron Resonance Mass Spectrometry FT-ICR-MS) revealed the presence of thousands hydrophilic species, non-detectable with other techniques.[59,94,95] Fluorescence spectroscopy and Parallel Factor Analysis (PARAFAC) were used as rapid and sensitive techniques to investigate its aromatic fraction.[83,96,130,132] These studies have noticeably increased the understanding on the chemical composition of BC WSOCs, however, the relationship with production parameters and especially with the composition of the pyrolysis vapors are poorly known. The present study was primary focused on the comprehensive characterization of BC WSOCs with spectroscopic, chromatographic and mass spectrometry techniques in relation to the composition of the water-soluble fraction of the pyrolysis vapors, condensed (bio-oils) during the pyrolyses for BC production. The linkage between WSOC patterns, BC bulk properties and their implications on seeds germination, could eventually shed light on the role of BC mobile organic compounds in the determination of its quality for environmental applications, and possible threshold levels can be proposed.

4.2) MATERIALS AND METHODS

Samples

BC were produced from pellettized corn stalks at the temperature of 350, 400, 450, 500, 550, 600, 650°C and characterized in the previous section (3.2). During pyrolysis experiments vapors were condensed in two ice/salt cold traps at -14°C to collect the pyrolysis liquids. The content of the two traps was merged to produce one bio-oil (OL) sample per temperature. In this study, the samples produced at the temperature *XXX* °C are named *BCXXX* and *OLXXX*.

Lipid extraction from corn stalk biomass

The total lipid fraction of the corn stalk biomass used in the pyrolysis experiments was determined by sequentially extracting the feedstock with $CHCl_3$-MeOH 2:1 (v/v) at 50°C for 1.5 hours (triplicate analysis). The profile of fatty acids was determined by GC-MS after methanolysis followed by the production of fatty acid methyl esters (FAME)[133].

Extraction of biochar WSOCs

An amount of BC was weighed (1 g ± 0.01 mg) into 20 ml vials and 10 ml of deionized water (DW, HPLC grade) was added. The vials were sealed with aluminum crimp seals with PTFE/rubber septa. The sealed vials were then placed on a mechanical shaker (IKA KS 260) covered with an aluminum foil, and left shaking at 150 rpm for 72 hours at ambient temperature. The resulting solutions were centrifuged at 3800 rpm for 10 min (ALC4232 centrifuge) to separate the solid material and filtered with PTFE syringe filters 0.45µm (Sartorius Minisart SRP) thereafter.

Analysis of BC WSOCs by direct immersion (DI)-SPME-GC-MS

Each BC extract (1ml) was added with 0.5 ml of 2M phosphate buffer (KH_2PO_4/Na_2HPO_4) at pH 5.7 in 1.5 ml vials. Carboxen-PDMS (Car-PDMS) SPME fiber was exposed to the solution under magnetic stirring for 30 minutes[55]. The thermal desorption of the analytes and GC-MS analysis were performed with the method developed in a previous study.[60] The amounts of WSOCs were

expressed as peak area counts normalized by the sample weight (*NA*). Blank analysis of phosphate buffer and DW were performed to check procedural contaminations. A calibration curve of volatile fatty acids (VFA) was performed with a standard VFA solution (0.1%) containing acetic acid, propanoic acid, methyl propanoic acid, butanoic acid, methyl butanoic acid and pentanoic acid in deionized water. Serial dilutions were prepared at 10, 5, 1 and 0.1 mg/l in phosphate buffer, spiked with 2-ethyl butyric acid 5mg/l in DW (internal standard) and analyzed in triplicate. The concentration of each VFA was calculated using the response factors from the calibration curve and expressed as ($\mu g/g_{biochar}$). Calibrations on model VFA solutions (10-0.1mg/l) demonstrated the suitability of DI-SPME to this purpose, as ordinary least square regressions led to values of R^2 and Pearson product-moment correlation coefficients (r) higher than 0.97 and 0.98 for each VFA. S/N at the lowest concentration was still about 400, indicating the possibility of quantification even at lower levels. A response factor f for each VFA was used in the quantification.

Analysis of BC WSOCs by ESI(-)FT-ICR-MS

BC extracts were diluted 1:10 in methanol and analyzed by negative electrospray ionization (capillary voltage 4kV) on a Bruker solariX 12T FT-ICR-MS. 500 scans were acquired for each spectrum (syringe infusion, $200\mu L \cdot h^{-1}$) using an 8 MW acquisition size (broadband). Ion accumulation time was set at 0.8sec. Samples were spiked with ES tuning mix (Agilent) and a starting calibration list was developed from single point correction on the m/z 301.998139. Mass spectra were analyzed using Data Analysis software (Bruker Daltonics). Peaks were assigned with a signal to noise threshold of 4 and absolute intensity threshold of $2 \cdot 10^6$. Calibration lists were developed over the m/z range 100-600 by Kendrick mass analysis. Mass spectra without the calibrant were recalibrated with a quadratic equation with a standard deviation < 100ppb (67 points). Calibrated mass lists were processed with PetroOrg software. Peaks were assigned with a threshold of 100ppb and molecular formulas within the range: $C_{1\text{-}100}$, $H_{4\text{-}200}$, $O_{1\text{-}20}$, $N_{0\text{-}4}$.

Analysis of BC WSOCs by fluorescence spectroscopy and PARAFAC

BC extracts were diluted in DW until the absorbance in the UV-Vis wavelength range 200-800 nm was <0.1[134], recorded with a PerkinElmer λ650 spectrophotometer, using quartz cells with 1.0 cm optical path. Fluorescence excitation/emission matrices (EEMs) were acquired (duplicate analysis) on an Edinburgh Instrument F900 with excitation and emission wavelengths in the range of 220-500 and 280-600 nm respectively, both at 5nm intervals. Solutions of 16 EPA PAHs (1µg/ml), IHSS Suwanee River Fulvic Acid (SRFA, 1mg/ml), *o*-cresol and *o*-eugenol (0.1 mg/ml) in DW were analyzed under the same conditions. PARAFAC was performed on the EEMs corrected for instrument bias and non-trilinear signals, with N-way toolbox[135], drEEM tool for Matlab [136].The number of PARAFAC components was selected considering the Stoke's shift, leverage values, analysis of residuals and core consistency diagnostic[82,136].

Analysis of bio-oil WSOCs

The OL samples were diluted 1:10 in DW and centrifuged (3800 rpm for 15 min) to precipitate the water-insoluble part, while the WSOCs were analyzed by DI-SPME-GC-MS, FT-ICR-MS and fluorescence-PARAFAC. An aliquot of 250µl was spiked with 150 µl of *o*-eugenol 10 µg/ml in DW, phosphate buffer and DW to a final volume of 1.5 ml. DI-SPME and GC-MS conditions were those used for BC. FT-ICR-MS was performed on solutions further diluted 1:100 in methanol. 500 scans were acquired for each spectrum using an 8 MW acquisition size (broadband). Ion accumulation time was set at 0.5sec. Peaks were assigned with a signal to noise threshold of 4 and absolute intensity threshold of $2 \cdot 10^6$. Mass spectra without the calibrant were recalibrated with a quadratic equation with a standard deviation < 100ppb (89 points). Calibrated mass lists were processed with PetroOrg software. Peaks were assigned with a threshold of 100ppb and molecular formulas within the range: $C_{1\text{-}100}$, $H_{4\text{-}200}$, $O_{1\text{-}20}$, $N_{0\text{-}4}$, $S_{0\text{-}2}$. Fluorescence-PARAFAC conditions were the same used for BC.

4.3) RESULTS AND DISCUSSION

Semi-volatile WSOCs (DI-SPME-GC-MS)

The corn stalk BC and the corresponding OL presented noticeable dissimilarities in the patterns of semi-volatile WSOCs. Representative examples are reported in the chromatograms of Figure 13, while all the compounds detected in BC and OL are listed in Table S5 and S6 respectively. The series of peaks in BC350 extracts were predominantly associated to carboxylic acids, which were the main components of the low molecular weight fraction of BC WSOCs, with $C_{1\text{-}12}$ straight-chain and branched, saturated and unsaturated aliphatic acids, and aromatic acids like benzoic acid and its $C_{1\text{-}2}$ alkylated derivatives.

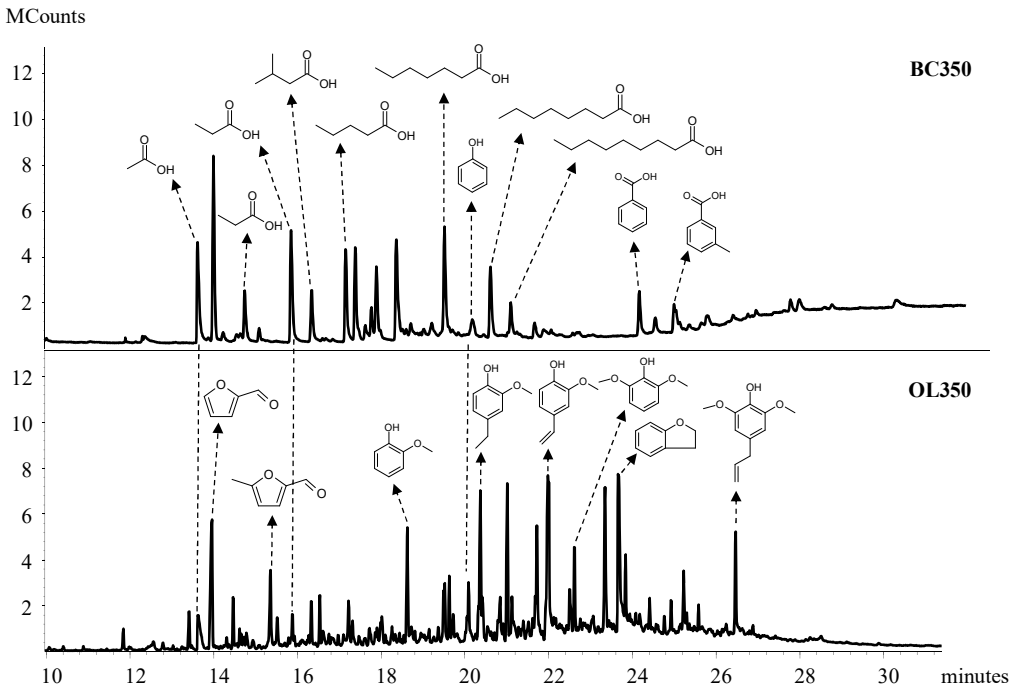

Figure 13: Total ion chromatograms of BC350 and OL350 WSOCs after DI-SPME-GC-MS analysis. Principal compounds are evidenced, while the complete lists of the volatile and semi-volatile WSOCs are reported in Tables S5 and S6

The composition of the OL was more complex (Figure 13), with 124 tentatively identified compounds in contrast with the 36 of BC350. OL profiles included primarily lignin markers (2-methoxy-, 2,6-dimethoxy- and $C_{1\text{-}3}$ alkyl substituted phenols) and typical degradation products of the cellulose and hemicellulose fractions of the parent corn stalk (5-6 membered rings heterocyclic

aldehydes ketones and diketones, C_{1-3} alkyl substituted and hydroxyl substituted cyclopentenones, and furans). Only 5 lignin markers characterized BC WSOCs out of the 15 of OL (phenol, C_{1-2} phenols, guaiacol and 4-methyl guaiacol). Their signals became negligible in the BC produced above 500°C. However, traces of alkylated phenols were observed in all the BC WSOCs, possibly indicating their stronger interaction with the aromatic structure of the BC compared to the methoxylated homologues. Furthermore, WSOCs of BC produced below 450°C featured 8 proxies of hemicellulose (furfural and methyl furfural, benzaldehyde and hydroxyl benzaldehyde, 2-acetyl furan and C_{1-3} cyclopentenones) compared to the 34 of OL. A series of compounds generated by the progressive carbonization of the biomass inside the reactor during the pyrolysis were detected in all the OL, like monoaromatic hydrocarbons (benzene, toluene and C_{1-5} alkylated derivatives), and low molecular weight PAHs. None of these species characterized the BC WSOCs, but minor contribution of some of these compounds was evidenced in other studies.[59] Low molecular weight aliphatic aldehydes (C_{3-4}), ketones and diketones (C_{4-6}) were detected in the OL, but not in BC WSOCs, indicating that, if retained by the BC after their production, they could be released preferentially as VOCs.[60] OL composition included also nitrogen containing aromatic compounds deriving from the protein fraction of the biomass feedstock (pyridines, pyrazines, aromatic nitriles quinolines and indoles). Interestingly, BC WSOCs did not present any of these species, indicating an effective removal as pyrolysis vapors or a stronger interaction with BC. Finally, VFA, C_{3-5} unsaturated and higher molecular weight aromatic acids were present in the OL as free carboxylic acids but also in the form of methyl esters, probably originated from the reactivity with methylating products (e.g. methanol) at low pH. OL WSOCs lacked in the C_{4-12} and the methyl substituted homologues of carboxylic acids, indicating their possible formation and preferential adsorption onto the biochar surface during pyrolysis. However, it cannot be excluded that the mass spectra of the missing aliphatic acids, were covered by the dominance of other more intense signals from lignin. The formation of low molecular weight fatty acids during pyrolysis (acetic and propanoic) is associated with the thermal decomposition of the hemi/cellulose fraction. Nevertheless, the higher

molecular weight fatty acids could form from the fragmentation of the parent corn stalk lipid fraction. The lipids accounted for 7.0 ± 0.7 % of the biomass dry weight. The pattern of FAME by GC-MS revealed a total of 14 compounds (Table 5), ranging from saturated (8:0-30:0) to unsaturated species (16:1, 18:1 and 18:2). Palmitic, stearic, linoleic and oleic acids were the principal constituents of the FAME in corn seeds,[137] whose residues left in the field could contribute to the composition of the collected corn stalk.

Table 5: FAME profile of corn stalk biomass: Average concentrations (µg/$g_{biomass}$), SD (n=3), m/z quantitation ion, r.t. retention time (min)

Acronym	r.t	m/z	Compound name	µg/g	SD
8:0	7.45	74	octanoic acid, methyl ester	180	40
12:0	12.81	74	dodecanoic acid, methyl ester	26	5.9
14:0	15.11	74	tetradecanoic acid, methyl ester	47	7.6
16:1	17.01	55	9-hexadecenoic acid, methyl ester	74	15
16:0	17.22	74	hexadecanoic acid, methyl ester	1831	329
18:2	19.02	67	9,12-octadecadienoic acid, methyl ester	237	53
18:1	19.11	55	9-octadecenoic acid, methyl ester	561	148
18:0	19.37	74	octadecanoic acid, methyl ester	938	139
20:0	21.75	74	eicosanoic acid, methyl ester	81	23
22:0	24.31	74	docosanoic acid, methyl ester	57	17
24:0	26.90	74	tetracosanoic acid, methyl ester	61	23
26:0	28.96	74	hexacosanoic acid, methyl ester	14	6.0
28:0	30.70	74	octacosanoic acid, methyl ester	14	6.2
30:0	32.17	74	triacontanoic acid, methyl ester	14	5.7
			Total GC-MS detected	4134	755

A net decrease of the fatty acids was observed in the WSOCs of BC with increasing carbonization degree, measured by the H/C atomic molar ratio. Trace amounts were released even by highly carbonized BC (H/C 0.32), while branched and C_{3-7} unsaturated homologues were typical of less carbonized ones (H/C 0.80-0.59). In accordance with their presence in the water extracts, fatty acids were also volatilized by BC in the form of methyl esters.[60] Rombolà et al.[55] evidenced the inhibiting activity of WSOCs of poultry litter BC on the germination of cress and VFA were the potential cause. Due to their mobility in air and solubility in water, VFA could play a considerable role in the agronomic/environmental performance of BC application to soil and their quantification could be

useful for the determination of its quality. Total VFA concentrations decreased with the increasing BC production temperature from 3.0 ± 0.3 mg/g of CS350 to 35 ± 14 µg/g of CS650 and statistically significant correlations ($r > 0.9$, $p < 0.01$) were observed between the values of each single and total VFA (Figure 14/TableS7) and the decreasing H/C values of the BC. This correlation is in line with the decreasing amount of VOCs.[60] In summary, the great majority of species detected in the OL WSOCs were not found in the water extracts of BC. While OL WSOCs featured mostly lignocellulosic derived pyrolysis products, BC was dominated by carboxylic acids from hemi/cellulose and lipids.

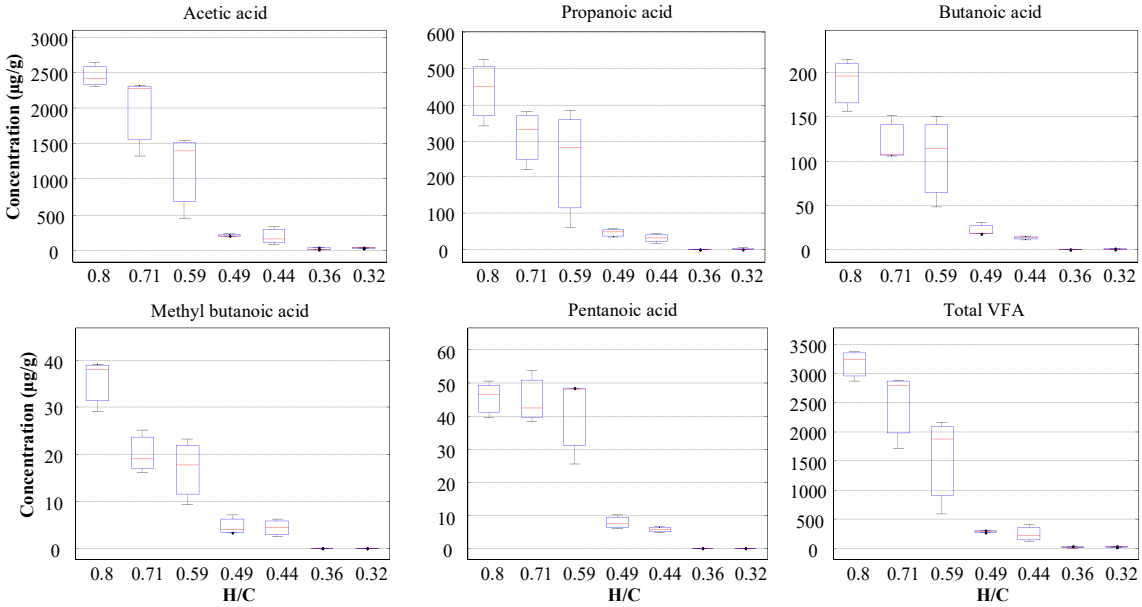

Figure 14: Quantitative analysis of VFA in BC WSOCs (µg/g$_{biochar}$) depending on BC carbonization degree (H/C atomic molar ratio), (n=3)

Hydrophilic WSOCs (ESI-FT-ICR-MS)

BC contamination could occur if pyrolysis vapors are not correctly swept from the reactor during its production.[58,59] Given the divergent patterns of BC and OL WSOCs discussed in the previous section, the comparison was extended to the less-volatile components that could be detected by ESI(-)FT-ICR-MS. Because ionization with ESI is suitable for polar compounds with both acidic and basic functionalities,[89] the fraction investigated was categorized as hydrophilic. The mass spectra of the OL WSOCs confirmed the complex composition evidenced in other studies on similar

feedstocks,[138] as molecular formula assignment allowed to identify up to 4000 peaks (Table S8 and S9). Oxygenated ($C_cH_hO_x$) and nitrogen ($C_cH_hN_yO_x$) species together accounted for more than 60% of the total intensity. Trace contribution of sulfur was observed. The $C_cH_hO_x$ distributions were similar in all the OL, encompassing oxygen atoms in the range $O_{1\text{-}16}$, with O_5 and O_6 as most abundant classes (Figure 15). Interestingly, the N_1O_x class, followed the same pattern with NO_6 as most abundant group, while for the minor N_2O_x and N_3O_x classes, O_4 and O_3 species had the highest abundance (Figure 16).

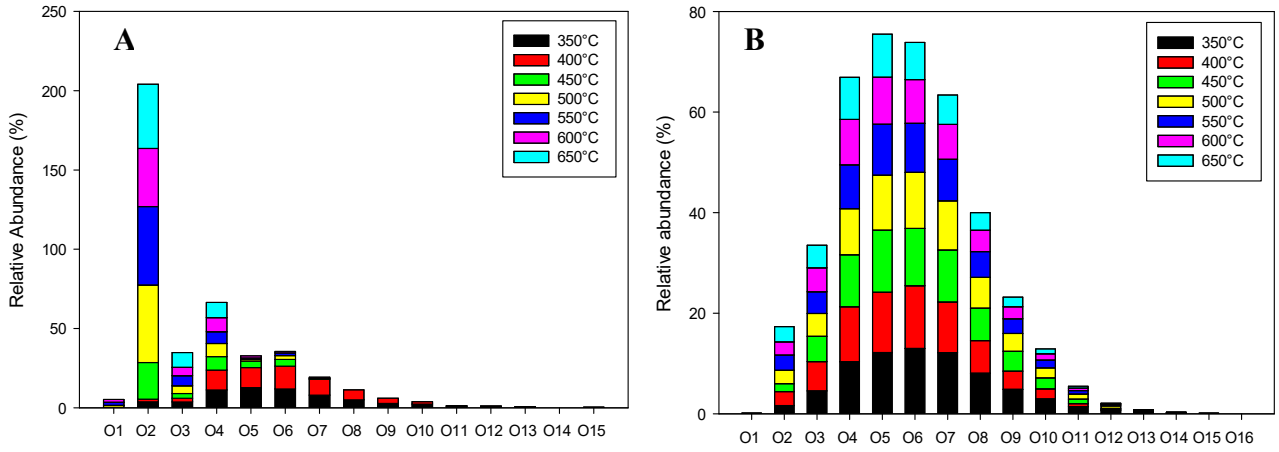

Figure 15: Distribution of oxygenated species ($C_cH_hO_x$) in BC (A) and OL (B) WSOCs by ESI(-)FT-ICR-MS

The number of identifiable peaks in the WSOCs of BC350 and BC400 was comparable to that of the OL (about 2000) but sharply decreased to 40 in BC650 (Table S10 and S11). In contrast to the dissimilarities evidenced in the GC detectable fraction, the distribution of WSOCs in BC and OL pictured by ESI presented common features, with $C_cH_hO_x$ compounds as most abundant, followed by the $C_cH_hN_yO_x$ distributions (Table S10 and S11). The same range of oxygen atoms characterized BC350 and BC400 with O_5 as most abundant group, but from BC450 the distributions progressively shifted to the prevalence of O_2 species (Figure 15).

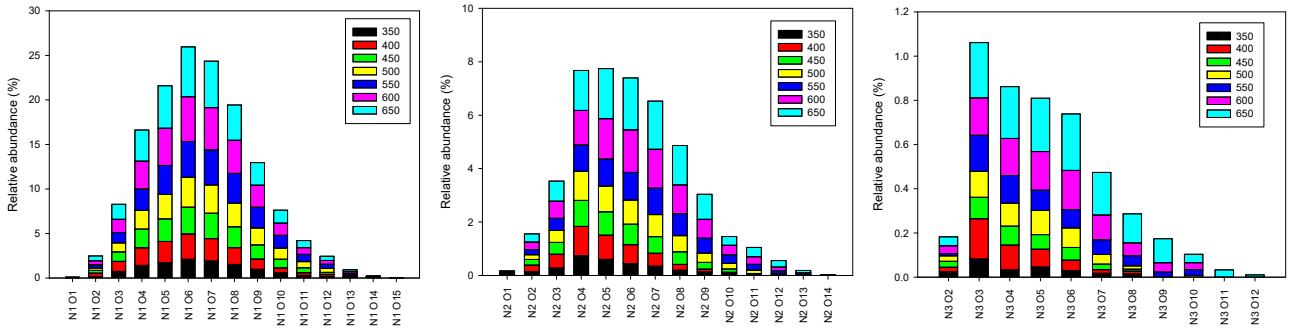

Figure 16: Distribution of nitrogen species ($C_cH_hN_yO_x$) in OL WSOCs by ESI(-)FT-ICR-MS

The oxygenated species of BC WSOCs revealed a high bioactivity, as some carboxyl and hydroxyl functionalities were the main source of toxicity on algal growth.[94,129] Given their prevalence in both the BC WSOCs and the OL, the attention was focused primarily on $C_cH_hO_x$ compounds. Van Krevelen diagrams are useful to understand the nature of these species as the molecular formula assigned in the mass spectra can be compared to the major biochemical classes of compounds.[139] To highlight the considerable changes occurring in the WSOCs of OL and BC due to the pyrolysis temperature, Van Krevelen plots of the samples produced at 350, 450, 650°C are reported in Figure 17. The patterns of OL350, 450 and 650, suggest that the pyrolysis temperature did not affect OL composition. Contrarily, those of BC450 and BC650 were distinctly different compared to the corresponding OL, and the increasing BC production temperature caused a net decrease in the number of WSOCs. However, BC350 and OL350 were highly similar, with the series of O_x classes shifting towards higher values of O/C, as consequence to the increasing number of oxygen atoms. Linear regression of the data points in Figure 17 revealed two main pathways: series with an intercept of 2 and those aligning along the equation y=2x.

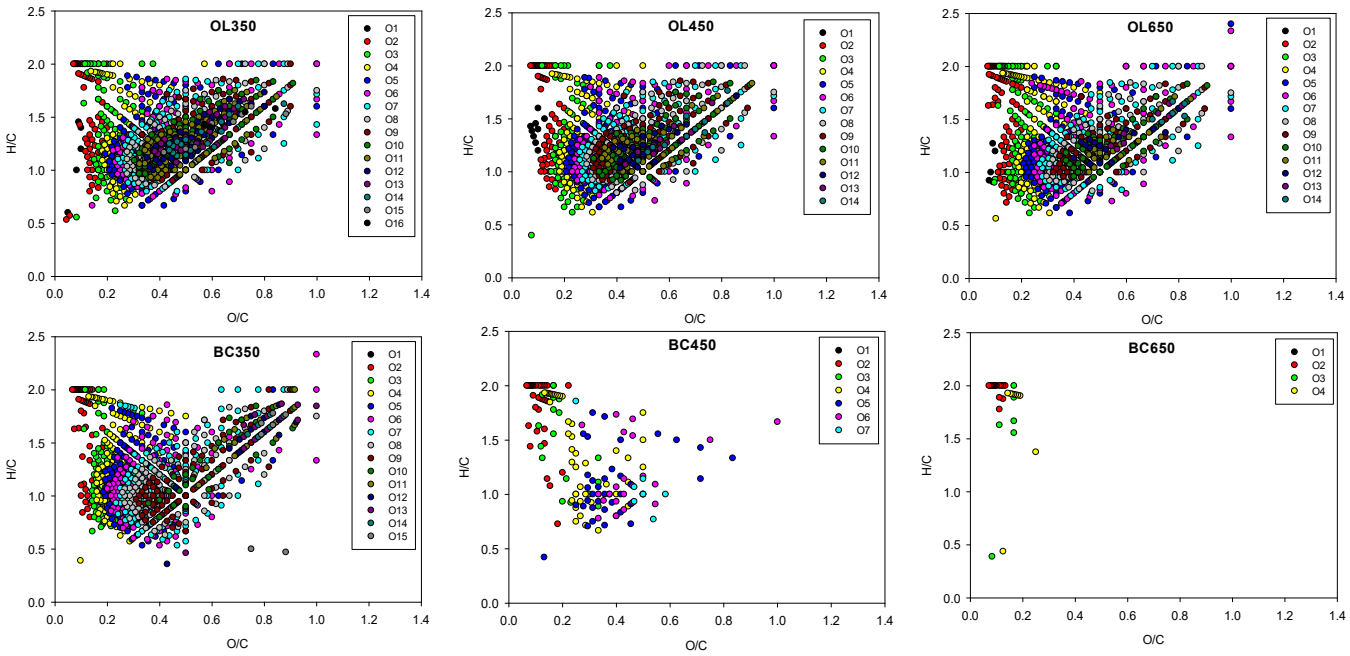

Figure 17: Van Krevelen diagrams of WSOCs by ESI(-)FT-ICR-MS, in BC produced at 350, 450, 650 °C and the corresponding OL

The first one is associated to species differing by units of CH_2.[139] Coherently, alkyl chain elongation was observed also in all the principal compound classes of the volatile and semi-volatile fractions of BC and OL WSOCs (organic acids class, aldehydes, ketones, phenols and mono-aromatic hydrocarbons), as evidenced in Figure 18, where Van Krevelen plots of BC350 and OL350 mass spectra by GC-MS were produced.

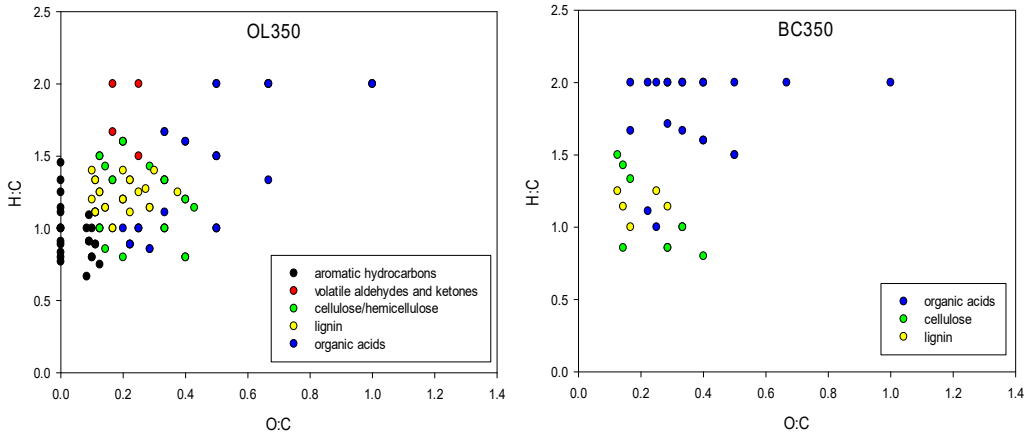

Figure 18: Van Krevelen plots of OL350 and BC350 by DI-SPME-GC-MS

The latter pathway is indicative of dehydration reactions. Noteworthily, BC350, BC400 and all the OL displayed a point with coordinates (1,2) and molecular formula $C_6H_{12}O_6$, that could be tentatively attributed to glucose or one of its isomers. The dehydration pathway could play an important role in the formation of BC and OL WSOCs from the pyrolysis products of the cellulose/hemicellulose, as many data points in Figure 17 fall in the region conservatively attributed to carbohydrates (0.67<O:C<1.2, 1.5<H:C<2.4).[92] Differently, Kendrick mass defect analysis, revealed chain elongation of molecular formulas ascribed to guaiacols and syringols, which were confirmed by DI-SPME analysis. Therefore, the O_2 and O_3 species appearing in the region attributed to lignin structures 0.1<O:C<0.7, 0.7<H:C<1.5,[90] could be assigned to higher molecular weight phenolic functionalities originated from the pyrolysis of the corn stalk lignin fraction. Similarly, those with higher oxygen content within the same range of H:C and O:C values could represent dimers, trimers or higher molecular weight homologues. Their presence in the BC WSOCs could led to the release of lighter monomers in water by photochemical degradation.[140] Several peaks fell in the region indicative of lipids (1.6<H:C<2, 0<O:C<0.2),[139] and especially the O_2 species can be correlated to analogues of the fatty acids composing the lower molecular weight WSOCs. Generally, the carbon numbers of all the OL WSOCs were comparable to those observed by Hertzog et al in the OL of a lignocellulosic material.[141] Likewise, the values ranged from 5 to 35 in BC350 and BC400 (Figure 19), corroborating the similarity between the WSOCs of poorly carbonized BC and those of OL. Double bond equivalent (DBE), or degree of unsaturation is the number of rings and double bonds and can provide information on the aromaticity of the WSOC species. The DBE values ranged from 1 to 18 in BC350, BC400 and all the OL. For DBE>2 an increase in the carbon number was associated to an increase of the number of oxygens (Figure 19). In summary, BC350 and BC400 WSOCs resembled those of the OL, but for BC>450 the cellulose and hemi-cellulose derivatives disappeared (Figure 17 and 18), while lignin degradation products could be detected until 550°C (BC550). The trend was associated to a sharp decrease of the more aromatic species with DBE>10 (Figure 19). At higher pyrolysis temperatures (> BC550), WSOCs

tended to have increased H:C and low DBE values (<5) ascribable to organic acids, still detectable at the highest pyrolysis temperature (BC650) (Figures 16-19).

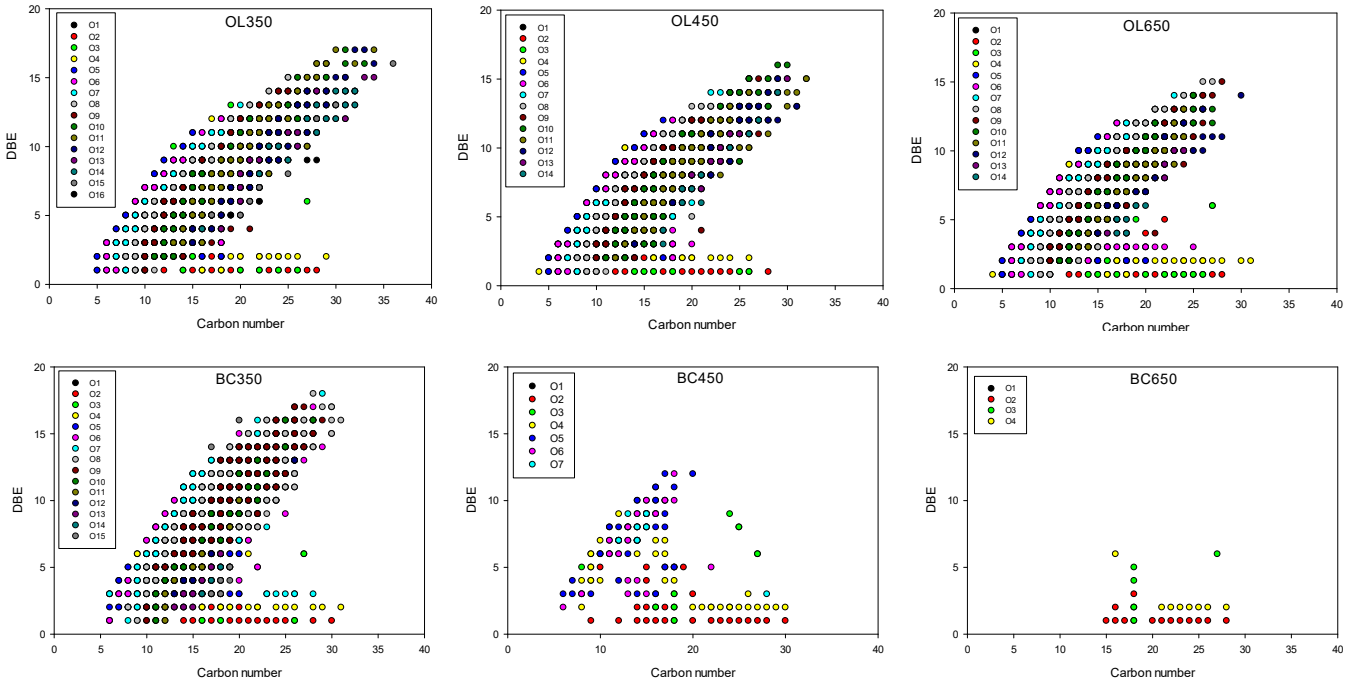

Figure 19: Plots of DBE vs Carbon number of BC 350, 450, 650 WSOCs and the corresponding OL

Aromatic structures of biochar WSOCs (Fluorescence-PARAFAC)

All the aqueous extracts of BC and OL exhibited fluorescence indicative of the occurrence of aromatic functionalities. Figure 20 reports the EEMs of the BC and OL WSOCs, and those of the standard compounds, that were acquired to qualitatively compare known chemical species with the aromatic structures recurring in biochar WSOCs. PAHs were selected for their high fluorescence even though detected only in traces in the chromatograms of the OL (Table S6), alkylated and methoxylated phenols (*o*-cresol and *o*-eugenol) as lignin derivatives, and IHSS-SRFA as model humic substance. A PARAFAC model with 4 components (C1-4) suitably represented the dataset (95% of the variance explained) and is reported in Figure 21. C1 and C2 presented excitation/emission maxima at 320/405 and 350/470 nm respectively. Fluorophores of the natural organic matter (NOM) are characterized by broad excitation/emission spectra, with representative peaks in the wavelength range of 300-370/400-500 nm.[93]

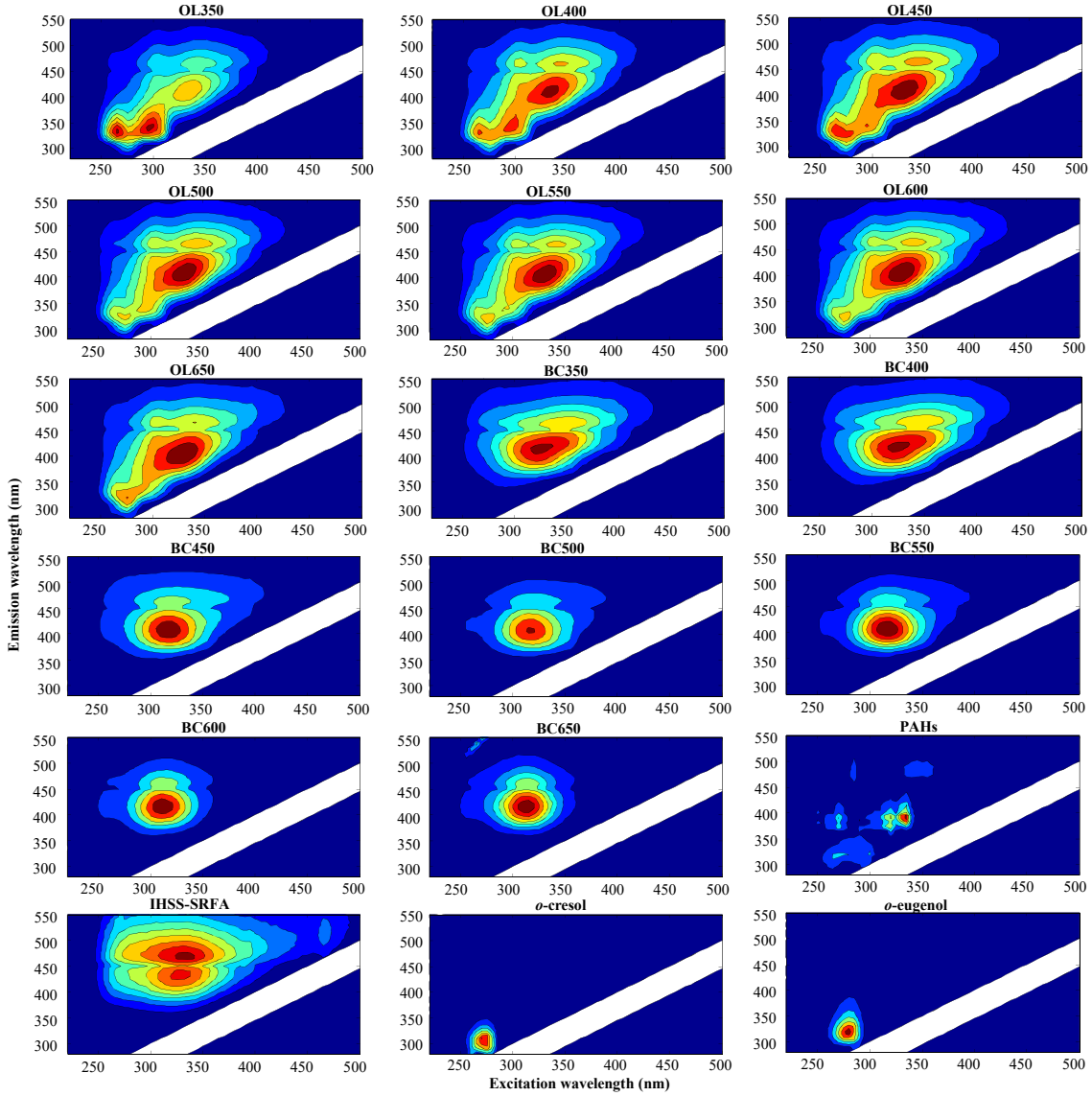

Figure 20: EEMs of BC, OL WSOCs and standard solutions of EPA PAHs, IHSS-SRFA, o-cresol and o-eugenol

Zhongqui et al.[142] characterized 13 IHSS standard humic substances (aquatic and soil derived humic and fulvic acids) with PARAFAC, and two components resembled C1 and C2, while the spectral characteristics of IHSS-SRFA in Mobed et al.[143] showed the same peaks at 320/405 and 350/470. C3 and C4 featured maxima at 285/335 and 275/310 nm respectively. Similar peaks in NOM were associated to protein-like structures.[93] The intensities of the PARAFAC components are reported in Table S12, and their relative percent contributions to the total signal of each sample are presented in Figure 22. The BC WSOCs were mainly composed by C1 and C2 and lacked in the C3 and C4 structures, that featured the OL.

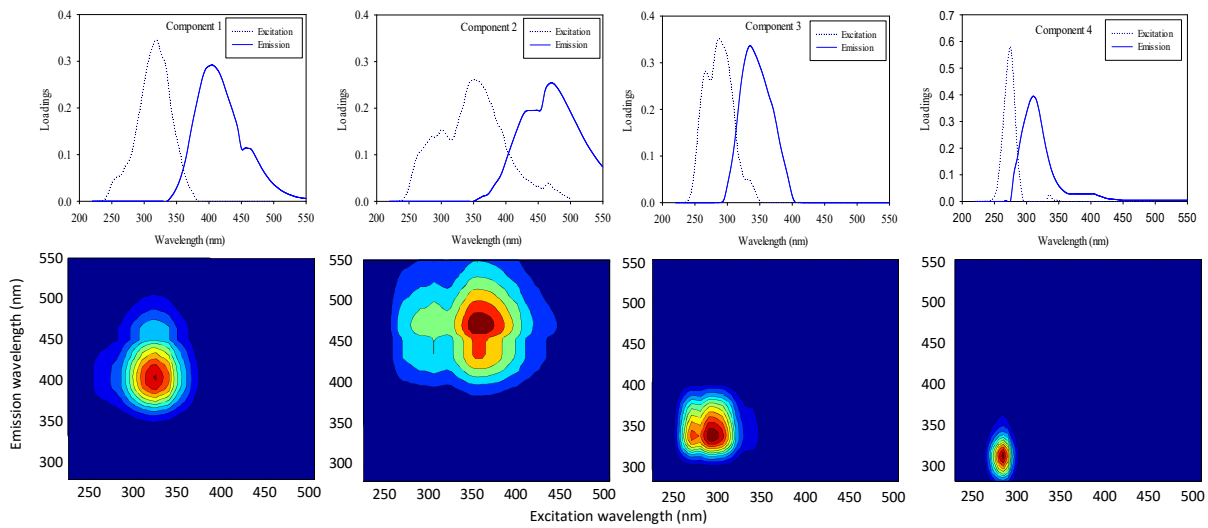

Figure 21: Spectral characteristics of the four components (C1-4) PARAFAC modeling of BC and OL WSOCs

Overall, the total signal intensity of BC WSOCs sharply decreased from BC450 and likewise that of C1 and C2, even though were still detectable at 650°C (Table S12). Contrarily, the total EEM intensity of the OL was not dependent on the pyrolysis temperature and showed values one order of magnitude higher than that of the BC (Table S12). The percent contribution of C1 sharply increased with the pyrolysis temperature in the BC WSOCs while C2 decreased accordingly. Similar trends were observed by Uchimiya et al.[83] in which pyrogenic DOC of lignocellulosic and animal based BC were investigated: two peaks (310/420 and 350/470 nm) were attributed to polyphenolic pyrolysis products and aromatic humic-like compounds that decomposes above 350°C, with the first one increasing and the second one decreasing with the pyrolysis temperature. C3 presented a maximum in OL350 and decreased at higher temperatures, while C4 smoothly increased. A high contribution of C3-C4-like components was observed in non-completely pyrolyzed BC from sawmill waste feedstocks[132] and the pyrolysis of lignocellulosic biomass with low nitrogen and sulfur content is known to produce phenolic species. Given the low nitrogen content in the BC (1%),[60] and their similarity with the EEMs of the lignin markers (Figure 20), C3 and C4 could be associated to phenolic-like species. However, it cannot be excluded that protein-like structures could contribute to C3, as several nitrogen-containing compounds were detected in the OL.

Moreover, the standard PAHs solution showed a similar peak at 280/335 nm, that could be attributed the naphthalene or fluorene.[144] Nevertheless, PAHs exhibited distinctively narrower peaks compared to the broader ones observed for C1 and C2. Noteworthily, C3 and C4 had lower emission wavelengths than C1 and C2. A red shift in the excitation/emission maximum can be associated to an increased aromaticity and higher molecular weight,[96,132] therefore C3 and C4 presented a lower degree of aromaticity compared to C1 and C2. Similarly, Uchimiya et al.[96] observed a component comparable to C2 (380/460 nm) that was associated to recalcitrant polyaromatic fraction substituted with carboxyl and phenolic functionalities, especially in low temperature BC (350-500°C). In summary, BC WSOCs were composed of fulvic-like structures and depleted in the phenolic-like less aromatic functionalities C3 and C4. Interestingly C1 and C2 were also primary components of the OL suggesting that biomass pyrolysis could intrinsically produce aromatic NOM-like moieties.

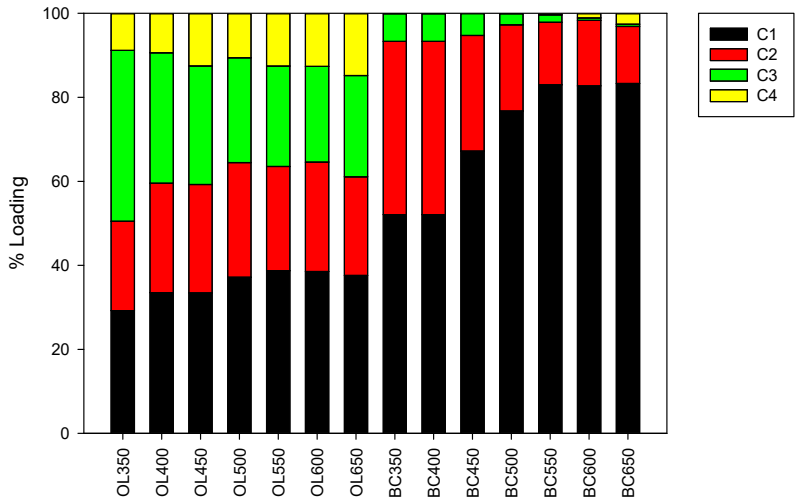

Figure 22: Score loadings of the four PARAFAC components (C1-4) in BC and OL WSOCs expressed as relative percent contribution (%) to the total intensity of each sample.

In this study it was shown that after three days in water at r.t, even highly carbonized BC released WSOCs. Interestingly, in a leaching study lasted for 17 days, most of the BC WSOCs were released within the first 3 days of the experiment.[132] Thus, under environmental conditions, a wide array of compounds can contribute to the pool of natural organic matter in soil. Overall, SPME-GC-MS, ESI(-)FT-ICR-MS and fluorescence-PARAFAC indicated that the release of WSOCs from BC was

strongly reduced above 450°C, in agreement with the trend observed for VOCs, that began to decrease above 400°C.[60] The investigation of OL WSOCs revealed original clues about the formation and release of those from BC. Organic acids were the main semi-volatile components released in water, suggesting that the more abundant OL components of lignin were strongly adsorbed onto the biochar matrix or efficiently volatilized during pyrolysis. Given the porous structure of biochar, pores could be accessible by water solutions and the retention of phenolic compounds could possibly occur due to a hydrophobic effect, or by π-π interactions,[145] that become more pronounced as the matrix gets more carbonized.[14] Previous studies categorized biochar water-extractable organic compounds into classes used to describe NOM and evidenced that low molecular weight acids were important species even in BC produced at higher temperatures, while humic acids and low molecular weight neutral species were the principal components of the lower temperature BC (<450°C).[72,73] The higher molecular weight and aromatic structures of BC WSOCs were comparable to the species recurring in NOM. In D'Andrilli et al.[93] the standard IHSS-SRFA was dominated by $C_cH_hO_x$ species by ESI(-)FT-ICR-MS and likewise BC and OL WSOCs of this study, that displayed similar structures in the lignin region of the Van Krevelen diagrams, but unique formulas in that of carbohydrates and lipids. Besides, the principal mass spacing patterns in the mass spectra (ESI-FT-ICR-MS) were alkyl chain elongation (14.01565 Da) and substitution of CH_4 versus O (0.0364 Da)[146] in both SRFA and WSOCs. Fluorescence EEMs further confirmed the fulvic-like nature of the BC WSOCs, that was composed of labile (C1) less aromatic, and more recalcitrant polyaromatic (C2) structures substituted with carboxyl and hydroxyl groups, in agreement with previous hypotheses.[75,96,130] The bioactivity of biochar WSOCs were tested by germination experiments on cress seeds, revealing stimulating effects. In conclusion, WSOCs influence the suitability of BC for environmental applications. Previous studies proposed that high amounts of PAHs,[54] phenolic and carboxylic acids[55,59] in BC WSOCs caused harmful effects on cress seeds. In this study, fulvic-like WSOCs and concentrations of VFA< 3mg/g induced statistically significant positive effects on the seedlings of cress, corroborating the hypothesis that

the complex biological effects of BC WSOCs are the results of an interaction between contrasting factors.[56,57] However, this study demonstrated that BC to soil application can be sustainable when BC contamination (organic and inorganic) is limited.

5) APPLICATIONS OF THE DEVELOPED ANALYTICAL METHODS

5.1) Process conditions: profiles of VOCs and WSOCs in biochar produced from a pilot scale pyrolysis reactor

In the previous sections (Chapter 3 and 4) it was evidenced that corn stalk biochars produced at increasing pyrolysis temperature can release variable amounts of organic compound in air (VOCs) and water (WSOCs). The correlation between the profiles of VOCs/WSOCs and biochar bulk parameters was therefore fundamental to understand the mechanism of release by the biochar matrix. Higher pyrolysis temperature and residence time increase the carbonization of biomass producing biochar with increasing aromatic ring condensation. Aromaticity, porosity and surface functionalities are among the most important parameters for the adsorption of organic compounds.[14] Biochar aromaticity was investigated by means of different techniques as outlined in section 1.2. It was demonstrated that atomic ratios determined by elemental analysis can be a suitable parameter for aromatic polycondensation.[36] Recently H/C ratio was proposed as fundamental parameter that links biochar production conditions, aromaticity and sorption capacity.[44] Finally, the aromaticity of the biochar structure is directly connected to its thermal stability and ultimately the stability in soil (environmental stability).[41,147] To this purpose, the patterns of VOCs and WSOCs were correlated to the H/C values of the corn stalk thermosequence biochars. The levels of VOCs and WSOCs decreased sharply with the decreasing carbonization degree indicating progressively lower release as the biochar becomes more carbonized. The data obtained from biochar produced with a bench scale quartz reactor in laboratory showed that the emission of VOCs resulted minimal when H/C<0.70 (section 3.3), while WSOCs release was strongly reduced for H/C < 0.59 (section 4.3). The application of the developed analytical methods to biochar produced at larger scale is important to test the incidence of process on biochar quality. This section deals with the characterization of biochar produced with a pilot plant pyrolysis reactor named thermocatalytic refoming (TCR), developed at the Fraunhofer Institute for Environmental, Safety, and Energy Technology

(UMSICHT). The technology is based on intermediate pyrolysis followed by the reforming of the biochar at high temperature.[148,149] The profiles of VOCs and WSOCs were then correlated to the biochar charring degree in order to verify the applicability of the methods for the determination of biochar quality.

MATERIALS AND METHODS

Samples

Biochars were produced from digestate pellets from anaerobic digestion plants (maize silage (62%), cattle and pig slurry (34%) and cereals (4%)) and sewage sludge with the TCR process. Four samples were produced with the reforming step of the biochar and one without (V66). The characteristics of the process and samples are reported in Table 6.

Table 6: Feedstocks and pyrolysis conditions used for the production of the TCR biochar samples

Name	Feedstock	Pyrolysis temperature (°C)	Reforming temperature (°C)
V66	digestate	400	non-reformed
V40	digestate	390	720
V44	digestate	500	720
DIG	digestate	400	700
SEW	sewage sludge	400	700

Elemental analysis of biochar samples

Elemental analysis of the biochar samples was performed in triplicate as described in section 3.2

Characterization of mobile organic compounds

The profiles of VOCs in biochar samples were determined with the HS-SPME-GC-MS method reported in section 3.2. WSOCs were extracted from the biochar and analyzed with DI-SPME-GC-MS and Fluorescence-PARAFAC according with the procedures described in section 4.2.

RESULTS AND DISCUSSION

Profiles of mobile organic compounds in biochar and relationships with thermal stability

The elemental composition and atomic molar ratios of the biochar samples are reported in Table 7. Overall, the digestate biochars that underwent the reforming step during pyrolysis presented higher C and lower O content compared to the non-reformed biochar (V66). Sewage sludge biochar had lower values of C and high ash content compared to the digestate biochars. These results are in accordance with previous studies on the composition of biochar produced from the TCR process.[148–150]

Table 7: elemental composition of digestate and sewage sludge biochars produced by the TCR process

Sample	C		H		N		O	Ash		**H/C**	**O/C**
	%	SD	%	SD	%	SD	%	%	SD		
V40	62	2.6	1.0	0.056	0.91	0.08	5.7	31	0.64	**0.20**	**0.069**
V44	64	1.4	1.0	0.059	1.1	0.079	2.8	30	0.57	**0.18**	**0.033**
V66	59	0.51	4.0	0.082	2.3	0.042	18	17	0.67	**0.82**	**0.22**
TCR-DIG	44	3.1	0.9	0.22	1.0	0.242	7.6	46	0.13	**0.24**	**0.15**
TCR-SEW	23	0.52	0.72	0.022	1.9	0.060	3.0	71	0.37	**0.38**	**0.10**

The increasing carbonization of the biochar due to the effect of the reforming step in the TCR is evidenced by the lower H/C and O/C values exhibited by the reformed biochars compared to the non-reformed sample (V66). The pattern of VOCs and WSOCs released from the biochar samples was investigated in relation with their aromaticity, in turn affected by the reforming step of TCR. Chromatograms of VOCs released from non-reformed and reformed biochars sorted by decreasing values of H/C are reported in Figure 23. The non-reformed digestate biochar (V66) presented intense signals of VOCs released from the poorly carbonized biochar matrix. The most intense peaks are associated to the degradation products of the lignin fraction of the digestate, and include alkylated and methoxy phenols. The non-complete carbonization of the biochar is also suggested by the presence of degradation products of hemi/cellulose, like cyclopentenones. Volatile and straight

chain fatty acids were detected as proxies of lipids while nitrogen compounds (indoles) as representative of the protein fraction of the digestate. All these molecular markers were detected during HS-SPME method development on thermosequence corn stalk biochars and proposed as important proxies for biochar quality. On the other hand, the reformed biochars from digestate and sewage sludge presented simplified chromatograms, especially in the regions associated with the aforementioned molecular markers, but with more intense peaks at the earlier retention times. For example, sewage SEW biochar displayed only traces of alkylated phenols and no methoxy phenols. Despite the different feedstock, this is in accordance with the corn stalk biochars having similar H/C values (0.36). The VOCs profile of SEW still included signals of nitrogen and sulfur species (benzonitrile, pyridine, thiophene), but the most abundant compounds were the monoaromatic hydrocarbons (benzene and C_{1-3} alkylated derivatives) and low molecular weight PAHs (naphthalene, methyl naphthalene). DIG biochar, with H/C 0.24, also presented these characteristic peaks but without contributions of PAHs (Figure 23). In summary, as observed for VOCs in thermosequence biochar samples, carbonization degree decreased the release of VOCs from biochar produced by the scaled-up TCR pyrolyser. Similarly, the carbonization degree influenced also the release of WSOCs. The chromatograms in Figure 24 show the semi-volatile fraction by DI-SPME-GC-MS analysis.

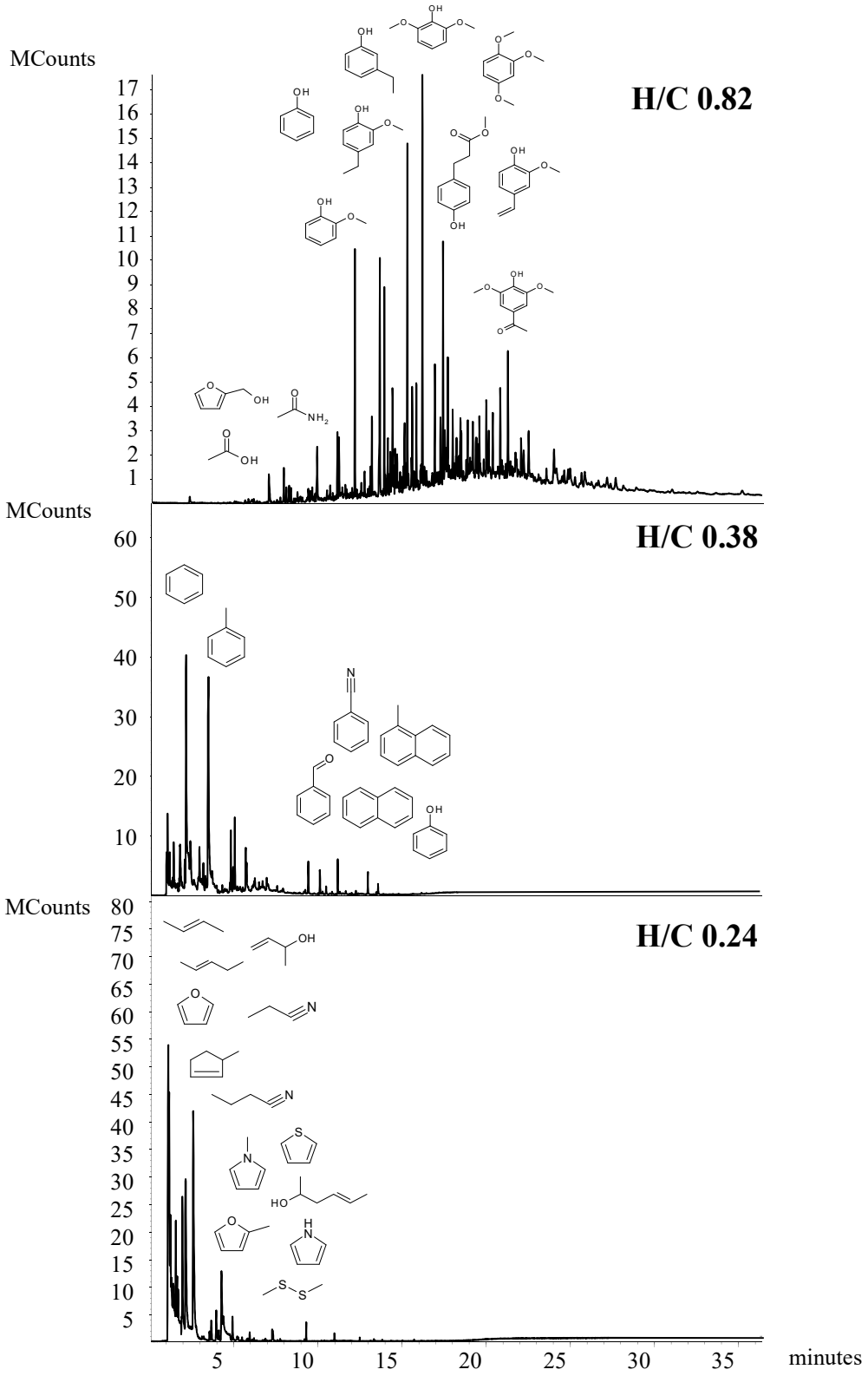

Figure 23: Chromatograms of VOCs released by digestate and sewage sludge biochars produced with the TCR process in relation to their increasing aromaticity measured by the decreasing H/C values. At high values of H/C, the distribution of VOCs was mainly composed by semi-volatile thermal degradation products of lignocellulose. For higher carbonized biochars the profile shifted towards lower molecular weight volatile compounds.

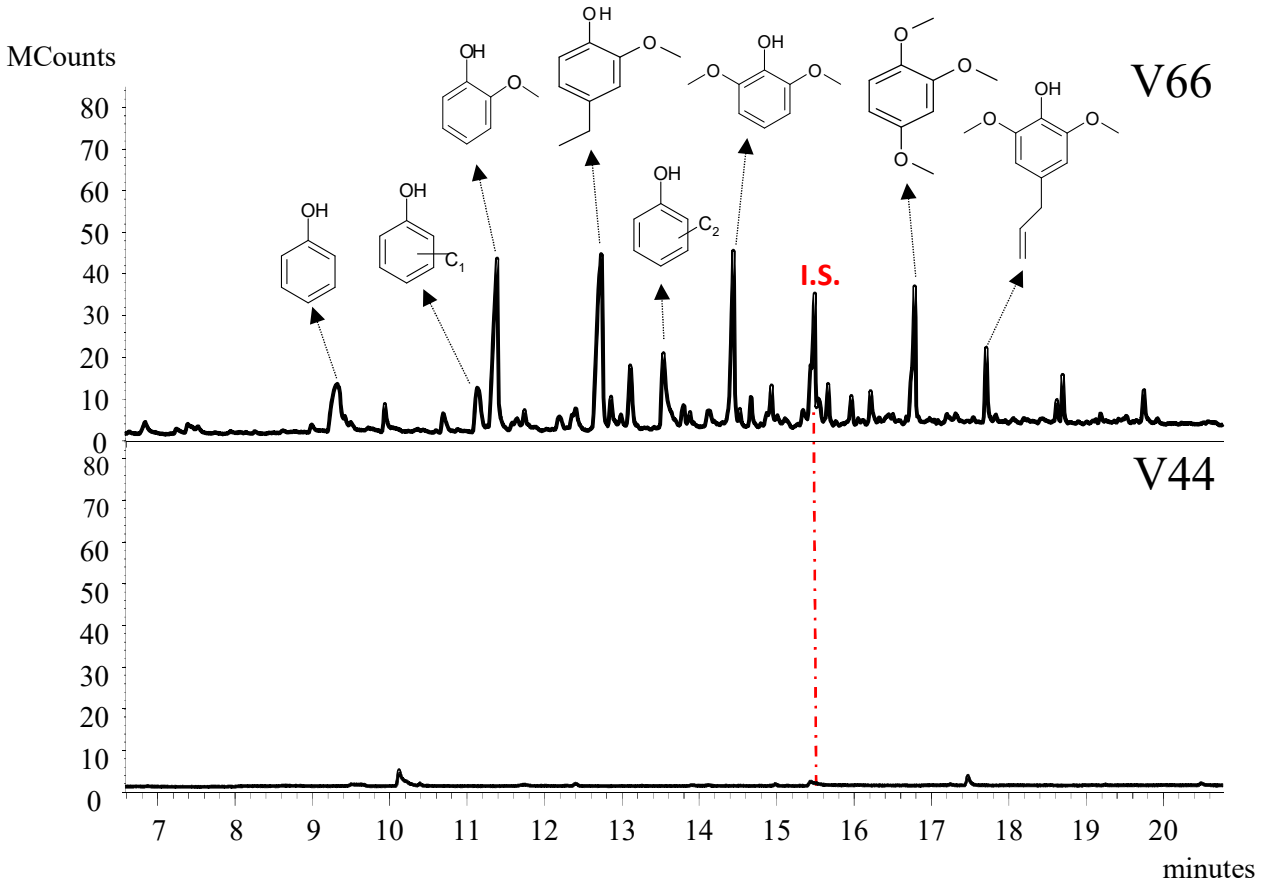

Figure 24: Chromatograms of WSOCs released from non-reformed (V66) and reformed (V44) digestate biochars. The dotted red line was used to indicate the suppression of the peak of the internal standard interpreted by the effect of the carbonization in the reformed sample (V44).

Non-reformed digestate biochar (V66) presented the pattern of lignin phenols as the most abundant compounds released in water. These species were already evidenced for VOCs. Instead, the reformed biochar (V44) did not present detectable signals of WSOCs, confirming the hypothesis that highly carbonized biochars are less prone to release WSOCs. In fact, the high adsorption affinity of the reformed biochars was confirmed, as the peak of the internal standard (*o*-eugenol) was strongly suppressed in Figure 25. The fluorescence spectra of WSOCs highlighted considerable dissimilarities in the aromatic structures released from the reformed versus the non-reformed biochars. Examples of the marked changes occurring in the fluorophores of WSOCs due to the biochar carbonization degree are reported in Figure 25, that represents the EEMs of V66 and V44.

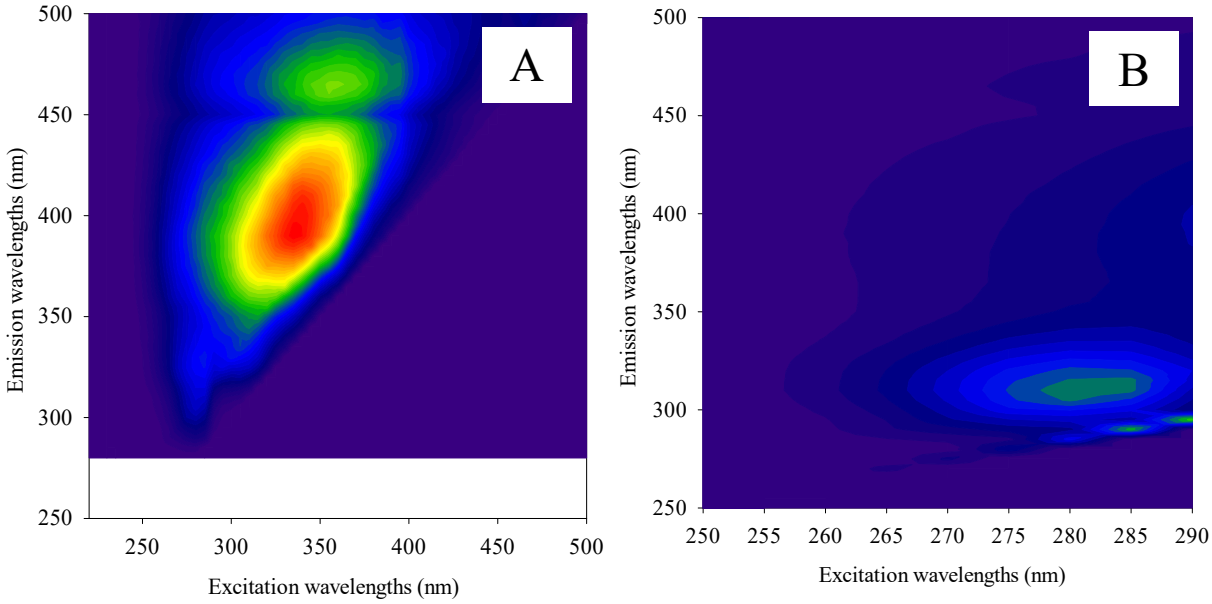

Figure 25: EEMs of WSOCs extracted from non-reformed V66 (A) and reformed V44 (B) biochars.

Non-reformed biochar presented two peaks at 325/400 and 350/470 nm. The aromatic functionalities associated with these fluorophores were detected in the characterization of corn stalk biochar WSOCs and representatives of fluvic-like structures (section 4.3). These moieties were not detected in the EEMs of reformed biochars, that were characterized by a peak at 280/310 nm possibly representing protein-like structures or low molecular weight polyaromatic hydrocarbons. In conclusion, the characterization of biochar samples produced with a pilot plant pyrolysis reactor revealed that the increasing carbonization degree induced by severe reforming conditions of the biochar in the TCR notably affected the release of VOCs and WSOCs from the resulting biochar. Highly carbonized biochars are less prone to release organic compounds in air and water and presented the opposite tendency to adsorb organic compounds (internal standard suppression). Besides, the analytical methods developed for the characterization of mobile organic compounds in biochar successfully correlated with the thermal stability measured with the H/C molar ratio.

5.2) Biological response: relationships of VOCs and WSOCs with seed germination

In the previous sections, analytical methods were developed for the determination of VOCs and WSOCs in biochar produced in a bench scale quartz reactor and applied to the characterization of biochar produced from a pilot plant pyrolysis reactor. Strong correlations between the quantity of these mobile organic species released from biochar and its carbonization degree emerged, indicating that less carbonized biochars are more prone to release organic contaminants than the more carbonized ones. As discussed in section 1.5 organic species released from biochar can induce biological activity, ultimately affecting biochar performance in soil. Germination tests were proposed as a rapid and easy tool to screen biochar toxicity. It was evidenced that soil-less germination tests can successfully evaluate the performance of the biochar for environmental application[104] and that cress (*Lepidium sativum*) is a suitable specie for the evaluation of harmful effects due to organic compounds.[58] To this purpose germination tests on cress seeds were conducted to evaluate the performance of biochar in relation to the presence of organic compounds released into water (WSOCs) and volatilized in the air (VOCs).

MATERIALS AND METHODS

Biochar materials

Poultry litter biochar was produced from the apparatus described in section 3.2 at 400°C with 20 min residence time and named PL400. The production of the thermosequence of corn stalk biochar was already discussed in section 3.2. TCR-DIG and V66 digestate biochars from TCR pyrolysis process were described in the previous section (5.1).

Characterization of biochar mobile organic compounds

VOCs and WSOCs were characterized by means of HS- and DI-SPME-GC-MS with the procedures described in Chapters 3 and 4.

Germination tests

The germination tests were conducted in four replicates by incubating 50 seeds of cress (*L. sativum*) with 5 g of a mixture containing biochar and deionized water onto sterilized cellulose filter paper (Whatman no. 1) placed in a Petri dish sealed with para-film. Two levels of biochar concentration were tested 5 and 40 g/L. These rates were equivalent to 5, and 40 t/ha on an area basis of 10 cm soil depth and a dry bulk density of 1.5 kg/m^3. Before incubation, the samples were shaken at 150 rpm on a platform shaker at room temperature for 24 h. All Petri dishes were covered and incubated at room temperature, 25 ± 2 °C, for 72 ± 0.5 h in the dark. Similarly, a control was prepared with deionized water. After 72 h of exposure, visible root development was used as the operational definition of seed germination. Data were reported as relative seed germination (RSG) percentage with respect to the control (deionized water): RSG = (number of germinated seeds in the sample/number of germinated seeds in control) ×100. Fifteen seeds per Petri dish were sampled and seedlings elongation was measured (root and shoot lengths in cm). The following statistics were performed with the software PAST (Paleontological Statistic vers. 2.16): Kruskal-Wallis test (non-parametric), one-way ANOVA (after data transformation with Box-Cox, to achieve normality of the distributions and homogeneity of the variance), post-hoc tests (Mann-Whitney and Tukey test).

RESULTS AND DISCUSSION

Poultry litter biochar

VOCs and WSOCs of PL400 biochar are reported in Figure 26. The biochar from poultry litter released a wide range of VOCs and WSOCs. VOCs included compounds deriving from the thermal degradation of polysaccharides (e.g., cyclopentenones, furans), lignin (e.g., 4-vinylphenol, guaiacol), proteins (e.g., pyrroles, pyridines, indole), lipids (e.g., VFAs; acetic acid is also derived from hemicellulose). Alkylated pyrazines and acetamide were probably derived from Maillard reactions between carbohydrates and proteins. Notably, a suite of short-chain n-alkanes/alkenes were identified, supporting previous studies on the occurrence of aliphatic components in poultry litter biochar.[151] It is expected that the polar fraction of VOCs will be preferentially distributed into

the aqueous phase in comparison to nonpolar constituents. In fact, the SPME-GC-MS analysis of the PL400 water extract showed a predominance of organic acids, including C_2 −C_{10} aliphatic and C_7 −C_9 aromatic acids. PL400 significantly inhibited seed germination at 5 and 40 g/L in water suspensions. Further investigation allowed to conclude that the inhibition of germination was suppressed after solvent extraction or treatment of biochar with active sludge, and that the phytotoxicity could be attributed to hydrophilic biodegradable substances derived from lipids or proteins.[55]

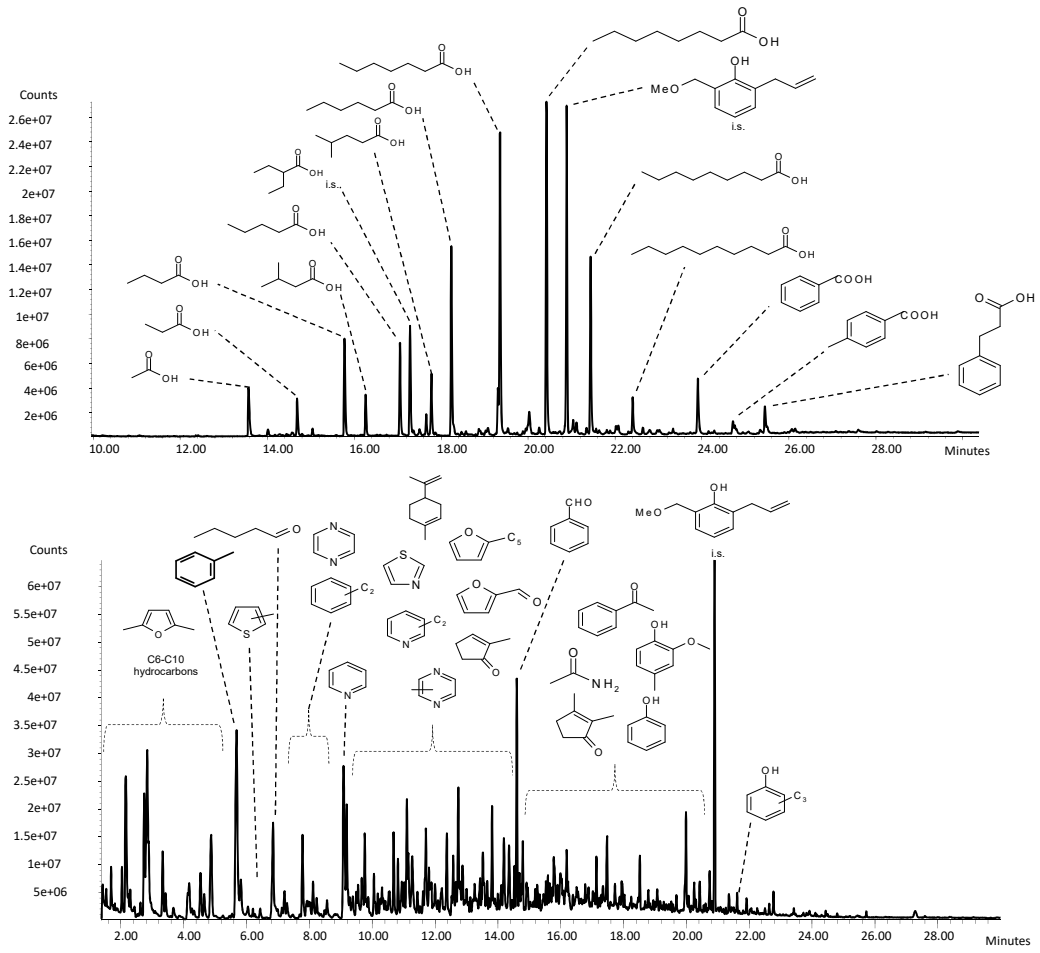

Figure 26: Total ion chromatograms obtained after (A) DI-SPME of WSOCs and (B) HS-SPME of VOCs of poultry litter biochar (PL400).

TCR biochar samples

Figure 27 represents the results of the germination tests conducted on the digestate biochars produced by the TCR process with (V66) and without the reforming step (TCR-DIG) versus the control with deionized water only.

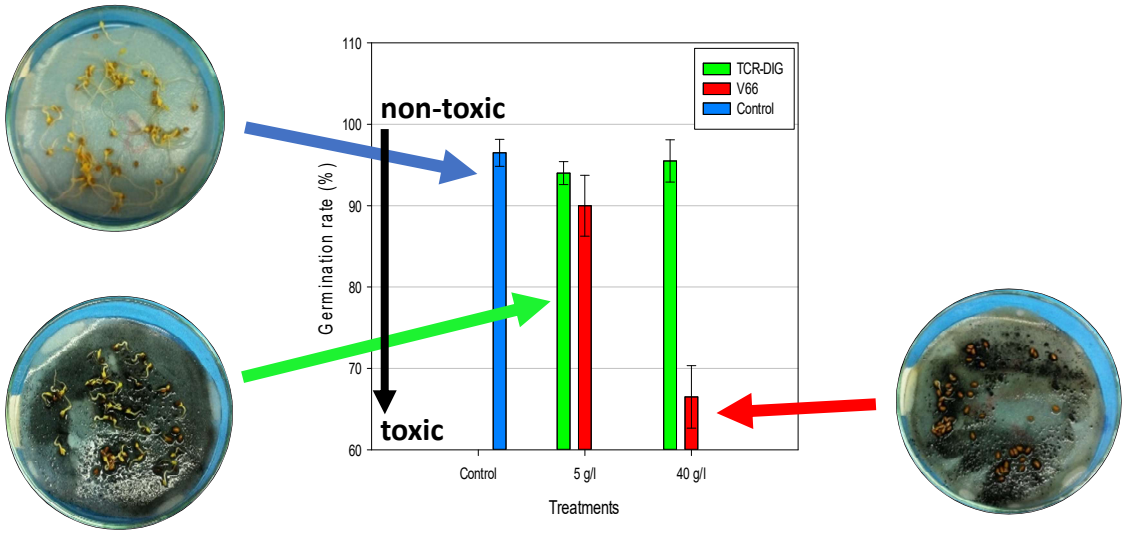

Figure 27: Results of phytotoxicity tests of TCR digestate biochars in water suspension at 5 and 40 g/l. Average values of germination rate (n=4) are expressed as percentage relative seed germination (% RSG) with respect to the control (no biochar) ± standard deviation.

TCR-DIG biochar (produced at temperature 400°C and reformed at 700°C) led to average germination rates of 94±1 and 96±3% (5 and 40 g/l treatment solutions), V66 (non-reformed biochar) to 90±4 and 67±4% in the same concentration range, while the average germination rate of the control (no biochar) was 97±2%. TCR biochar had a germination rate very similar to the control without biochar in both treatments, while V66 was toxic even at the lowest treatment. As outlined in section 5.1, the profiles of VOCs determined by HS-SPME-GC-MS showed that V66 featured mainly phenolic species, while the reformed biochar volatilized protein-derived aromatic compounds. The germination inhibition observed with V66 could be due to alkyl and methoxy phenols that are no longer present after TCR process.[103] Alkylated monoaromatic hydrocarbons detected in both cases seemed not to be associated with toxic effect. The reduced occurrence of VOCs in the reformed biochar could explain the suppression of the potential toxicity. However, other causes could also support this observation, such as the nutrient release associated to the

increased ash content with the carbonization intensity. The effect of the charring degree was examined in the next section.

Thermosequence of corn stalk biochar samples

Germination tests on the thermosequence of corn stalk biochar were carried out to evaluate the performance of biochar containing variable amounts of WSOCs in consequence of the pyrolysis temperature, and to correlate these effects with the charring intensity. In total, seven biochar samples with values of H/C ranging from 0.8 to 0.3 were examined (section 3.2). The germination rates of the biochar samples were not significantly different from the controls (p> 0.05) and the average value of all the treatments was 97 ± 2.5 %. In agreement with previous studies on corn stalk biochar with high VOC content,[60] WSOCs did not present inhibiting effect. Surprisingly, the seedlings emerged after the germination showed significantly longer shoots in all the biochar treatments versus the controls without BC ($p < 0.001$), and the values for the biochar with greater WSOCs content (BC 350-500) were higher ($p < 0.05$) than those of the more carbonized ones (BC 550-650) (Figure 28 and 29). Apparently, biochar released compounds that promoted the shoot growth. Among the possible candidates, karrikins are a group of smoke derived compounds that originate during the combustion of cellulose and act as potent plant stimulants, particularly KAR1 (3-methyl-2*H*furo[2,3-*c*]pyran-2-one).[152] Recently KAR1 was determined in slow pyrolysis biochar from green waste and in the corresponding pyrolysis water, and induced longer shoot lengths of tomato and lettuce seedlings in germination tests.[57] Interestingly, ESI(-)FT-ICR-MS mass spectrum of all the bio-oils collected during biochar synthesis presented a peak at m/z 149.02442 $[M-H]^-$ with molecular formula $[C_8H_5O_3]^-$, that could be associated to KAR1. However, the peak was not revealed in the mass spectra of the water extracts of biochar samples. The stimulant could be absent or present at undetectable concentration. Prior studies indicated that KAR1 in biochar presented trace values (0.8 ng/g) and its determination required a tailored method, while pyrolysis water showed markedly higher values (70ng/ml).[57] The highly carbonized biochar caused significantly shorter root lengths in comparison with the controls ($p < 0.001$), especially BC550-650 ($p < 0.001$).

In summary, less carbonized biochar favoured shoot against root growth in comparison with the more carbonized samples. The difference could be caused by a series of concomitant factors. A higher supply of micronutrients could be associated to the increasing ash content of the biochar with the charring degree, ranging from 24 (BC350) to 35% (BC650)[60]. However, the promotion of shoots could be also derived from the release of trace organic compounds such as karrikins or fulvic-like aromatic structures.

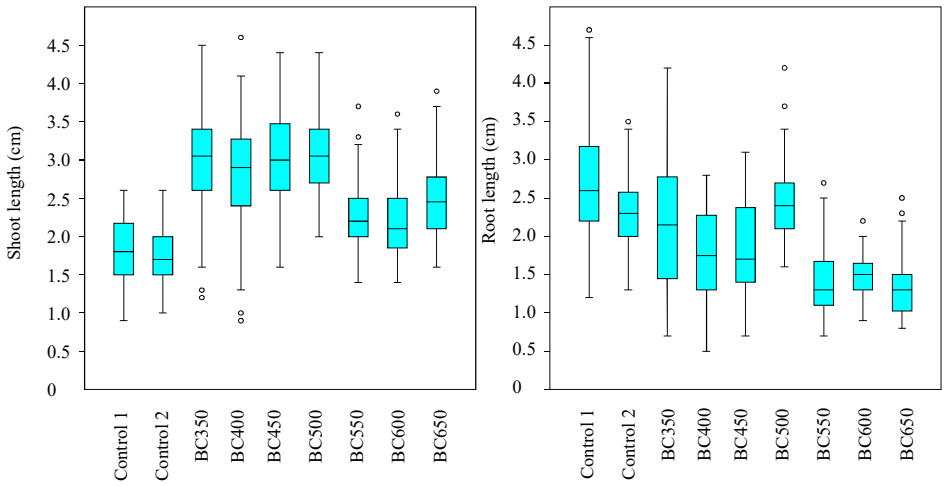

Figure 28: Box and whisker plots of shoot and root lengths (cm) of the cress seedlings for each treatment with biochar (BC) and without biochar (Controls). 25-75 percent quartiles are drawn using a box. The median is shown with a horizontal line inside the box. Horizontal lines outside the box represents the variability outside the lower and upper quartiles. Values >1.5 times the box height are reported as circles.

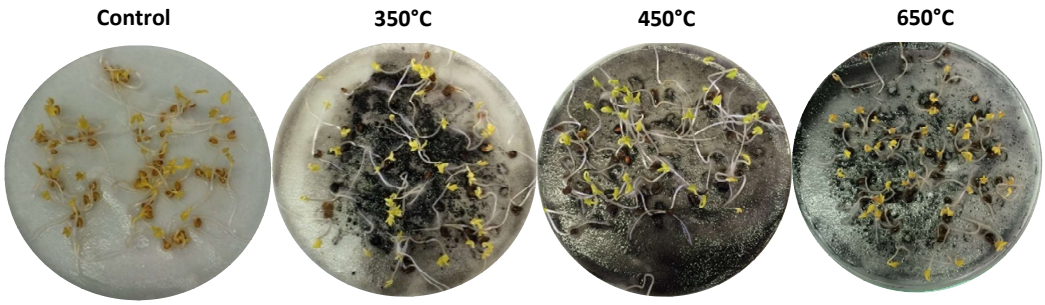

Figure 29: Examples of cress seedlings emerged after the germination tests with thermosequence corn stalk biochars versus the control with only deionized water.

5.3) Relationships of VOCs and WSOCs with corn growth at different stages

The results of germination tests of cress seeds in soil-less conditions highlighted the importance of mobile organic species in the determination of biochar quality. Therefore, it is likely that also biochar application to soil can influence the growth of plants. Depending on soil type and condition, biochar application can impede or facilitate root growth, which can impact aboveground biomass.[153–156] Furthermore, VOCs or WSOCs contained in biochars may indirectly affect plant growth by affecting microbial mediated processes in soil such as C and N cycling.[8] In this section the properties of three biochar materials were compared to the biological effects induced on corn growth at different stages, as the combination and interaction of biochar bulk properties, VOCs, WSOCs and physicochemical properties on plant physiology and growth in biochar-amended soils remain unclear.

MATERIALS AND METHODS

Biochar samples

Three biochar materials were characterized: a softwood chip biochar prepared by slow pyrolysis at 500 °C for 12 minutes (**PYR**) (Pyrovac, Jonquière, Québec, Canada) and two biochars prepared by slow pyrolysis from biosolids at 270 or 320 °C by Anaergia Inc. (**ALT** and **AHT**, respectively) (Burlington, Ontario, Canada). It has to be noted that, given the low content of carbon (<50%), **ALT** and **AHT** should be named pyrogenic carbonaceous materials according to EBC Guidelines.[18]

Biochar characterization

VOCs and WSOCs were determined for each biochar with HS- and DI-SPME according with the methods developed in Chapter 3 and 4. Biochar bulk chemical properties, specifically, fixed C, VM, ash content, carbonization degree (H/C), pH, EC, and its mobile species, that included available nutrients were also determined.

Corn growth at different stages

A germination assay determined whether biochar effects on root length resulted from (1) direct contact of seeds with biochar, due to exposure to VOCs, WSOCs, salts and water retention, (2) only water extract of biochar, containing WSOC and salts or (3) only VOCs emitted at 25 ˚C. Moreover, a greenhouse experiment tested the effects of the three biochars (**ALT**, **AHT** and **PYR**) on corn early root growth, biomass accumulation and N uptake. The experiment was performed on two sandy loam soils with an application of biochar of 2 % (w/w) or 26 Mg ha^{-1} (based on a depth of incorporation of 10 cm and 2.24 x 10^6 kg soil ha^{-1}). Corn was grown until the V3 stage, that represents a method for the evaluation of corn growth by measuring the stages of leaves.[157] The experiments were conducted at the Department of Plant Science of the McGill University, Quebec, Canada.

RESULTS AND DISCUSSION

The feedstock type and pyrolysis temperature highly influenced the bulk chemical characteristics of the biochars, that could in turn affect soil properties and plant growth after biochar amendment. The physicochemical and bulk chemical properties of biochar samples are reported in Table 8. In order to evaluate the potential influence of biochar mobile organic species on corn growth at different stages, i.e. germination and V3, the compounds that can be volatilized (VOCs) and those that can be released in water (WSOCs) were determined by SPME-GC-MS. VOCs were sampled in the head space (HS-SPME) of biochar samples, while WSOCs in the water extracts, by means of direct immersion (DI)-SPME-GC-MS. Representative chromatograms obtained from the analysis of **ALT**, **AHT** and **PYR** are reported in Figure 30.

Table 8: physicochemical properties of biochar samples. Average values of triplicate analysis are reported with standard deviations (tests were performed at the Department of Plant Science of the McGill University, Quebec, Canada)

	ALT	**AHT**	**PYR**
pH	5.8	6.4	8.7
TGA, % dry weight			
VM	67.2 (2.94)	43.1 (4.10)	33.0 (2.05)
Fixed C	7.86 (0.67)	16.8 (0.72)	57.3 (0.18)
Ash	26 (1.3)	41 (0.046)	8.7 (0.099)
Available elements, mg kg^{-1}			
N	494 (2.94)	10.8 (0.138)	12.3 (0.349)
P	759 (8.13)	565 (26.2)	111 (2.24)
K	377 (29.8)	45 (0.693)	464 (5.38)
Na	358 (5.56)	51 (2.27)	39 (0.119)
Elemental composition, % dry weight			
N	5.9 (0.034)	5.4 (0.017)	0.50 (0.0028)
C	38 (0.134)	39 (0.20)	62 (0.057)
H	5.8 (0.029)	3.8 (0.026)	3.2 (0.041)
S	2.5 (0.15)	1.9 (0.059)	0 (0)
O	21 (1.4)	9.2 (0.27)	26 (0.19)
Atomic molar ratios			
H/C	1.8 (0.0026)	1.2 (0.011)	0.63 (0.0074)
O/C	0.41 (0.029)	0.18 (0.0061)	0.31 (0.0026)

The GC traces of **ALT** and **AHT** presented intense peaks associated to WSOCs and VOCs, while the signals of **PYR** were significantly reduced. A total of 111 compounds were detected and tentatively identified in the WSOCs and VOCs extracted from the three biochars (Table S13). **ALT** and **AHT** WSOCs were mainly characterized by aldehydes and ketones, accounting for 53 % of the total amounts for both biochars. The principal constituents of this class were typical pyrolysis products of hemi/cellulose evolved from poorly carbonized biochars, such as furfural, C_{1-3} alkyl substituted cyclopentenones, benzaldehyde and its alkylated/hydroxylated derivatives.[42] Benzaldehyde was the most abundant compound in both samples, while furfural was the second in **ALT**, and 2-butanone in **AHT**. C_{4-9} straight-chain and branched ketones were also detected, especially in **AHT**. Carboxylic acids were the second most abundant class of WSOCs in **ALT** (23%) and included C_{2-12} saturated, unsaturated (2-butenoic acid) and aromatic acids (benzoic acid

and its $C_{1\text{-}3}$ alkylated derivatives), with *n*-octanoic and *n*-decanoic acids as the most prominent species. Their content sharply decreased in **AHT** and the total amounts of organic acids decreased accordingly (13%). The presence of carboxylic acids can explain the slightly acidic pH of **ALT** (5.8) and **AHT** (6.4) compared to **PYR**, that was weakly basic (8.7). Carboxylic functional groups on the biochar surface can also contribute to this effect.[158] In an experiment with oak, pine and grass biochars prepared at three different temperatures (250, 400 and 650 °C), an increasing pyrolysis temperature increased the loss of acidic surface functional groups which were transformed to give neutral or basic groups[159] which explains the higher pH of **PYR**. **ALT** and **AHT** presented significant contributions of nitrogen containing compounds (13 and 15 % respectively), that were the second most abundant class in **AHT**.

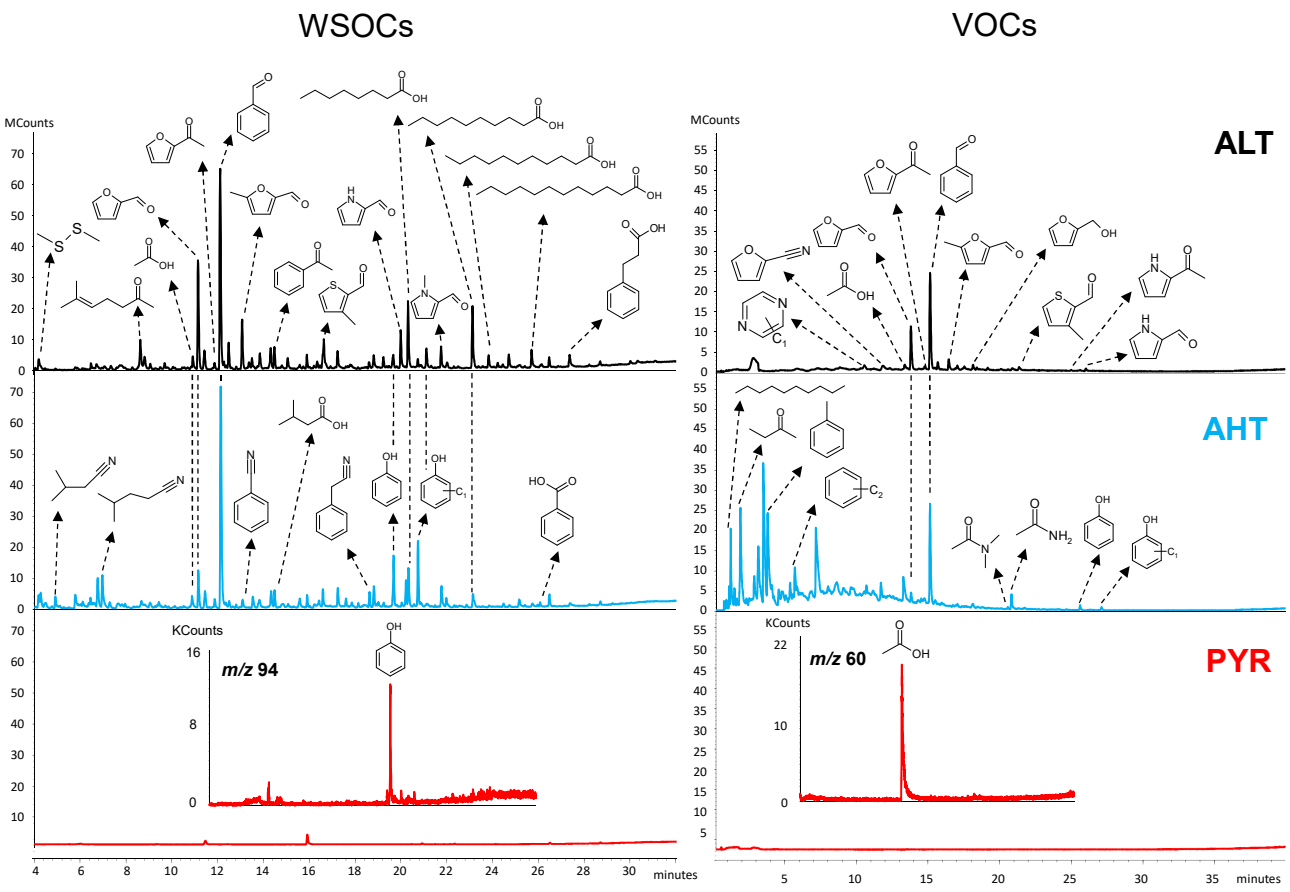

Figure 30: total ion chromatograms of WSOCs and VOCs released at 25°C from ALT, AHT and PYR. The most abundant common compounds in the chromatograms are indicated by the dotted lines.

Their distributions differed between the two biochars, as pyrrolic species (pyrrole-2-carboxaldehyde) and C_{1-3} pyrazines were the most abundant in **ALT**, while aliphatic/aromatic nitriles were the most important in **AHT**. The presence of moieties like aromatic nitriles (benzonitrile, 1,2-benzenedicarbonitrile, benzenepropanenitrile), indoles, pyrroles and pyrazines indicated the significant protein content of the feedstock biomass.[55] The presence of sulfur species in **ALT** and **AHT** (8 and 2% respectively) is in accordance with the sulfur content of biochar[42] (Table 8) and S-containing compounds sharply decreased in **AHT** compared to **ALT**, probably due to protein degradation at the higher pyrolysis temperature. Derivatives of thiophene and thiazole were the principal components of this class, with methyl-thiophencarboxaldehyde as most abundant compound. Phenolic species were detected in both **ALT** and **AHT** WSOCs as markers of the lignin fraction.[42,60] The content of alkyl substituted phenols and methoxy phenols significantly increased in **AHT** (14%) compared to **ALT** (2%), possibly due to incomplete degradation of lignin at 270°C that becomes more severe at 320°C.[160] The occurrence of monoaromatic hydrocarbon units in the volatilome of biochars was associated to the extent of carbonization.[43] Despite their solubility in water, trace amounts were detected in the WSOCs of **ALT** (1 %) and **AHT** (2%) with toluene as most representative species. The majority of the compounds detected in **ALT** and **AHT** WSOCs were also identified as VOCs evolved from biochars heated at 150°C. However, the VOC profiles at 150°C presented additional species that were not (or barely detected) in the WSOCs, including monoaromatic (C_{3-8} alkylated benzenes) and low molecular weight polyaromatic hydrocarbons (C_{1-3} alkylated naphthalenes), amides, C_{14-16} fatty acids, benzenediols, volatile sulfur compounds (carbon disulfide) and chlorobenzenes (Table S13). The release of VOCs was tested also at ambient temperature (25°C) and confirmed that many of the aforementioned species were released preferentially in the air, like amides, C_{3-6} benzenes, naphthalene, carbon disulfide and chlorobenzenes. On the other hand, VOC profiles at 25°C included several compounds detected in the WSOCs (aromatic aldehydes, ketones phenols, volatile fatty acids, nitrogen and sulfur containing compounds), confirming that the mobility of organic species in biochar can occur both in

air and water phase.[55] The VOC pattern of **PYR** was characterized by signals more than one order of magnitude lower than those of **ALT** and **AHT** (Table S13), and only 12 species could be identified. Among the compounds detected, only 5 were released as WSOCs, acetic acid, 2-butanone, methyl phenol, phenol and furfural, respectively in order of abundance. These species were also detected in the VOC profiles at 150°C, while only acetic acid was volatilized at 25°C. Traces of other volatiles were detected at 150°C including benzaldehyde and 2-pentanone, while benzene, toluene, ethylbenzene, trimethyl benzene and indane are representative of the higher carbonisation degree of the biochar.[60] The absence of nitrogen and sulfur compounds in **PYR** is indicative of the lower protein content in the feedstock material, compared to that of biosolids, as highlighted by the elemental composition (Table 8). The much lower content of VOCs and WSOCs released from PYR and the presence of aromatic hydrocarbons in VOCs due to the effect of the carbonization degree is in accordance with Ghidotti et al.[60] The increasing pyrolysis temperature is known to increase fixed C, decrease VM and concentrate ash in the resulting biochar.[30,108,159] The effect of pyrolysis temperature on the pH, FC, VM, H/C and ash content in the biochar samples (Table 8) is in accordance with other studies in literature.[42,43] The values of H/C (Table 8) confirmed that the degree of carbonization decreased in the order **PYR**>**AHT**>**ALT**. There was a large reduction in available N, P, K and Na when pyrolysis temperature was increased from 270 °C (**ALT)** to 320 °C (**AHT)**. The higher ash content of **AHT** did not result in higher available N, P, K and Na concentrations, which suggests loss of these nutrients by volatilization with increasing pyrolysis temperature. The reduction in available Na concentration is desirable due to the potential phytotoxicity of this element. The reduction in available N, P and K, however, reduces the fertilizer value and nutrient recovery of **AHT** *versus* **ALT**. **PYR** had a similar available N and Na as **AHT**, higher K and lower P concentrations than **AHT** and **ALT**. Germination tests on corn seeds revealed that biochar treatments did not alter the number of seeds germinated compared to the control without biochar, in accordance with Ghidotti et al.[60] In contrast, shoot and root lengths were markedly affected, highlighting the importance of biochar characteristics and its mobile species on

the biological response induced on corn. The exposure to VOCs released by **ALT** led to longer root lengths, while in the case of **PYR**, trace amounts of 2-butanone, 2-pentanone, benzaldehyde, acetic acid and phenols could explain the increased shoot growth. Contrarily, the species released in water by **ALT** reduced the root length, and can be possibly explained by the phytotoxic activity of WSOCs and the high concentrations of available N and K. Under greenhouse conditions, corn plants were grown to the V3 stage on two coarse-textured temperate zone soils, amended with 26 Mg ha^{-1}. In accordance with the results of germination tests, ALT and AHT reduced root length compared to the control soil without biochar application, while the more carbonized PYR increased root length. AHT also improved biomass accumulation and N uptake. In conclusion, VOCs, WSOCs, available nutrient concentration and degree of carbonization are biochar characteristics that should be taken into consideration to predict effects on plant growth. Biochars with low H/C could help plants develop longer root systems and therefore higher resilience to drought and nutrient stress. Biochars with higher H/C and available N and K concentrations may provide fertilization value and consequently reduce root development but increase N uptake.

6) CONCLUSIONS

The application of biochar to soil for agronomic and environmental purposes requires the comprehensive investigation on its chemical properties. Priority contaminants (polycyclic aromatic hydrocarbons, polychlorinated dibenzofurans, dioxins and heavy metals) that could limit biochar utilization were established and threshold values for the determination of its suitability were proposed. Apart from this suite of regulated compounds, a wide range of other organic species can be retained onto the biochar aromatic and porous structure, in consequence of re-condensation of pyrolysis vapors during its production, or adsorption during its storage. These compounds can be subsequently mobilized in the environment and could impact plants, soil organisms or affect the pool of natural organic matter. The present study provided insights into the chemical nature of organic compounds released from biochar into air as volatile organic compounds (VOCs), and water, as water-soluble organic compounds (WSOCs). For the first time, solid-phase microextraction (SPME) was applied to the sampling of VOCs and WSOCs in biochar. The relationship between these species and the carbonization degree was evidenced through the analysis of a set of biochar from corn stalks produced at increasing temperatures under reproducible conditions. A fast and solvent-less method for the analysis of VOCs was developed, based on head space (HS)-SPME sampling of compounds volatilized when biochar was heated at 150°C, and detection by gas chromatography mass spectrometry (GC-MS). The procedure suitably revealed VOCs at the sub-ng level. The principal classes of VOCs identified were aldehydes and ketones, especially benzaldehydes and furaldehydes, aromatic hydrocarbons (benzene, toluene, C_2 benzenes), phenols and methyl esters of fatty acids. The %RSD of the intensities ranged from 14 to 34%. Considering the complexity of the matrix and the number of compounds constituting the biochar volatilome, the method resulted adequate to the scope of evidencing the relationship between the molecular pattern and relative intensities of VOCs and the biochar carbonization degree, expressed by the H/C atomic ratios and volatile matter (VM). Total VOCs concentrations were significantly correlated with VM, indicating that highly carbonized biochar should be

produced to reduce the risk of VOCs emission. Overall, when H/C<0.70 VOCs emission was sharply reduced compared to the samples with lower carbonization degree (H/C 0.80) and resulted minimal at ambient temperatures (25, 50°C). The organic compounds released in water (WSOCs) exhibited a complex composition and required the integration of different analytical techniques for a detailed characterization. Direct immersion (DI)-SPME-GC-MS was employed for the analysis of the semi-volatile WSOCs, high resolution mass spectrometry (Fourier Transform Ion Cyclotron Resonance Mass Spectrometry, FT-ICR-MS) to investigate the hydrophilic fraction ionized by negative electrospray ionization ESI(-), and fluorescence spectroscopy with Parallel Factor Analysis (PARAFAC) for the characterization of aromatic structures. The pattern of WSOCs was compared to that of the pyrolysis vapours collected into bio-oil during pyrolysis. The bio-oil WSOCs were composed mainly by lignocellulosic-derived compounds (alkylated and methoxy phenols) and their composition did not change with the increasing pyrolysis temperature. On the contrary, biochar WSOCs distribution presented remarkable differences. Interestingly, biochar water extracts featured carboxylic acids, principally volatile fatty acids, that ranged from 3 mg to 14µg per gram of biochar. The pattern of higher molecular weight species (<600 Da) in poorly carbonized biochar was comparable to that of the corresponding bio-oils, but the increasing charring degree noticeably reduced the homologues with higher degree of aromaticity. The aromatic functionalities of biochar WSOCs resembled those of natural organic matter (fulvic acids), while lignin-like moieties characterized mostly the bio-oils. Overall, SPME-GC-MS, ESI(-)FT-ICR-MS and fluorescence-PARAFAC indicated that the release of WSOCs from biochar was strongly reduced for biochar with H/C < 0.59, in agreement with the trend observed for VOCs. A mechanism of released of WSOCs was hypothesized related to the biochar structure: the fractionation of carboxylated, lipid-derived aliphatic structures into water of the more carbonized biochar could be attributed to a combination of porosity, hydrophobic effect and π-π interactions, favouring their release while retaining phenolic species. The methods developed were employed to characterize biochars from different feedstocks (spent mushroom substrate, digestate from anaerobic digestion, sewage sludge,

poultry litter, biosolids, softwood) and pyrolysis units. The profiles of VOC and WSOCs differed markedly and presented a variety of thermal degradation products of biomass biopolymers (lignin, hemi/cellulose, lipids and proteins). The extent of carbonization, in turn related to biochar recalcitrance in soil, highly affected the mobility of organic species in air and water. Biochar reformed under the severe temperature conditions of the thermo-catalytic reforming process (TCR) not only were less prone to release VOCs/WSOCs but exhibited the opposite tendency to strongly adsorb organic compounds. The information gathered on the molecular composition could be utilized to interpret potential bioactivity of the organic species retained in the biochar matrix. The biological response was evaluated by means of germination assays and early stage growth tests. VOC and WSOCs induced contrasting biological effects. Generally, carbonized biochars which were less prone to release organic species did not suppress the growth of the investigated plant species. Noticeably, corn stalk biochar improved the shoot lengths of cress seedlings, regardless of the carbonization extent and the presence of mobile organic species, and the effect was more pronounced for less carbonized samples that released WSOCs. This effect could be correlated to the occurrence of growth promoters such as karrikins or fulvic-like aromatic structures mimicking dissolved organic matter in soil. On the contrary, poorly carbonized biochar from digestate, poultry litter and biosolids released organic compounds that could be involved in the inhibition of germination and growth of cress and corn seedlings, possibly phenolic species, carboxylic acids, nitrogen and sulfur containing compounds. These results support the view that VOCs and WSOCs are crucial parameters in the assessment of biochar quality in environmental applications. This study provided new insights into the source and release of VOCs and WSOCs, but a comprehensive understanding of the role played by these species in the induction of biological effects requires further investigation. Fast analytical methods based on SPME developed in this study are suitable for screening the effect of pyrolysis process conditions on the mobile compounds in the resulting biochar and their potential impact in the soil system.

7) SUPPLEMENTARY MATERIAL

Table S1. Quantitative HS-SPME analysis of VOCs in CS biochars with H/C 0.80-0.49. Average quantities and SD were expressed as normalised areas (NA), (n=3), m/z quantitation ion.

compound classes	H/C			0.80		0.71		0.59		0.49	
	Compound	*m/z*	*r.t (min)*	*NA*	*SD*	*NA*	*SD*	*NA*	*SD*	*NA*	*SD*
alkyl amides	formamide	45	17.44	8651	1732	12338	2544	19377	5396	19545	1344
	formamide-N-methyl	59	15.77	22867	6757	6148	3759	-	-	-	-
	acetamide	59	17.17	17647	5318	10526	3491	8186	2476	4005	1296
	propanamide-N-methyl-	87	16.09	2439	937	-	-	-	-	-	-
	acetamide-N,N-dimethyl-	87	17.05	3663	469	1625	215	301	264	200	196
aldehydes and ketones	propanal	58	1.987	259902	6410	99685	9993	13364	3099	5200	1146
	butanal	72	2.524	132772	23578	56327	11499	4747	1556	2671	771
	2-butanone	84	9.106	210989	29490	106291	16693	23798	8518	10169	2796
	2-butenal-3-methyl-	72	2.759	-	-	24687	5669	6959	2967	1360	339
	2-pentanone	86	3.820	61231	15550	51309	43653	-	-	-	-
	benzaldehyde	106	14.29	620496	120782	138350	31516	15088	3220	7622	1869
	benzaldehyde, 4-methyl-	120	15.49	21369	4337	5457	1422	595	347	160	55
	benzaldehyde, 2-hydroxy-	134	16.93	9448	6877	2234	568	342	592	310	367
	Benzaldehyde, 2,5-dimethyl-	122	16.24	2950	620	-	-	-	-	-	-
	furfural	96	13.50	406962	108427	27761	7265	2776	1262	3776	2627
	2-furancarboxaldehyde, 5-methyl-	110	14.95	69007	22950	5026	1526	716	403	607	727
	cyclopentanone	84	8.703	-	-	-	-	-	-	562	169
	cyclohexanone	98	10.70	-	-	-	-	1143	1286	1384	487
	ethanone, 1-(2-furanyl)-	120	15.88	42870	12418	2455	704	342	200	288	333
	ethanone, 1-(2-hydroxyphenyl)-	136	17.66	3023	798	440	125	-	-	-	-
	acetophenone	110	14.04	18606	3654	3792	1257	605	106	396	83
aromatics	benzene	78	3.237	40567	9108	89177	21019	172635	60304	75247	21683
	toluene	91	5.238	943133	190185	1071113	214917	942228	289812	289861	70102
	C2 benzene	106	7.668	63834	10398	113922	21118	61837	17230	14652	2719
	C3 benzene	120	10.54	36463	6448	49156	11742	12551	3107	3095	722
	C4 benzene	134	13.00	10745	1835	10050	2806	3058	1113	594	148
	C5benzene	148	12.45	5375	1055	7310	2048	-	-	-	-
	benzene, methoxy-	108	11.63	28770	6073	5776	1266	1329	439	807	262
	benzene, 1-methoxy-4-methyl-	122	13.08	54210	14557	3778	1131	-	-	-	-
	benzene, 1-ethyl-4-methoxy-	136	13.96	9939	1949	-	-	-	-	-	-
	benzene, 1,4-dimethoxy-2-methyl-	152	19.33	4812	1238	-	-	-	-	-	-
furans	Furan, 2-pentyl-	81	9.644	29137	8773	3442	1202	-	-	-	-
	3-methyl-furan	82	2.472	25347	4480	8396	982	6355	1430	3123	488
	furan, 2,5-dimethyl-	96	3.424	30683	7674	11894	2749	6478	1901	1632	335
cyclopentenones	2-cyclopenten-1-one	82	11.86	87072	22123	31571	7684	8486	3334	3215	931
	2-cyclopenten-1-one-2-methyl-	96	12.04	59959	16494	14667	4204	2578	949	658	570

	2-cyclopenten-1-one, 2,3-dimethyl-	110	14.49	44030	12991	6791	2143	457	179	195	176
	2-cyclopenten-1-one, 2,3,4-trimethyl-	124	13.87	11839	3219	2316	735	-	-	-	-
lactones	butyrolactone	86	15.69	21079	6349	1771	313	-	-	-	-
	2(5H)-furanone, 3-methyl-	98	16.74	24944	6354	9964	2024	1568	246	349	121
	2-furanone, 2,5-dihydro-3,5-dimethyl	112	17.60	6004	4117	1084	266	130	136	-	-
phenols	phenol	94	19.70	537960	117085	471769	75607	362405	58862	144423	46590
	methyl phenol	108	20.45	89238	25413	53543	12172	35659	3903	6260	2616
	C2 phenol	122	21.23	49420	16788	23057	6562	6104	1899	1699	1272
	C3 phenol	136	20.60	10191	5088	5073	2917	506	186	78	136
	C4 phenol	150	21.61	1930	552	1699	2091	-	-	-	-
	phenol, 2-methoxy-	124	18.22	120773	31991	5062	1652	1005	525	1473	861
	phenol, 2-methoxy-4-methyl-	138	19.23	26519	7084	907	334	396	273	520	327
	phenol, 4-ethyl-2-methoxy-	152	19.96	11729	3437	411	273	153	264	271	367
anhydrosugars	1,4:3,6-dianhydro-alfa-d-glucopyranose	69	23.40	5034	1993	765	211	192	41	-	-
organic acids	formic acid-methyl ester	60	1.875	187339	37241	23971	5615	9941	1134	9382	3864
	acetic acid-methyl ester	74	2.187	850572	38731	623686	60141	194077	53699	68049	21150
	butanoic acid methyl ester	88	2.816	161466	44323	53043	16834	3823	1671	1808	442
	Propanoic acid, 2-methyl-, methyl ester	87	3.000	3190	831	1433	1274	-	-	-	-
	2-hydroxy-propanoic acid-methyl ester	45	11.24	12459	3508	6139	2213	1232	510	-	-
	Butanoic acid, methyl ester	74	3.997	45788	13334	15305	4935	-	-	-	-
	Pentanoic acid, methyl ester	87	6.447	10670	3120	3407	1259	-	-	-	-
	Pentanoic acid, 3-methyl-, methyl ester	87	8.796	8219	2155	1594	428	-	-	-	-
	Heptanoic acid, methyl ester	87	10.68	3984	521	490	164	-	-	-	-
	Octanoic acid, methyl ester	87	12.30	4747	1191	-	-	-	-	-	-
	benzoic acid methyl ester	136	15.52	248246	53071	114174	31570	16466	6369	6021	2051
	methyl benzoic, methyl ester	150	17.12	9519	2512	1773	544	413	140	-	-
alcohols	benzyl alcohol	82	12.49	6445	1730	1073	349	217	50	-	-
	2-furanmethanol	98	15.92	12650	4309	1049	230	-	-	83	144
	Cyclohexanol	108	18.38	-	-	-	-	1340	703	1679	718
nitrogen compounds	carbamic acid, methyl ester	75	15.60	14152	2389	15366	3968	10502	3124	7696	2393
	Benzonitrile	103	15.35	-	-	5568	1246	2746	413	1456	293
low molecular weight PAHs	Naphtalene	128	16.95	14246	698	5255	1207	2293	415	1114	468
	naphthalene, 1-methyl-	142	18.58	7260	268	2859	707	731	145	197	60
indane/benzofuran derivatives	Indane	117	12.00	21123	3365	22330	5180	6084	1512	1169	267
	Benzofurane	132	13.90	27685	5152	12941	3050	3805	661	1313	372
	benzofuran, 2,3-dihydro-	132	19.91	6737	3016	4714	5591	5314	7202	10619	15460
	indan, 1-methyl-	146	16.54	8591	2563	8985	2008	1014	188	128	113
	Benzofuran, 2-methyl-	118	14.03	22583	3722	29437	38771	1105	232	244	251
	1-indanone	120	23.30	4848	1401	453	225	240	188	293	407
	C2 indane	132	15.18	9895	2346	3861	1213	279	83	-	-
	benzofuran, 4,7-dimethyl-	146	16.19	11625	1539	4307	1183	396	98	-	-
TOTAL VOCs	-	-	-	6014633	1041954	3521363	642344	1986275	530254	717657	167229

Table S2. Quantitative HS-SPME analysis of VOCs in CS biochars with H/C 0.44-0.32. Average quantities and SD were expressed as normalised areas (NA), (n=3), m/z quantitation ion.

compound classes	H/C			0.44		0.36		0.32	
	Compound	*m/z*	*r.t (min)*	*NA*	*SD*	*NA*	*SD*	*NA*	*SD*
alkyl amides	formamide	45	17.44	20126	4575	22131	10682	19169	4353
	formamide-N-methyl	59	15.77	-	-	-	-	-	-
	acetamide	59	17.17	1882	1892	548	949	-	-
	propanamide-N-methyl-	87	16.09	-	-	-	-	-	-
	acetamide-N,N-dimethyl-	87	17.05	210	60	-	-	-	-
aldehydes and ketones	propanal	58	1.987	1363	148	-	-	-	-
	butanal	72	2.524	722	134	-	-	-	-
	2-butanone	84	9.106	3530	1089	-	-	-	-
	2-butenal-3-methyl-	72	2.759	318	77	-	-	-	-
	2-pentanone	86	3.820	-	-	-	-	-	-
	benzaldehyde	106	14.29	3147	746	1718	232	1544	250
	benzaldehyde, 4-methyl-	120	15.49	-	-	-	-	-	-
	benzaldehyde, 2-hydroxy-	134	16.93	500	866	-	-	-	-
	Benzaldehyde, 2,5-dimethyl-	122	16.24	-	-	-	-	-	-
	furfural	96	13.50	5369	6867	2094	967	2017	834
	2-furancarboxaldehyde, 5-methyl-	110	14.95	1314	2119	289	500	-	-
	cyclopentanone	84	8.703	245	72	-	-	-	-
	cyclohexanone	98	10.70	402	140	105	183	-	-
	ethanone, 1-(2-furanyl)-	120	15.88	565	859	112	194	-	-
	ethanone, 1-(2-hydroxyphenyl)-	136	17.66	132	228	-	-	-	-
	acetophenone	110	14.04	290	502	-	-	-	-
aromatics	benzene	78	3.237	38448	10187	25339	6932	10619	3564
	toluene	91	5.238	93970	29202	24205	6056	22632	10197
	C2 benzene	106	7.668	3707	1349	697	218	315	112
	C3 benzene	120	10.54	568	198	-	-	-	-
	C4 benzene	134	13.00	-	-	-	-	-	-
	C5benzene	148	12.45	-	-	-	-	-	-
	benzene, methoxy-	108	11.63	-	-	-	-	-	-
	benzene, 1-methoxy-4-methyl-	122	13.08	-	-	-	-	-	-
	benzene, 1-ethyl-4-methoxy-	136	13.96	-	-	-	-	-	-
	benzene, 1,4-dimethoxy-2-methyl-	152	19.33	-	-	-	-	-	-
furans	Furan, 2-pentyl-	81	9.644	-	-	-	-	-	-
	3-methyl-furan	82	2.472	1041	157	-	-	-	-
	furan, 2,5-dimethyl-	96	3.424	855	93	-	-	-	-
cyclopentenones	2-cyclopenten-1-one	82	11.86	1297	430	-	-	-	-
	2-cyclopenten-1-one-2-methyl-	96	12.04	-	-	-	-	-	-
	2-cyclopenten-1-one, 2,3-dimethyl-	110	14.49	110	191	-	-	-	-
	2-cyclopenten-1-one, 2,3,4-trimethyl-	124	13.87	-	-	-	-	-	-
lactones	butyrolactone	86	15.69	-	-	-	-	-	-
	2(5H)-furanone, 3-methyl-	98	16.74	-	-	-	-	-	-
	2-furanone, 2,5-dihydro-3,5-dimethyl	112	17.60	-	-	-	-	-	-
phenols	phenol	94	19.70	33396	12587	6535	3316	5106	1635
	methyl phenol	108	20.45	2278	2560	694	468	662	306
	C2 phenol	122	21.23	2017	3190	490	596	346	311

	C3 phenol	136	20.60	312	540	-	-	-	-
	C4 phenol	150	21.61	-	-	-	-	-	-
	phenol, 2-methoxy-	124	18.22	1794	2104	953	415	1047	299
	phenol, 2-methoxy-4-methyl-	138	19.23	771	819	495	180	529	92
	phenol, 4-ethyl-2-methoxy-	152	19.96	586	1014	-	-	-	-
anhydrosugars	1,4:3,6-dianhydro-alfa-d-glucopyranose	69	23.40	-	-	-	-	-	-
organic acids	formic acid-methyl ester	60	1.875	7143	1598	10103	1345	8275	788
	acetic acid-methyl ester	74	2.187	19549	4089	7614	1339	6996	189
	butanoic acid methyl ester	88	2.816	416	94	-	-	-	-
	Propanoic acid, 2-methyl-, methyl ester	87	3.000	-	-	-	-	-	-
	2-hydroxy-propanoic acid-methyl ester	45	11.24	-	-	-	-	-	-
	Butanoic acid, methyl ester	74	3.997	-	-	-	-	-	-
	Pentanoic acid, methyl ester	87	6.447	-	-	-	-	-	-
	Pentanoic acid, 3-methyl-, methyl ester	87	8.796	-	-	-	-	-	-
	Heptanoic acid, methyl ester	87	10.68	-	-	-	-	-	-
	Octanoic acid, methyl ester	87	12.30	-	-	-	-	-	-
	benzoic acid, methyl ester	136	15.52	1037	951	-	-	-	-
	Methyl benzoic acid, methyl ester	150	17.12	190	330	-	-	-	-
alcohols	benzyl alcohol	82	12.49	-	-	-	-	-	-
	2-furanmethanol	98	15.92	217	376	-	-	-	-
	cyclohexanol	108	18.38	662	260	902	469	1378	597
nitrogen compounds	carbamic acid, methyl ester	75	15.60	6010	1307	4715	2669	3952	345
	benzonitrile	103	15.35	1045	527	642	97	816	186
low molecular weight PAHs	naphtalene	128	16.95	977	1294	269	138	161	147
	Naphthalene, 1-methyl-	142	18.58	229	397	-	-	-	-
indane/benzofuran derivatives	indane	117	12.00	-	-	-	-	-	-
	benzofurane	132	13.90	594	401	125	217	-	-
	benzofuran, 2,3-dihydro-	132	19.91	27629	44867	3395	5096	2790	3734
	indan, 1-methyl-	146	16.54	-	-	-	-	-	-
	Benzofuran, 2-methyl-	118	14.03	219	380	-	-	-	-
	1-indanone	120	23.30	687	1190	170	197	-	-
	C2 indane	132	15.18	-	-	-	-	-	-
	benzofuran, 4,7-dimethyl-	146	16.19	-	-	-	-	-	-
TOTAL VOCs	-	-	-	287870	88827	114339	38378	88355	12579

Table S3: VOC quantities (NA) released by CS thermosequence biochars at ambient temperatures (25 and 50°C).

	H/C	**0.8**		**0.71**		**0.59**		**0.49**		**0.44**		**0.36**		**0.32**	
	Sampling temperature	**25°C**	**50°C**	**25°C**	**50°C**	**25°C**	**50°C**	**25°C**	**50°C**	**25°C**	**50°C**	**25°C**	**50°C**	**25°C**	**50°C**
Compounds classes	Compound	NA													
alkyl amides	formamide	-	5288	-	-	-	-	-	-	-	-	-	-	-	-
	acetamide	639	1888	-	-	-	-	-	-	-	-	-	-	-	-
aldehydes and ketones	propanal	101173	301871	18918	30320	-	-	-	-	-	-	-	-	-	-
	butanal	-	63000	-	-	-	-	-	-	-	-	-	-	-	-
	2-butenal-3-methyl-	-	364	-	-	-	-	-	-	-	-	-	-	-	-
	2-butanone	-	37107	-	-	-	-	-	-	-	-	-	-	-	-
	benzaldehyde	560	957	-	-	-	-	-	-	-	-	-	-	-	-
	furfural	425	2737	-	-	-	-	-	-	-	-	-	-	-	-
furans	3-methyl-furan	-	6127	-	-	-	-	-	-	-	-	-	-	-	-
cyclopentenones	2-cyclopenten-1-one	373	2533	-	-	-	-	-	-	-	-	-	-	-	-
	2-cyclopenten-1-one-2-methyl-	-	597	-	-	-	-	-	-	-	-	-	-	-	-
	2-cyclopenten-1-one, 2,3-dimethyl-	-	122	-	-	-	-	-	-	-	-	-	-	-	-
lactones	butyrolactone	549	1682	-	2210	790	-	-	-	-	-	-	-	-	-
	2(5H)-furanone, 3-methyl-	-	192	-	-	-	-	-	-	-	-	-	-	-	-
phenols	phenol	5858	4460	7885	13659	5410	-	-	-	-	-	-	-	-	-
	methyl phenol	223	122	-	-	-	-	-	-	-	-	-	-	-	-
	phenol, 2-methoxy-	-	169	-	-	-	-	-	-	-	-	-	-	-	-
organic acids	formic acid-methyl ester	57755	291146	17593	10535	11650	7118	24241	35556	-	6772	-	3519	-	-
	acetic acid-methyl ester	15365	399609	2615	36836	-	-	-	8891	-	914	-	-	-	-
	propanoic acid-methyl ester	-	34717	-	-	-	-	-	-	-	-	-	-	-	-
alcohols	cyclohexanol	1001	374	-	-	-	-	-	-	-	-	-	-	-	-
Total VOCS	-	183922	1155061	47011	93561	17850	7118	24241	44447	-	7686	-	3519	-	-

Table S4: Quantitative HS-SPME analysis of VOCs in SM500 biochars. Average quantities and SD were expressed as normalised areas (NA), (n=3), m/z quantitation ion.

Compound classes	Compound name	m/z	r.t	NA	SD
alkyl amides	formamide	45	17.42	53500	19702
	formamide, N-methyl-	59	15.84	10440	3236
	acetamide	59	17.18	26811	4231
	acetamide, N-methyl-	73	15.76	3127	1542
	acetamide, N,N-dimethyl-	87	17.05	2781	818
nitriles	methyl isocyanide	41	4.866	592347	39667
	propanenitrile	54	5.399	19929	2097
	butanenitrile	41	6.861	7803	870
	isobutyronitrile	68	4.354	1428	181
	benzonitrile	103	15.35	18202	3178
	methyl-benzonitrile	117	16.44	1340	348
aldehydes and ketons	2-propenal, 2-methyl-	70	2.571	22615	4041
	butanal	72	2.531	16085	943
	hexanal	82	6.366	1131	261
	2-butenal	70	5.375	28900	2104
	2-butenal, 2-methyl-, (E)-	84	6.692	12250	2573
	2-butenal, 3-methyl-	84	9.106	19458	4058
	2-pentenal, 2-methyl-	98	8.376	1004	237
	benzaldehyde	106	14.29	169170	34038
	benzaldehyde, 3-methyl-	120	15.89	7498	1404
	3-furaldehyde	96	13.00	7738	1707
	2-furancarboxaldehyde, 5-methyl-	110	13.55	3175	777
	2,5-furandicarboxaldehyde	124	14.81	293	128
	3-thiophenecarboxaldehyde	111	16.43	1660	434
	methyl vinyl ketone	70	3.601	4937	822
	2-butanone	72	2.765	87877	5783
	3-penten-2-one, (E)-	84	7.526	7234	1806
	2-pentanone	86	3.837	13739	3162
	2,3-butanedione	86	3.920	8260	401
	methyl isobutyl ketone	100	4.424	2250	234
	2,3-pentanedione	100	5.950	1231	135
	2-hexanone	100	5.600	6112	400
	cyclopentanone	84	8.708	3292	78
	cyclohexanone	98	11.02	8330	384
aromatics	benzene	78	3.453	93278	4048
	toluene	91	5.245	1276935	58050
	C2 benzene	106	7.668	26170	3440
	benzene, methoxy-	108	11.64	2800	774
	C3 benzene	120	10.55	10250	988
	C4 benzene	134	11.30	1412	170
	C5 benzene	162	13.51	379	43
	benzene, 1-ethyl-4-methoxy-	136	13.90	1376	370
furans	furan, 2-methyl-	82	2.481	15300	515
	vinylfuran	94	12.78	2990	684
	furan, 2,5-dimethyl-	96	3.439	13787	487
	furan, 2-methoxy-	98	8.023	5368	934
	furan, 2,3,5-trimethyl-	110	5.727	5408	227
cyclopentenones	2-cyclopenten-1-one	82	15.64	25710	5519
	2-cyclopenten-1-one, 2-methyl	96	12.05	6426	1654
	2-cyclopentene-1,4-dione	96	15.13	1528	588
	2-cyclopenten-1-one, 2,3-dimethyl	110	16.71	1213	420
lactones	4-methyl-5H-furan-2-one	98	16.74	12601	2340
	2-furanone, 2,5-dihydro-3,5-dimethyl	112	16.19	3271	502
phenols	phenol	94	19.69	547040	49144
	methyl phenol	108	19.66	75519	10745
	C2 phenol	122	21.33	8457	1431
	C3 phenol	136	22.10	947	243

	phenol, 2-methoxy-	124	18.23	717	351
alcohols	cyclohexanol	82	12.47	5220	459
	benzyl Alcohol	108	18.39	578	226
heterocyclic compounds	pyridine	79	8.752	20934	7880
	pyrazine	80	9.343	754	124
	pyridine, 3-methyl-	93	9.414	15946	3487
	pyridine, 2,3-dimethyl-	107	10.03	3494	955
	thiophene	84	4.817	3086	338
	thiazole	85	10.05	5218	1144
	thiophene, 3-methyl-	97	7.221	3260	710
	1,3-benzodioxole	121	14.43	5524	1398
	1,3-benzodioxole, 5-methyl-	135	15.46	1278	354
	benzofurane	118	14.03	8082	1374
	benzoxazole	119	15.84	7265	1125
other compounds	indane	117	12.01	2996	255
	naphtalene	128	16.96	2208	357
	carbamic acid, methyl ester	75	15.59	41018	15538
	cyclohexene	82	2.075	5029	189
Total VOCs	-	-	-	3443760	236006

Table S5: BC volatile and semi-volatile WSOCs by DI-SPME-GC-MS. Average NA are reported with standard deviation (SD), (n=3), m/z quantitation ion, r.t. retention time (min)

			BC350	BC350	BC400	BC400	BC450	BC450	BC500	BC500	BC550	BC550	BC600	BC600	BC650	BC650
r.t.	m/z	Compound	*Mcounts*	*SD*	*Mcounts*	*SD*	*Mcounts*	*SD*	*Mcounts*	*SD*	*Mcounts*	*SD*	*Mcounts*	*SD*	*Mcounts*	*SD*
14.18	46	formic acid	29	25	31	27	44	42	27	46	56	61	16	28	38	35
13.29	60	acetic acid	1104	770	903	474	600	454	80	19	92	91	14	17	18	8.3
15.49	60	butanoic acid	1766	1184	1190	653	1129	741	175	13	120	57	0	0	6.1	11
15.97	60	methyl butanoic acid	407	285	237	131	217	131	45	7.5	42	3.9	0	0	0	0
16.67	60	pentanoic acid	1262	921	1246	633	1288	853	182	17	140	33	0	0	0	0
17.37	60	methyl pentanoic acid	240	174	176	75	123	77	25	8.7	90	104	0	0	0	0
17.95	60	hexanoic acid	1005	782	1345	509	1471	1060	191	55	551	603	22	28	12	8.7
18.36	74	methyl hexanoic acid	43	28	82	14	73	31	9.4	9.0	19	21	0	0	0	0
19.07	60	heptanoic acid	758	619	1133	266	761	415	59	88	224	171	0	0	0	0
20.15	60	octanoic acid	395	320	452	62	221	167	15	27	79	107	18	25	4.4	7.7
21.19	60	nonanoic acid	24	39	28	49	18	18	3.2	5.6	2.9	5.0	1.9	3.3	3.8	6.6
22.33	60	decanoic acid	4.5	7.8	4.2	7.2	0	0	0	0	0	0	0	0	0	0
24.15	60	dodecanoic acid	4.8	8.3	7.4	12.8	5.7	9.9	0	0	0	0	0	0	0	0
14.40	74	propanoic acid	623	416	467	261	411	317	58	12	44	33	1.2	2.1	2.9	5.0
16.23	86	2-propenoic acid, 2-methyl-	28	20	12	5.3	11	8.5	0.67	1.2	0	0	0	0	0	0
17.22	86	2-butenoic acid	115	91	37	22	23	25	1.7	2.9	0	0	0	0	0	0
18.01	100	2-butenoic acid, 3-methyl-	51	26	49	19	22	23	0	0	0	0	0	0	0	0
18.30	100	2-pentenoic acid	66	54	15	6.4	0	0	0	0	0	0	0	0	0	0
18.78	114	2-pentenoic acid, 2-methyl-	39	43	16	15	1.4	2.5	0.74	1.3	0	0	0	0	0	0
19.75	68	3-heptenoic acid	34	32	10	18	0	0	0	0	0	0	0	0	0	0
23.67	122	benzoic acid	260	225	187	28	400	300	60	74	23	40	3.2	5.6	2.3	3.9
24.49	136	benzoic acid, 3-methyl-	169	92	179	67	369	213	44	76	16	27	1.4	2.4	0	0
24.84	150	benzoic acid, 3,5-dimethyl-	25	24	21	26	29	25	8.4	15	2.6	4.6	0	0	0	0
14.05	110	ethanone, 1-(2-furanyl)-	7.0	5.4	2.4	2.5	1.2	2.1	0.92	1.6	0	0	0	0	0	0
12.11	96	2-cyclopenten-1-one, 3-methyl-	11	7.2	0	0	0	0	0	0	0	0	0	0	0	0
14.52	110	2-cyclopenten-1-one, 2,3-dimethyl-	7.8	6.5	1.1	2	0.51	0.89	0	0	0	0	0	0	0	0
13.88	124	2-cyclopenten-1-one, 2,3,4-trimethyl-	2.8	3.2	0	0	0	0	0	0	0	0	0	0	0	0
13.76	96	furfural	62	45	19	21	12	21	1.0	1.8	0.67	1.2	4.6	8.0	2.2	3.8
14.98	110	2-furancarboxaldehyde, 5-methyl-	13	11	4.2	7.2	3.5	3.4	0	0	0	0	0	0	0	0
14.26	106	benzaldehyde	50	37	27	23	15	13	0	0	0	0	0	0	0	0
16.22	122	benzaldehyde, 2-hydroxy-	8.2	8.6	16	9.9	24	21	0	0	0	0	0	0	0	0
19.70	94	phenol	86	46	84	33	148	63	61	7.9	39	21	21	17	21	14
20.44	108	methyl phenol	11	8.5	10	4.7	11	2.2	3.0	0.93	2.3	2.9	1.8	2.3	1.8	1.6
21.32	122	C2 phenol	6.3	7.5	6.2	3.2	6.1	3.6	1.3	0.69	1.4	2.5	0.89	1.5	0.48	0.83
18.22	124	phenol, 2-methoxy-	17	15	4.6	5.4	5.2	4.7	3.1	3.5	0	0	0	0	0	0
19.22	138	phenol, 2-methoxy-4-methyl-	5.2	5.7	3.3	4.1	3.8	4.3	2.3	2.5	0	0	0	0	0	0

Table S6: OL volatile and semi-volatile WSOCs by DI-SPME-GC-MS. Average NA are reported (n=2), m/z quantitation ion, r.t. retention time (min)

			OL350	OL400	OL450	OL500	OL550	OL600	OL650
m/z	r.t	Compound	*Mcounts*	*Mcounts*	*Mcounts*	*Mcounts*	*Mcounts*	*Mcounts*	*Mcounts*
46	14.52	formic acid	48	63	89	41	53	57	12
60	1.86	formic acid, methyl ester	49	45	64	20	49	50	12
60	13.59	acetic acid	1477	1494	1893	1182	1549	1890	1186
74	2.27	acetic acid, methyl ester	15	38	40	17	39	33	11
74	14.79	propanoic acid	157	160	194	119	161	203	111
88	3.02	propanoic acid, methyl ester	10	6.8	13	2.7	5.2	4.9	1.0
72	16.20	2-propenoic acid	26	35	53	32	50	69	47
55	3.58	2-propenoic acid, methyl ester	10	12	47	6.7	24	20	0
69	7.60	2-butenoic acid, methyl ester,	40	36	54	27	22	21	22
114	9.56	2-Pentenoic acid, methyl ester,	1.8	1.7	2.1	6.4	1.1	1.0	4.8
122	15.88	benzoic acid	197	201	180	187	186	0	0
136	15.91	benzoic acid, methyl ester	145	126	148	115	127	121	111
166	20.27	benzoic acid, 3-methoxy-, methyl ester	116	108	144	117	71	78	35
168	23.37	benzoic acid, 3-hydroxy-4-methoxy-	524	609	669	485	155	0	0
148	20.14	cinnamic acid	0	0	113	522	1315	985	826
162	24.57	4-methylcinnamic acid	96	136	199	190	174	235	164
53	4.72	2-propenenitrile	0	0	12	6.6	16	15	18
67	8.72	2-butenenitrile	0.91	2.0	3.5	4.3	5.5	7.1	44
54	5.64	propanenitrile	4.2	6.4	16	15	18	17	23
103	15.74	benzonitrile	34	78	154	190	282	332	327
117	16.83	benzonitrile, 3-methyl-	0	55	114	110	120	102	125
117	19.39	benzyl nitrile	0	82	0	155	190	274	277
79	9.79	pyridine	25	28	29	70	35	0	162
93	10.29	pyridine, 2-methyl-	3.7	6.9	13	17	13	0	73
105	13.19	pyridine, 2-ethenyl-	0	3.3	0	0	0	14	49
80	9.99	pyrazine	0	31	0	0	0	0	62
94	10.90	pyrazine, methyl-	46	55	0	76	104	65	147
107	11.94	pyrazine, ethyl-	0	0	0	20	0	0	26
129	19.54	quinoline	48	61	79	113	123	227	233
143	20.74	quinoline, 6-methyl-	30	45	80	109	98	122	148
130	24.61	indole, 4-methyl-	131	147	221	233	140	230	127
145	25.26	indole, 2,3-dimethyl-	30	33	0	97	58	103	80
56	2.46	2-propenal	4.6	7.9	15	6.6	62	105	116
70	5.98	2-butenal	435	414	521	407	478	377	420
84	7.26	2-butenal, 2-methyl-	8.3	10	18	13	17	19	23
72	2.94	2-butanone	32	42	26	23	54	47	18
100	14.73	3-pentanone, 2-methyl-	0	0	0	41	47	49	38
84	8.13	3-penten-2-one	28	29	36	36	54	59	71
69	9.48	4-hexen-3-one	16	20	28	139	49	50	291
86	4.38	2,3-butanedione	52	57	75	63	75	74	80
100	6.57	2,3-pentanedione	25	26	34	27	31	28	34
114	8.34	3,4-hexanedione	1.9	1.6	2.2	1.6	1.6	1.5	1.4
74	11.59	2-propanone, 1-hydroxy-	164	173	267	139	225	280	153
88	12.66	2-butanone, 1-hydroxy-	44	38	53	30	40	54	29
82	12.38	2-cyclopenten-1-one	364	434	507	456	577	649	651
96	12.52	2-cyclopenten-1-one, 2-methyl-	208	289	323	314	396	436	472
110	14.92	2-cyclopenten-1-one, 2,3-dimethyl-	154	242	314	267	305	385	369
124	14.31	2-cyclopenten-1-one, 2,3,4-trimethyl-	40	68	86	83	89	81	100
98	17.63	2-cyclopenten-1-one, 2-hydroxy-	149	160	200	93	111	129	53
112	18.26	2-cyclopenten-1-one, 2-hydroxy-3-methyl-	227	268	369	241	256	361	253
126	18.95	2-cyclopenten-1-one, 2-hydroxy-3-ethyl-	116	133	172	105	117	123	112
96	15.53	2-cyclopentene-1,4-dione	324	381	439	416	464	520	466
110	18.44	2-cyclohexene-1,4-dione	282	311	96	82	265	345	86
124	17.24	2-cyclohexen-1-one, 4,4-dimethyl-	503	460	520	407	383	417	380
82	10.07	furan, 2-methyl-	85	51	3.6	14	12	0	0
96	11.42	furan, 2,5-dimethyl-	21	26	33	50	134	151	187
94	13.22	vinylfuran	67	80	89	84	111	144	189
110	14.47	ethanone, 1-(2-furanyl)-	690	698	733	637	705	788	814
124	15.31	1-propanone, 1-(2-furanyl)-	0	0	166	0	0	128	0
98	16.33	2-furanmethanol	572	539	597	453	513	600	478

140	14.81	2-furanmethanol, acetate	65	53	56	42	43	45	32
112	10.92	furan, 2-(methoxymethyl)-	40	29	39	26	21	20	12
126	15.38	3-furancarboxylic acid, methyl ester	285	252	263	212	212	230	216
96	13.96	furfural	4341	4475	4764	4415	4308	4712	4372
110	14.36	2-furancarboxaldehyde, 5-methyl-	1249	1344	1439	1352	1360	1535	1482
84	17.59	2(5H)-furanone	112	127	183	137	106	100	47
84	9.29	cyclopentanone	4.7	5.8	7.6	29	6.9	0	44
86	16.16	butyrolactone	42	43	56	35	52	69	0
106	14.69	benzaldehyde	195	222	283	274	363	364	341
120	15.96	benzaldehyde, 4-methyl-	80	84	115	121	155	173	139
122	16.63	benzaldehyde, 2-hydroxy-	157	181	241	375	762	862	815
136	17.02	benzaldehyde, 2-hydroxy-4-methyl-	62	59	85	161	345	391	236
148	23.07	1,4-benzenedicarboxaldehyde, 2-methyl-	228	194	223	182	95	149	88
120	16.29	acetophenone	78	178	123	150	171	208	332
134	14.50	acetophenone, 3-methyl-	0	0	145	0	186	215	182
121	18.04	acetophenone, 2-hydroxy-	0	0	307	314	0	0	342
105	17.13	1-propanone, 1-phenyl-	173	176	0	0	276	311	232
160	24.16	2H-1-benzopyran-2-one, 3-methyl-	114	108	123	104	72	121	60
94	20.08	phenol	1713	2001	2280	2269	2400	2652	2742
108	20.85	phenol, 4-methyl-	508	866	989	1027	1094	1350	1408
122	21.72	phenol, 4-ethyl-	1174	1294	1550	1411	1471	1755	1693
136	21.62	phenol, 2-ethyl-5-methyl-	385	338	535	512	613	940	707
150	18.81	phenol, 2-ethyl-4,5-dimethyl-	71	88	0	0	312	412	239
134	23.55	phenol, 4-(2-propenyl)-	221	207	275	267	437	674	488
148	24.64	2-allyl-4-methylphenol	0	51	228	86	159	235	167
124	18.62	phenol, 2-methoxy-	1940	1995	2299	1741	1234	1011	466
138	19.63	phenol, 2-methoxy-4-methyl-	976	1129	1302	995	570	422	173
152	20.37	phenol, 2-methoxy-4-ethyl-	1725	1719	1827	1420	816	633	227
166	21.13	phenol, 2-methoxy-4-propyl-	250	239	306	243	98	80	18
150	21.97	2-methoxy-4-vinylphenol	5500	5549	5781	5095	3609	3513	1236
154	22.61	phenol, 2,6-dimethoxy-	1504	1577	1699	1280	644	505	156
194	26.46	phenol, 2,6-dimethoxy-4-(2-propenyl)-	2346	2149	2249	1434	355	249	38
182	23.84	5-tert-butylpyrogallol	735	700	744	515	145	117	23
78	3.46	benzene	3.2	4.3	12	17	26	32	10
91	5.81	toluene	71	107	252	266	241	241	153
106	8.95	benzene, 1,4-dimethyl-	3.4	5.1	10	9.4	15	18	20
120	10.84	benzene, 1,2,4-trimethyl-	7.3	13	28	25	23	26	14
148	23.96	benzene, pentamethyl-	0	0	0	90	155	205	148
104	10.51	styrene	74	75	125	111	119	94	79
108	12.04	benzene, methoxy-	66	88	67	62	43	39	33
122	13.47	benzene, 1-methoxy-4-methyl-	256	331	443	335	286	284	132
136	14.55	benzene, 1-methoxy-4-ethyl-	111	119	143	108	91	93	0
134	16.57	benzene, 1-methoxy-4-ethenyl-	859	586	750	596	465	520	190
152	18.51	3,5-dimethoxytoluene	62	215	105	218	107	117	57
116	14.09	indene	70	147	66	197	279	112	164
130	15.61	2-methylindene	24	39	44	64	77	84	42
117	12.22	indane	10	24	46	42	34	39	25
128	17.43	naphthalene	74	92	221	382	724	1046	537
142	18.59	naphthalene, 1-methyl-	29	48	133	203	267	340	179
166	23.35	fluorene	0	0	0	0	60	89	37
118	14.41	benzofuran	102	114	184	196	257	254	185
132	15.56	benzofuran, 2-methyl-	118	158	270	221	271	312	170
146	16.88	benzofuran, 4,7-dimethyl-	37	65	121	102	66	136	86
144	18.47	benzofuran, 2-ethenyl-	0	0	34	0	105	149	79
120	23.67	benzofuran, 2,3-dihydro-	7357	7138	6970	7077	5801	8223	6498
168	22.69	dibenzofuran	0	0	0	38	49	74	35
154	20.01	biphenyl	0	0	0	35	23	51	40
132	20.33	1-indanone	511	620	719	677	694	938	854
146	20.44	1-indanone-7-methyl	335	363	433	401	387	504	364
160	21.28	1-indanone, 3,3-dimethyl-	117	120	167	160	148	219	110
148	21.52	1-indanone-7-hydroxy	214	216	259	236	228	227	277
144	28.77	1-naphthalenol	30	60	89	87	86	135	81
172	28.00	1-naphthol, 6,7-dimethyl-	8	10	21	20	25	39	22
158	26.93	1-naphthalenol, 2-methyl-	46	50	80	79	72	150	74

Table S7: Concentrations of VFA in biochar and bio-oil WSOCs. Values are expressed as µg/g biochar, with SD (n=3), m/z quantitation ion, r.t. retention time (min)

	BIOCHAR WSOCs													
	BC350		BC400		BC450		BC500		BC550		BC600		BC650	
	(µg/g)	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*
acetic acid	2459	173	1973	564	1135	601	208	16	196	129	20	15	33	12
propanoic acid	439	92	312	82	243	167	47	11	31	12	0.40	0.69	1.8	3.2
butanoic acid	189	30	122	26	104	51	23	7.4	13	2.6	0	0	0.59	1.0
methyl butanoic acid	35	5.4	20	4.5	17	6.9	4.8	2.0	4.4	1.9	0	0	0	0
pentanoic acid	46	5.5	45	7.9	41	13	8.0	2.1	5.7	0.86	0	0	0	0
	BIO-OIL WSOCs													
	OL350		OL400		OL450		OL500		OL550		OL600		OL650	
	(µg/g)	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*	*(µg/g)*	*SD*
acetic acid	7065	773	8089	95	7931	2151	6434	484	7200	312	6683	2016	5314	343
propanoic acid	211	30	256	8.9	237	57	187	18	233	36	204	63	151	3.9
butanoic acid	8.9	0.84	12	2.4	11	2.2	9.5	1.71	13	3.80	13	3.3	11	0.39

Table S8: Main classes of compounds identified in OL WSOCs by ESI(-)FT-ICR-MS. The number of peaks assigned with a molecular formula are reported with their percent abundance relative to all the peaks in the mass spectra (%R.A.)

	OL350		OL400		OL450		OL500		OL550		OL600		OL650	
Group	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A
O_x	1133	55	1004	40	1027	53	1012	45	1026	45	932	37	917	36
N_xO_y	1210	11	1288	12	1452	19	1548	19	1733	26	1775	28	1947	35
O_xS_y	130	1.1	100	0.79	147	1.2	109	1.5	184	2.6	115	0.91	118	1.1
$N_xO_yS_z$	68	0.27	120	0.47	97	0.38	92	0.34	140	0.47	164	0.59	179	0.69
Isotopic peaks	726	8	674	6	748	8	711	7	815	8	722	6.999	764	7
Total identified	3267	76	3186	60	3471	81	3472	73	3898	83	3708	74	3925	80

Table S9: Average errors (ppb) in the molecular formula assignment of the compound classes identified in OL WSOCs by ESI(-)FT-ICR-MS.

Group	OL350	OL400	OL450	OL500	OL550	OL600	OL650
Ox	8	5	12	17	19	11	4
NxOy	7	3	8	7	9	3	4
OxSy	3	0.2	5	1	3	1	5
NxOySz	3	9	9	11	8	15	5
Ox13Cy	8	5	10	11	11	7	1
NxOy13Cz	10	3	11	5	4	1	6
OxSy13Cz	11	0.5	18	0	6	23	7

Table S10: Main classes of compounds identified in BC WSOCs by ESI(-)FT-ICR-MS and percent abundance relative to all the peaks in the mass spectra (%R.A.)

Biochar	BC350		BC400		BC450		BC500		BC550		BC600		BC650	
Group	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A	N° Peaks	% R.A
Ox	993	13	924	12	161	1.8	122	2.5	2	2	106	1.3	30	0.20
NxOy	874	5.6	511	2.4	17	0.92	5	0.046	11	0.44	17	0.59	n.d	n.d
OxSy	33	0.17	8	0.051	14	0.29	12	0.37	8	0.18	9	0.14	4	0.051
Isotopic peaks	307	1.7	276	1.4	22	0.39	42	0.49	27	0.30	32	0.32	6.0	0.023
Total identified	2207	21	1719	16	214	3	181	3	150	3	164	2	40	0.28

Table S11: Average errors (ppb) in the molecular formula assignment of the compound classes identified in BC WSOC with ESI(-)FT-ICR-MS

Group	BC350	BC400	BC450	BC500	BC550	BC600	BC650
Ox	0.3	1	6	5	7	1	10
NxOy	3	5	6	23	8	14	n.d
OxSy	9	7	12	5	23	6	9
Ox13Cy	1	1	22	1	1	5	1
NxOy13Cz	4	17	24	n.d	n.d	4	n.d

Table S12: Score loading results for 4 component PARAFAC modelling of BC and OL WSOCs, and standard solutions. Average values are reported with standard deviations (SD), (n=2)

Samples	***C1***		***C2***		***C3***		***C4***		***TOTAL INTENSITY***	
	Loading ($x10^6$)	SD	Loading ($x10^6$)	SD	Loading ($x10^6$)	SD	Loading ($x10^6$)	SD	Loading ($x10^6$)	SD
Bio-oils WSOCs										
OL350	28903	313	20966	6169	40251	4021	8787	4894	98907	2433
OL400	32086	1927	25097	8559	29622	1512	8864	4500	95669	4473
OL450	33899	562	26113	7833	28621	2706	12726	6894	101358	1206
OL500	39262	3995	28352	5244	26515	5610	11507	7343	105637	11703
OL550	40978	3690	25810	5076	25426	5081	13705	8598	105920	12293
OL600	40839	3887	27136	5200	24287	4980	13811	8718	106073	12385
OL650	37431	1552	23368	7573	24009	**1663**	14733	7620	99541	157
Biochar WSOCs										
BC350	2261	98	1831	595	282	20	4.5	6	4378	666
BC400	3744	162	3031	985	467	34	7.5	10	7250	1103
BC450	3405	593	1374	157	263	76	4.8	0.17	5047	511
BC500	1393	82	373	85	47	8.5	2.3	0.36	1815	5.7
BC550	908	269	157	1.9	20	8.6	4.7	6.6	1089	286
BC600	50	20	10	6.4	0.36	0.085	0.59	0.02	60	26
BC650	33	26	5.9	5.8	0.090	0.13	0.76	0.18	39	32
Standard solutions										
16 EPA PAHs	138	138	52	43	97	102	98	119	385	403
IHSS fulvic acid	2908	1433	7231	5243	45	16	133	2.0	10316	6690
o-cresol	143	202	41	59	1471	2080	61100	14022	62755	16363
o-eugenol	11	4.3	14	8.9	166	45	369	74	560	16

*Table S13:Mobile compounds **ALT**, **AHT** and **PYR** biochars. Normalised area (NA) of Water Soluble Organic Compounds (WSOC) sorted by functional groups were reported (triplicate analysis) with standard deviation (SD), retention time (r.t) and characteristic ions (m/z). VOCs directly released from biochars were qualitatively investigated at 25 °C ('V' = detected, '-'= not detected).*

r.t.	m/z	compound	ALT				AHT				PYR			
			Average	SD	VOC 150°C	VOC 25°C	**Average**	SD	VOC 150°C	VOC 25°C	**Average**	SD	VOC 150°C	VOC 25°C
1.86	72	2-butanone	**142**	27	V	-	**691**	219	V	V	**4.1**	1.9	V	-
4.48	84	2-butenal, 2-methyl-	**34**	16	V	-	**46**	12	V	-	**-**	**-**	-	-
2.77	86	2-pentanone	**14**	4.7	V	-	**118**	31	V	V	**-**	**-**	V	-
4.21	100	2-hexanone	**-**	-	-	-	**15**	2.6	V	-	**-**	**-**	-	-
5.81	114	2-hexanone, 5-methyl-	**4.6**	1.6	V	-	**4.9**	0.8	V	-	**-**	**-**	-	-
6.73	128	2-heptanone, 6-methyl-	**2.2**	0.93	V	-	**4.4**	0.69	V	-	**-**	**-**	-	-
8.65	126	5-hepten-2-one, 6-methyl-	**19**	10	V	-	**-**	-	-	-	**-**	**-**	-	-
9.51	142	2-nonanone	**17**	18	V	-	**-**	-	V	-	**-**	**-**	-	-
11.16	96	furfural	**1487**	595	V	V	**254**	72	V	V	**0.87**	0.76	-	-
11.89	110	ethanone, 1-(2-furanyl)-	**36**	12	V	V	**34**	10	V	V	**-**	**-**	-	-
12.30	140	2-furanmethanol, acetate	**15**	7.2	V	V	**-**	-	-	-	**-**	**-**	-	-
13.09	110	2-furancarboxaldehyde, 5-methyl-	**567**	223	V	V	**73**	22	V	V	**-**	**-**	-	-
9.37	96	2-cyclopenten-1-one, 2-methyl-	**-**	**-**	-	-	**39**	13	V	V	**-**	**-**	-	-
12.45	110	2-cyclopenten-1-one, 2,3-dimethyl-	**-**	**-**	-	-	**24**	7.0	V	-	**-**	**-**	-	-
11.59	124	2-cyclopenten-1-one, 2,3,4-trimethyl-	**-**	**-**	-	-	**8.2**	2.5	-	-	**-**	**-**	-	-
12.11	106	benzaldehyde	**2956**	1487	V	V	**2186**	537	V	V	**-**	**-**	V	-
13.73	120	benzaldehyde, 2-methyl-	**73**	37	V	V	**15**	5.0	V	V	**-**	**-**	-	-
15.76	134	benzaldehyde, 4-ethyl-	**18**	10	V	V	**-**	-	-	-	**-**	**-**	-	-
14.81	122	benzaldehyde, 2-hydroxy-	**35**	18	V	V	**8.4**	2.6	V	-	**-**	**-**	-	-
26.89	152	benzaldehyde, 3-hydroxy-4-methoxy-	**33**	9.2	-	-	**-**	-	-	-	**-**	**-**	-	-
14.33	120	acetophenone	**158**	70	V	V	**72**	20	V	V	**-**	**-**	-	-
16.36	134	acetophenone, 4-methyl-	**42**	18	V	V	**-**	-	-	-	**-**	**-**	-	-
		aldehydes and ketones	**5652**	2528			**3592**	954			**4.9**	2.7		
19.69	94	phenol	**129**	30	V	V	**444**	136	V	V	**1.2**	0.52	V	-
20.76	108	methyl phenol	**68**	22	V	V	**388**	122	V	V	**1.8**	1.4	V	-
22.00	122	phenol, 4-ethyl-	**20**	7.0	V	-	**37**	13	V	V	**-**	**-**	**-**	-
21.43	136	phenol, 3-ethyl-5-methyl-	**-**	-	V	-	**6.1**	1.8	V	-	**-**	**-**	**-**	-
17.62	124	phenol, 2-methoxy-	**26**	8.4	V	-	**51**	17	V	V	**-**	**-**	**-**	-
19.02	138	phenol, 4-methoxy-3-methyl-	**20**	7.6	V	-	**35**	12	V	V	**-**	**-**	**-**	-
20.05	152	phenol, 4-ethyl-2-methoxy-	**-**	-	V	-	**16**	5.0	V	-	**-**	**-**	**-**	-
42.05	110	1,4-benzenediol	**-**	-	**-**	-	**-**	-	V	-	**-**	**-**	**-**	-
42.45	124	1,4-benzenediol, 2-methyl-	**-**	-	**-**	-	**-**	-	V	-	**-**	**-**	**-**	-
		phenols	**263**	75			**977**	306			**3.0**	1.9		
11.11	60	acetic acid	**169**	88	V	V	**56**	46	V	V	**6.5**	7.6	V	V
12.39	74	propanoic acid	**36**	10	V	V	**28**	12	V	V	**-**	**-**	-	-
13.85	60	butanoic acid	**84**	14	V	V	**45**	14	V	V	**-**	**-**	-	-
14.50	60	butanoic acid, 2-methyl-	**106**	13	V	V	**75**	26	V	V	**-**	**-**	-	-
16.23	86	2-butenoic acid	**33**	1.6	V	-	**14**	11	V	-	**-**	**-**	-	-
15.61	60	pentanoic acid	**49**	7.2	V	V	**49**	16	V	V	**-**	**-**	-	-
16.61	60	pentanoic acid, 4-methyl-	**23**	3.0	V	-	**19**	6.6	-	-	**-**	**-**	-	-
17.25	60	hexanoic acid	**198**	34	V	-	**107**	36	V	-	**-**	**-**	-	-
18.83	60	heptanoic acid	**123**	25	V	-	**115**	41	V	-	**-**	**-**	-	-
20.33	60	octanoic acid	**522**	178	V	-	**176**	60	V	-	**-**	**-**	-	-
21.77	60	nonanoic acid	**198**	82	V	-	**89**	29	V	-	**-**	**-**	-	-
23.15	60	decanoic acid	**496**	262	V	-	**35**	10	-	-	**-**	**-**	-	-
23.85	60	undecanoic acid	**69**	35	V	-	**4.3**	0.54	-	-	**-**	**-**	-	-
25.72	60	dodecanoic acid	**142**	87	V	-	**8.8**	1.6	-	-	**-**	**-**	-	-
37.39	60	tetradecanoic acid	-	-	V	-	-	-	-	-	**-**	**-**	-	-
38.21	60	pentadecanoic acid	-	-	V	-	-	-	-	-	**-**	**-**	-	-
40.49	60	hexadecanoic acid	-	-	V	-	-	-	-	-	**-**	**-**	-	-
25.18	122	benzenecarboxylic acid	**38**	3.6	V	-	**47**	19	V	-	**-**	**-**	-	-
26.58	136	benzeneacetic acid	**15**	4.0	V	-	**-**	-	-	-	**-**	**-**	-	-
27.39	150	benzenepropanoic acid	**150**	46	V	-	**15**	6.6	V	-	**-**	**-**	-	-
		organic acids	**2451**	884			**882**	315			**6.5**	7.6		
0.98	76	carbon disulfide	-	-	V	V	-	-	V	V	**-**	**-**	-	-
4.07	94	disulfide, dimethyl	**46**	24	V	-	**-**	-	-	-	**-**	**-**	-	-
4.74	97	thiophene, 2-methyl-	**11**	5.1	V	-	**8.4**	1.6	V	V	**-**	**-**	-	-
10.27	114	thiophene, 3-methoxy-	**4.7**	2.5	V	V	**-**	**-**	-	-	**-**	**-**	-	-

17.81	140	2-acetyl-5-methylthiophene	**38**	17	V	-	**-**	**-**	V	-	**-**	**-**	-	-
15.08	111	2-thiophenecarboxaldehyde	**141**	58	V	V	**29**	8.2	V	V	**-**	**-**	-	-
16.66	125	3-methyl-2-thiophenecarboxaldehyde	**455**	220	V	V	**14**	3.9	V	-	**-**	**-**	-	-
6.66	73	thiocyanic acid, methyl ester	**9.0**	4.0	V	-	**18**	5.3	-	-	-	-	-	-
7.32	85	thiazole	**28**	8.2	-	-	**25**	7.3	-	-	-	-	-	-
7.09	99	thiazole, 2-methyl-	**42**	13	V	V	**35**	9.3	-	-	**-**	**-**	-	-
8.53	113	thiazole, 2,5-dimethyl-	**20**	10	V	V	**18**	4.8	V	-	-	-	-	-
9.61	127	thiazole, 2,4,5-trimethyl-	**12**	4.8	-	-	**6.2**	1.4	V	-	-	-	-	-
10.56	141	thiazole, 5-ethyl-2,4-dimethyl-	**10**	3.8	-	-	**-**	-	-	-	-	-	-	-
14.27	127	2-acetylthiazole	**-**	-	-	-	**4.3**	1.3	V	-	-	-	-	-
		organosulfur compounds	**816**	368			**159**	43			**0**	0		
7.76	94	pyrazine, methyl-	**126**	49	V	V	**38**	8.9	V	V	-	-	-	-
8.79	108	pyrazine, 2,3-dimethyl-	**88**	17	V	-	**43**	11	V	-	-	-	-	-
9.80	121	pyrazine, 2-ethyl-6-methyl-	**27**	5.4	V	-	**25**	8.5	V	-	**-**	**-**	**-**	-
10.70	136	pyrazine, 3-ethyl-2,5-dimethyl-	**12**	4.1	V	-	**-**	-	-	-	-	-	-	-
25.43	117	indole	**15**	5.8	V	-	**10**	3.4	V	-	-	-	-	-
25.98	130	indole, 2-methyl-	**10**	3.7	V	-	**15**	4.4	V	-	-	-	-	-
4.89	43	butanenitrile, 3-methyl-	**22**	5.3	-	-	**180**	58	-	-	-	-	-	-
4.24	55	butanenitrile, 2-methyl-	**-**	-	-	-	**168**	57	-	-	-	-	-	-
6.60	81	3-butenenitrile, 3-methyl-	**-**	-	-	-	**15**	3.3	-	-	-	-	-	-
6.83	55	pentanenitrile, 4-methyl-	**5.1**	2.3	-	-	**207**	51	-	-	-	-	-	-
7.91	96	hexanenitrile	**-**	-	-	-	**3.6**	0.78	-	-	-	-	-	-
9.86	82	heptanonitrile	**-**	-	-	-	**9.0**	1.5	-	-	-	-	-	-
13.52	103	benzonitrile	**179**	89	V	V	**63**	14	V	V	-	-	-	-
18.63	117	benzyl nitrile	**95**	41	V	V	**97**	27	V	V	-	-	-	-
20.23	131	benzenepropanenitrile	**11**	4.6	V	-	**87**	29	V	V	-	-	-	-
23.51	128	1,2-benzenedicarbonitrile	**-**	-	-	-	**7.1**	2.6	V	-	-	-	-	-
9.71	93	2-furancarbonitrile	**37**	19	V	V	**13**	3.1	V	V	-	-	-	-
26.10	92	1H-pyrrole-2-carbonitrile	**20**	5.3	V	-	**39**	13	V	-	-	-	-	-
20.01	95	1H-pyrrole-2-carboxaldehyde	**393**	91	V	V	**-**	-	V	-	-	-	-	-
21.13	109	1H-pyrrole-2-carboxaldehyde, 1-methyl-	**203**	59	V	V	**-**	-	-	-	-	-	-	-
16.74	123	2-formyl-4,5-dimethyl-pyrrole	**37**	14	V	-	**-**	-	-	-	-	-	-	-
19.25	109	ethanone, 1-(1H-pyrrol-2-yl)-	**79**	21	V	V	**-**	**-**	V	V	-	-	-	-
21.30	45	formamide	**-**	-	-	-	**-**	-	V	V	-	-	-	-
21.05	59	acetamide	**-**	-	V	V	**-**	-	V	V	-	-	-	-
21.81	73	propanamide	**-**	-	-	-	**-**	-	V	V	-	-	-	-
10.54	73	formamide, N,N-dimethyl-	**-**	-	V	V	**-**	-	V	V	-	-	-	-
18.08	73	acetamide, N-methyl-	**-**	-	V	-	**-**	-	V	V	-	-	-	-
12.54	87	acetamide, N,N-dimethyl-	**-**	-	V	V	**-**	-	V	V	-	-	-	-
18.80	87	propanamide, N-methyl-	**-**	-	-	-	**-**	-	V	V	-	-	-	-
		nitrogen containing compounds	**1360**	426			**1036**	299			**0**	0		
2.22	78	benzene	**9.2**	3.9	V	-	**9.3**	2.6	V	V	-	-	V	-
3.58	91	toluene	**13**	5.0	V	-	**106**	15	V	V	-	-	V	-
4.97	91	benzene, ethyl	**4.9**	1.6	V	V	**3.0**	0.69	V	V	**-**	**-**	V	-
7.32	91	benzene, propyl-	**-**	-	V	V	**-**	-	V	V	-	-	-	-
9.24	120	benzene, trimethyl	**-**	-	-	-	**-**	-	-	-	-	-	V	-
9.85	91	benzene, butyl-	**-**	-	V	V	**-**	-	V	V	-	-	-	-
12.38	91	benzene, pentyl-	**-**	-	V	V	**-**	-	V	V	-	-	-	-
14.96	91	benzene, hexyl-	**-**	-	V	V	**-**	-	V	V	-	-	-	-
17.43	91	benzene, heptyl-	**-**	-	V	-	**-**	-	V	-	-	-	-	-
19.84	91	benzene, octyl-	**-**	-	V	-	**-**	-	V	-	-	-	-	-
11.29	117	indane	**-**	-	-	-	**-**	-	-	-	-	-	V	-
		monoaromatic hydrocarbons	**27**	10			**119**	17			**0**	0		
20.11	128	naphthalene	**-**	-	V	V	**-**	-	V	V	-	-	-	-
23.25	142	naphthalene, methyl	**-**	-	V	V	**-**	-	V	V	-	-	-	-
24.81	156	naphthalene, dimethyl-	**-**	-	V	-	**-**	-	V	-	-	-	-	-
27.68	170	naphthalene,trimethyl-	**-**	-	V	-	**-**	-	V	-	-	-	-	-
		LMW PAHs	**0**	0			**0**	0			**0**	0		
16.73	160	benzene, 1,3-dichloro-5-methyl-	**-**	-	-	-	**-**	-	V	V	-	-	-	-
22.05	194	benzene, 1,2,4-trichloro-3-methyl-	**-**	-	-	-	**-**	-	V	V	-	-	-	-
		chlorobenzenes												
		total	**10569**	4282			**6764**	1921			**14**	12		

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