Research Article

Pyrolytic biochars from sunflower seed shells, peanut shells and Spirulina algae: their potential as soil amendment and natural growth regulators

Mariana P. Silva[1,2] · María L. Nieva Lobos[3] · Roxana V. Piloni[3] · Diego Dusso[3] · María E. González Quijón[4] · Ana L. Scopel[1,2] · Elizabeth L. Moyano[3]

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Abstract
Several studies have shown that pyrolysis conditions and feedstocks are the key factors influencing biochar chemical and physical properties. The information on the nature of biochar is quite important, especially when this carbonaceous material is intended to be used as a potential soil amendment. In this study, we investigated the formation and characterisation of biochars produced from vacuum pyrolysis of sunflower seed shells (SSS), peanut shells (PS) and Spirulina algae (Sp) at 280 °C (for SSS, PS and Sp) and 350 °C (for PS). As a proxy to test the potential of each biochar as soil amendment, we assessed the germination and growth effects of the biochar water-extractable substances (BWES) at different concentrations (10; 7.5; 5; and 2.5% w/v) on *Lactuca sativa*. Results showed that the biochar from pyrolysis of PS at 280 °C would be the most suitable soil amendment, since its BWES did not affect germination and exhibited a remarkable growth-promoting effect (50–100%) on roots and stems of *L. sativa*.
In contrast, BWES from SSS, Sp and certain concentrations of PS produced at 350 °C inhibited growth of *Lactuca sativa*, and particularly BWES of Spirulina dramatically reduced germination, posing a risk for direct application as soil amendment. The presence of carbonyl derivatives in the BWES from PS may be linked to the stimulatory effects of this extract. Aromatics could be responsible for the germination and growth inhibition in the BWES of SSS, while nitrogen organic compounds would enhance the inhibitory effect in BWES from Sp.

Keywords Vacuum pyrolysis · Biochar water-extractable substances · Germination · Growth promotion · Phytotoxicity

1 Introduction

Industrial waste products may cause serious contamination problems, and alternative uses are continuously being explored in order to improve the efficiencies of the productive chain, reduce carbon and water footprints and increase overall sustainability [77].

The production of energy is one of the major forms of utilisation of these waste products [74], and a variety of biomass conversion technologies (hydrochemical, biochemical and thermochemical) are being implemented

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✉ Ana L. Scopel, scopel.ana@gmail.com; ✉ Elizabeth L. Moyano, lauramoy@fcq.unc.edu.ar | [1]Estación de Biología Sierras, Facultad de Agronomía-Delegación Punilla, Universidad de Buenos Aires, Casilda S/N, Huerta Grande, 5174 Córdoba, Argentina. [2]Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (INBA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina. [3]INFIQC, Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina. [4]Departamento de Ingeniería Química, Universidad de La Frontera, Temuco, Chile.

and used as a source of energy to feed the same production system. Thermochemical conversion is increasingly being applied in industrial processes since it offers a clean and highly efficient technology that is easy to adapt to current energy infrastructures [35]. Among the thermochemical processes, pyrolysis has emerged as a promising front-end processing technology on the path to renewable fuels. During pyrolysis, biomass is rapidly heated in the absence of oxygen at temperatures between 300 and 600 °C and converted into three main product categories: a solid biochar, non-condensable pyrolytic gases (e.g. CO, H_2, CO_2) and a liquid phase including an aqueous fraction and water insoluble oil [12]. In particular, vacuum pyrolysis produces high yields of bio-oils (main product) and a reactive biochar as a secondary product [65]. Such behaviour is due to the short residence time of the organic vapour generated in the reactor, which reduces the occurrence and intensity of secondary reactions [66].

From the industrialisation process of several agronomic oil crops, lignocellulosic wastes like sunflower and peanuts (seed shells and husks) can account for as much as 18–30% of the total biomass used in the process. Even considering the best scenario, in which the major industrial companies re-utilise these wastes for energy production, a residue—biochar—will be produced. This biochar may represent between 20 and 40% of the initial biomass, although magnitude will depend on initial feedstock and pre-treatments, the energy conversion process and set-up conditions [36, 51]. Algal feedstocks are increasingly being used as a source of bio-oils and bio-products and can be produced under a wide range of biochemical and thermochemical technologies [5, 10]. Pyrolysis of algae can lead to the production of 10–35% of a biochar enriched in several nutrients including minerals [10, 27, 61]. From the above, the production of biochar can be only predicted to augment as more activities will engage in the re-utilisation of waste products. Indeed, biochar has been proposed as a win–win technology to mitigate climate global change without affecting food security [32]. It has been the focus of increased research due to its multiplicity of beneficial applications [15, 23, 31, 52, 71, 79]. Recent reviews on the agronomic benefits and drawbacks of biochar applications, including the effects on plant productivity, nutrient cycling, microbial interactions and carbon fate in the soil, conclude that biochar may be regarded as a promissory solution to energy, carbon sequestration and ecosystem function [11, 14, 39, 40, 79]. However, authors also warn on the risks of extrapolating results of untested materials and unexplored environments. Thus, before future large-scale application of biochar becomes a common practice, biochar toxic effects and any other short- and long-term threats on biological organisms and their processes in the soil should be investigated in detail.

The most common negative effects reported for biochars relate to adsorbed compounds such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals [24, 57, 63]. Although the extent to which these compounds are present in enough concentration and/or are bioavailable in the soil is somewhat controversial [40], some studies have shown that they may interfere with biological signalling within the rhizosphere and affect the soil biota [71].

Volatile organic compounds (VOCs) and particularly other potentially toxic elements and small organic compounds can be of further importance, since most of them remain in the solids during biochar production [14, 79] and could be easily leached when biochar is incorporated to the soil due to precipitation, or even by the presence of the water vapour-saturated atmosphere that is often present in the soil matrix [11, 29, 39, 81]. Studies on the effects of these leachates in bioassays have yielded mixed results and have been shown to depend on feedstocks, the thermo-conversion processes involved and production parameters [3, 9, 18, 20, 50, 51]. Alburquerque et al. [4] found that biochar water extracts (10% w/v) from five lignocellulosic agricultural and forest wastes increased seed germination of sunflower relative to the controls, with calculated germination indexes above 60%, typical of non-phytotoxic materials. In a previous work [68] in which we tested biochars' water extracts from pyrolysed leaves and shoots of *Flourensia oolepis* using *Lactuca sativa* as a test system, we found an amazing growth-promoting effect on roots (225%) and shoots (160%), and null or not permanent phytotoxic effect on germination. Lou et al. [48] found that biochar water extracts from wheat and maize significantly increased the yield and positively affected other ecophysiological parameters in potted cabbage experiments and concluded they had a great potential to be applied as liquid amendment. Rogovska et al. [63] reported no effect on corn seed germination but a decrease in seedling growth, in three out of six biochar extracts of different feedstocks obtained at the higher temperature treatments. Extracts from high volatile matter charcoal of macadamia nut shell (430 °C) reduced germination of radish and corn seeds [20]. Buss and Mašek [14] found that leachates of biochar produced from softwood pellets (550 °C) with high levels of VOCs induced heavy toxicity to germination of *Lepidium sativum*, while no phytotoxicity was observed for low-VOC biochars. Smith et al. [69] reported no phytotoxic effects of water-extractable substances form pyrolysed biochars of peanut seed husks and chicken litter on the growth of two blue-green algae, while negative effects were found for pinewood-derived biochar. Variable negative impacts on aquatic species of alga, bacteria, protozoa and crustaceans were also documented by Oleszczuk et al. [57]

when biochar extracts from different feedstocks were tested. In spite of these contributions, the number of studies evaluating the characteristics of pyrolytic biochars and the phytotoxicity of their extracts is still very scarce [69, 73].

Physico-chemical characteristics of lignocellulosic agricultural wastes may differ largely among feedstocks which in turn determine their potential applications. Sunflower seed husks have been traditionally used as feed additives in a variety of animal production systems—from broilers to dairy cattle—[59], as an effective and cheap source for dyes adsorption in water media [78], or burned to produce heat power in oil-producing refineries. However, information about the use of this waste as a source for renewable energy is very scarce. Few studies describe the biochar yields and elemental composition of pyrolysis of sunflower seed husks at varying temperatures, and other authors have tested its biosorbent capacity for Cu^{2+} and methylene blue from industrial wastewaters [67, 72].

Peanut husks-derived biochars obtained under a variety of pyrolysis methods and operating conditions have been characterised in terms of elemental composition, CEC values and BET [43]. Several studies demonstrated the capacity of peanut hull biochars to adsorb different dyes and contaminants from aqueous solutions [26], wastewaters [1, 67] and heavy metals in soils [41]. Other authors have also shown that when applied as soil amendment to different soils, they may improve soil properties [55] and increase growth and yields of tested crops [46]. Qian et al. [62] showed that when used as a compound fertiliser at very low rates (< 1 t ha^{-1}), it could effectively reduce GHG emissions of rice crops. In the case of the non-cellulosic algae Spirulina (*Arthrospira platensis*), the few reports that describe biochar yields and its ultimate analysis for pyrolysed materials under different methods and temperatures suggested that due to its high C content, the biochar could be suitable for soil amendment and C sequestration [27].

Although these residues are being extensively produced in major areas around the world, to the best of our knowledge no studies have yet evaluated the effects of these biochars' water extracts in bioassays as a proxy for their potential applications as soil amendments or to be incorporated in other soilless cultivation media. Therefore, the objectives of the present study were: (1) to determine product yields in the fast pyrolysis of sunflower seed shells, peanut shells and *Spirulina*, (2) to characterise the solid products (biochars) and evaluate their potential to be used as soil amendment through studying the effects of biochar water-extractable substances on germination and growth bioassays using *Lactuca sativa*, and (3) to identify the water-extractable organic compounds from the different biochars and correlate them with their bioactivity.

2 Material and methods

2.1 Biomass samples: origin, processing and characterisation

Sunflower seed shells (from now on SSS)—provided by Dr. M. A. Volpe (PLAPIQUI-UNS, Bahia Blanca, Argentina)—were pre-treated by growing *Ganoderma lucidum* on the shells, which allowed for a partial lignin degradation of the material [17]. Peanut shells (from now on PS) were obtained from AGD Company (Córdoba, Argentina). The blue-green alga *Spirulina* (*Arthrospira platensis*) (from now on Sp) was purchased to NuSci (USA) as dried powder (GB5009-2010). Characterisation of SSS, PS and Sp was performed by using various analytical techniques (Table 1). Elemental analysis was performed by a CHNS Elemental Analyzer 2400 Serie II (PerkinElmer Inc, USA). The lignin, hemicellulose and cellulose contents were determined by the Laboratorio de Servicios de Nutrición Animal (Faculty of Agronomy, University of Buenos Aires) using an ANKOM 200 Fiber Analyzer (ANKOM Technologies, USA), following the method described by Van Soest [82] as adapted by ANKOM ® 2005. Total ash content of each material was determined via combustion of the biomass at 575 °C according to standard test method for ash in biomass (ASTM E17551-01, 2015). The protein, lipids, moisture and carbohydrates contents were determined by the AOAC (2002) and FAO (2003) official methods of analysis.

Pyrolysis experiments The pyrolysis reactions were carried out in a horizontal quartz reactor under low pressures (0.01–0.05 Torr) and nitrogen flow of 0.05 L

Table 1 Physico-chemical composition of the raw materials used in the pyrolysis experiments

	SSS	PS	Sp
C (wt.%)[a]	50.51	46.15	35.89
N (wt.%)[a]	0.35	1.27	1.21
H (wt.%)[a]	5.72	3.07	7.11
O (wt.%)[b]	30.7	35.94	33.58
S (wt.%)[a]	0.09	0.08	0.01
Moisture (wt.%)[a]	11.2	8.8	3.6
Ash (wt.%)[a]	1.43	4.69	18.6
Cellulose (g kg^{-1})[c]	35	40.5	–
Hemicellulose (g kg^{-1})[c]	9	14.7	–
Lignin (g kg^{-1})[c]	14	26.4	–
Crude fat (wt.%)	–	–	3.01
Crude protein (wt.%)	–	–	51.3
Carbohydrate (wt.%)	–	–	27.1

[a]Dry basis, [b]calculated by difference, [c]biopolymer content determined by the reported method (wt% dry basis)

min^{-1} in a temperature range of 280–350 °C. Temperatures were selected based on our previous results [68] and preliminary experiments in which biochar bioactivity could be detected. The pyrolysis unit consisted of a feeding system, a vacuum pyrolysis system and a condensation system, as previously described [53]. Biomass samples (1.00 g) were crushed and sieved to obtain particles of 10–20 mesh size and placed in a sliding quartz boat, which was fed into the pyrolysis furnace when temperature and vacuum settings were reached, and kept at these conditions for 20 min. Due to the vacuum system, contact times of the generated products were very short (< 0.5 s), in the same range of fast pyrolysis experiments. After the experiments were completed, the pyrolysate was extracted from the condenser with organic solvents. The yield of the liquid and char products was calculated by the weight difference of the condenser and the quartz boat, respectively, before and after the experiment. The yield of gas was calculated by the difference of starting biomass and generated pyrolysis oil and char. All yields are informed as the average of at least three experiments to verify the reproducibility of the reported results. Biochar generated in the pyrolysis experiments was characterised by elemental analysis in a CHNS Elemental Analyzer 2400Serie II (PerkinElmer Inc., USA). The C/N and H/C atomic ratios were calculated from these results, and the content in oxygen was calculated by difference taking into account the ash content in the calculus. Ash content of biochar samples was determined by combustion method according to ASTM standards (ASTM D1762-84).

In the case of PS and SSS obtained at 280 °C, FT-IR spectrum was acquired using a microscope Thermo Scientific™ Nicolet™ iN^{TM}10 (Thermo Fisher Scientific, USA) in its reflection mode, to see the decomposition degree. The powder X-ray diffraction (XRD) patterns were recorded in a diffractometer Panalytical X'Pert Pro (The Netherlands), using a Cu Kα ($\lambda = 1.5418$ Å) radiation with current conditions at 40 mA and voltage at 40 kV. The patterns were collected using a PIXcel 1D detector with 230 canals; each pattern was recorded between 10 and 70° with a step of 0.026° and with a time for step of 92.95 s at room temperature. The samples were taken using a single-crystal silicon sample port. Microcrystalline cellulose (99.5%, Biopack, Argentina) was also measured for comparative purposes.

Nitrogen isotherms were determined at −196 °C using a Quantachrome Nova 1000e sorptometer (Anton Paar Quantatec Inc., USA) by adsorbing and desorbing nitrogen at 77 K on samples previously dried and out-gassed at 160 °C for 16 h. BET equation was used for surface area calculations.

2.2 Extraction and characterisation of biochar water-extractable substances

Extraction of water-soluble substances from biochar was performed at 10% (w/v) by soaking the solid sample material in distilled water. The biochar/water mixture was vortexed and placed at 22 °C for 24 h. This mixture was transferred to 15-mL centrifuge tubes and centrifuged at 1500 rpm and 15 °C for 5 min using a Heraeus Labofuge 400R (Thermo Fisher Scientific, USA). The supernatants and pellets were collected separately. Then, the biochar/water mixture was filtered through a—Whatman grade 1—qualitative filter paper (11 µm pore size) via a 12-cm-diameter Buchner funnel. After extraction, the remnant biochar was dried (50 °C) yielding ca. 90% of the original weight.

In order to characterise the water-soluble organic compounds, the extracts from different biochars were evaporated at vacuum to constant weight and the residue was redissolved in acetone/methanol for analysis by gas chromatography coupled to mass spectra (GC/MS) or dissolved in D_2O to perform the nuclear magnetic resonance experiments (^{1}H NMR).

GC/MS analyses of the samples were performed in a GC–MS-QP 5050 spectrometer (Shimadzu Corporation, Japan). The injector temperature was kept at 300 ºC, and the separation was performed using a VF-5 ms capillary column. Helium was used as a carrier gas with a constant flow rate of 0,5–1,0 µL/min. The oven temperature was programmed from 80 °C (3 min) to 280 °C (15 min) with a heating rate of 10 ºC/min. The temperature of the GC/MS interface was held at 280 °C, and mass spectrometer was operated at 70 eV under electron ionisation. The identification of chromatographic peaks corresponding to the main compounds was achieved according to NIST MS library (match > 90%). Also, the identification of phenols was established by comparison with authentic samples.

^{1}H NMR spectra were recorded on a 400 MHz spectrometer (Bruker Corporation, USA) at ambient temperature (^{1}H at 400.16 MHz and ^{13}C at 100.9 MHz). Solutions were typically prepared in deuterium oxide (D_2O), with chemical shifts referenced to deuterated solvent as an internal standard. The proportion of aromatic compounds was calculated through integration of all protons corresponding to phenol derivatives and polycyclic aromatic hydrocarbons (PAHs), while the proportion of non-aromatics (carbonyl and nitrogenated derivatives) was calculated as the total of protons minus the aromatic protons.

2.3 Bioassay of biochar water-extractable substances

The bioactivity of biochar water extracts was evaluated on seeds of lettuce (*Lactuca sativa*, Grand Rapids). Twenty-five

seeds were placed in a 5.0-cm Petri dish lined with one sheet of filter paper previously moistened with each test solution (1.5 mL) or distilled water in the case of controls and allowed to germinate in a growth chamber in the darkness at 22 °C. Three different bioassays with three replicates for each concentration were performed. Aqueous extracts were bio-assayed at 10% (w/v) as well as serial dilutions with distilled water at 7.5, 5, 2.5 and 1.25%. Seed germination was assessed at 24-h interval for three days as previously described [68]. A seed was considered germinated when root protrusion was evident (ca. 1 mm). At day 3, lengths of roots and shoots (hypocotyls) of 60% randomly chosen lettuce seedlings per Petri dish were determined, and relevant morphological features were also observed and annotated. Germination and growth responses expressed as a percentage of the controls were plotted against treatment concentrations. Where appropriate, effective concentrations capable of inhibiting 50% of germination, root growth or shoot growth were calculated as ECg50, ECr50 and ECs50, respectively. Additionally, seed viability of non-germinating seeds was tested in three to four seeds per Petri dish by means of the Tetrazolium test [19].

2.4 Statistical analysis

For the pyrolysis products yield, reported values correspond to the average of three replicate experiments and their respective standard deviation.

The bioassay results were analysed by ANOVA (REML) and DGCs test ($p < 0.01$), using InfoStat (National University of Córdoba, Argentina). Data are expressed as means of three independent bioassays (three replicates for each concentration (aqueous extracts) per bioassay) with their corresponding standard errors. Different letters (a–b) indicate significant differences between treatment effects when compared to the control (ANOVA, REML and DGC test, $p < 0.01$).

3 Results and discussion

3.1 Characterisation of raw materials

In lignocellulosic biomass feedstocks, the structural carbohydrates (cellulose and hemicellulose) and lignin polymers are the main organic constituents of plant cell walls, while fat, carbohydrates, and particularly proteins, are the key constituents of non-lignocellulosic materials as algae. Table 1 displays the contents of cellulose, hemicellulose and lignin of the sunflower and peanut seed shells (SSS and PS) and the fat, protein and carbohydrate values for *Spirulina* (Sp) used in this study. Elemental analyses, ash and moisture are also shown.

Differences in biopolymers content were detected between SSS and PS biomasses. In SSS, these components represented 58% of the dry material, while in PS ca. 82% of the weight is accounted for the three polymers, being cellulose the main component. The major differences between feedstocks were detected in the hemicellulose and lignin content, being 1.6 × and 1.9x (respectively) larger in PS relative to SSS.

For SSS, the values for the three biopolymers were identical to those obtained by Casoni et al. [17] for mushroom pre-treated husks. Cellulose content falls within those found in other studies with untreated husks (27–48%), but our values for hemicellulose and lignin were lower (9 vs. 13–35% and 14 vs. 17–37%, respectively) [7, 16, 21, 34]. A lower lignin value in our sample was expected based on the pre-treatment of the original material. Ash content was smaller than those previously reported [21, 47], Phyllis database (https://phyllis.nl/). In regard to the ultimate analysis of sunflower husks, values for C, H, N and S were within the range of those previously reported (C: 44–57.6 wt%, H: 5.6–6.5 wt%, N: 0.33–5.8 wt%, S: 0.05–0.31 wt%), while O was significantly lower (O: 41.4–49 wt%).

Peanut shells structural composition was very similar to that found by Jaishankar et al. [37], and within the range of those reported in previous papers [38, 84], Phyllis database). The ultimate analysis of PS showed that C and N values (Table 1) were similar to those found in the existing literature, while H and O were at the lower range of reported values (H: 2–7.50 wt%, O: 33.9–50.85 wt%) [38, 84], Phyllis database). Ash and moisture percentages were also within those reported previously.

In the case of *Spirulina*, protein levels fall within the 36–70 wt% range found in previous studies. Ortega-Calvo et al. [58] reported higher levels of fat (6.4–7.5 wt%) and lower amounts of carbohydrates (12.6–18.8 wt%). The ultimate analysis of our Sp sample showed H and O levels comparable with those previously reported [27, 58], while C contents were up to 30% lower (42.8–53.4 wt%). The major differences were detected in N and S contents, for which these authors reported up to 10 × higher N levels (4.1–12.4 wt%) and 90 × higher S (0.5–0.97 wt%). Moisture content was almost half of those previously reported (6.9–8 wt%), while ash was 30 to ca. 150% higher (7–14 wt%) (cf. values with Table 1).

3.2 Pyrolysis of biomass

The yields of the pyrolysis experiments for each type of the biomass are detailed in Table 2. For the lignocellulosic materials (SSS and PS), pyrolysis performed at these low temperatures resulted in high gas yields, followed

Table 2 Distribution of products in the pyrolysis of biomass

Biomass	T (°C)	Products		
		Bio-oil	Biochar	Gas
SSS	280	22 ± 1	29 ± 1	49 ± 3
PS	280	25 ± 2	32 ± 2	43 ± 4
	350	18 ± 3	20 ± 2	61 ± 4
Sp	280	14 ± 3	49 ± 1	37 ± 3

Mean ± standard deviation

by biochar and bio-oil, while for the non-lignocellulosic *Spirulina* the production of biochar was clearly favoured (ca. 50%).

Few reports have described the pyrolytic yields of SSS [47, 76] with highly varying results depending on the initial feedstock composition, pyrolysis system and temperatures. For instance, pre-treated SSS (w/ *Ganoderma lucidum*) transformed in a static system at 400 °C produced almost identical amounts of biochar (27%), but higher bio-oil yields (34%) and lower gas content (39%) [17]. Zabaniotou et al. [76] studied the pyrolytic behaviour of sunflower shells within the range of 300–600 °C and found that maximum yields for each product were attained at different temperatures: 21 wt% bio-oil at 400 °C, ca. 90 wt% biochar at 300 °C, and 53 wt% gas at 500 °C. Biochar and gas yields remained fairly constant within the 400–600 °C range, with 32–35% of biochar and 45–50% of gaseous products. Our reported values are almost identical to those found by these authors at a higher temperature (280 vs. 400 °C).

The yields for the pyrolysed PS were strikingly similar to those recently reported by Lazzari et al. [42] but at a much higher temperature (i.e. 700 °C, 32% bio-oil, 32% biochar, 40% gas). Bio-oil yields were higher than those stated by Xie et al. [84] where they obtained 12–22% of oils for peanut shells of different granulometry subjected to 400–600 °C. Biochar values reported here (32–20%) are also within the range of those previously reported by other authors [26, 43].

In the case of *Spirulina*, a high yield of biochar was obtained at 280 °C (49%, Table 2. This behaviour is different compared with previous studies where biochar yields did not exceed 31%, even when different pyrolysis treatments and temperatures were used [27]. This increase in char formation could be attributed to the higher ash content present in the starting alga; we found 18.6 wt% of ashes, while these authors found values close to 7 wt%. In fact, the promoting effect of higher percentages of ash—including metals and non-metal elements—in the original biomass in the formation of carbonaceous products has been previously reported [6].

Table 3 Characterisation of biochar derived from pyrolysis of the different biomasses

	B-SSS[a]	B-PS[b]	B-Sp[c]
C (wt.%)[d]	51	59	47
N (wt.%) [d]	2	2	8
H (wt.%) [d]	3	4	6
O (wt.%)[e]	22	32	13
Ash (wt.%)	2	3	26
Fixed carbon (wt.%)	54	58	43
Volatile matter (wt.%)[f]	44	39	31
H/C[g]	0.7	0.8	1.5
O/C[g]	0.3	0.4	0.2
S_{BET} ($m^2 g^{-1}$)	176.552	38.186	2.214
Pore volume ($cm^3 g^{-1}$)	0.038	0.007	0.003

[a]Biochar derived from pyrolysis of sunflower seed shells at 280 °C[b]Biochar derived from

pyrolysis of peanut shells at 280 °C

[c]Biochar derived from pyrolysis of *Spirulina* at 280 °C

[d]On dry basis

[e]Calculated by difference

[f]Calculated by difference

[g]Atomic ratio

3.3 Characterisation of biochars

The elemental composition, atomic ratios, ash content, fixed carbon, volatile matter, surface area and total pore volume of biochars are shown in Table 3.

Biochars from lignocellulosic materials (B-SSS and B-PS) showed similar elemental composition and atomic ratios compared to the non-lignocellulosic B-Sp (Table 1). B-SSS and B-PS showed higher O (wt%, 1.7 to 2.5x) and O/C ratios (1.5–2 x), while B-Sp showed much higher levels of N (4x) and of H/C ratio (ca. 2x). Ash content in B-Sp was 9 to 13 × higher than in B-PS and B-SSS, respectively.

Typically, during pyrolysis an initial loss of surface functional –OH groups due to dehydration is expected, followed by C-bound O and H atoms due to structural core degradation, although the rate and total losses of functional groups are strongly temperature dependent [6, 79]. At the low temperatures used in these experiments, cellulose-type components may be expected in the biochars; however, the transformation of aliphatic cellulose molecules to aromatic components within the range of 250–400 °C has also been reported (Wang and Xing [83]). FT-IR spectra and XRD profiles of PS- and SSS-derived biochars indicated that the cellulose component in original biomasses was transformed under pyrolysis conditions (Fig. 1 i, ii, Supplementary information). In IR spectra of biochars, the broadband between 3200–3650 cm^{-1} associated with O–H stretching

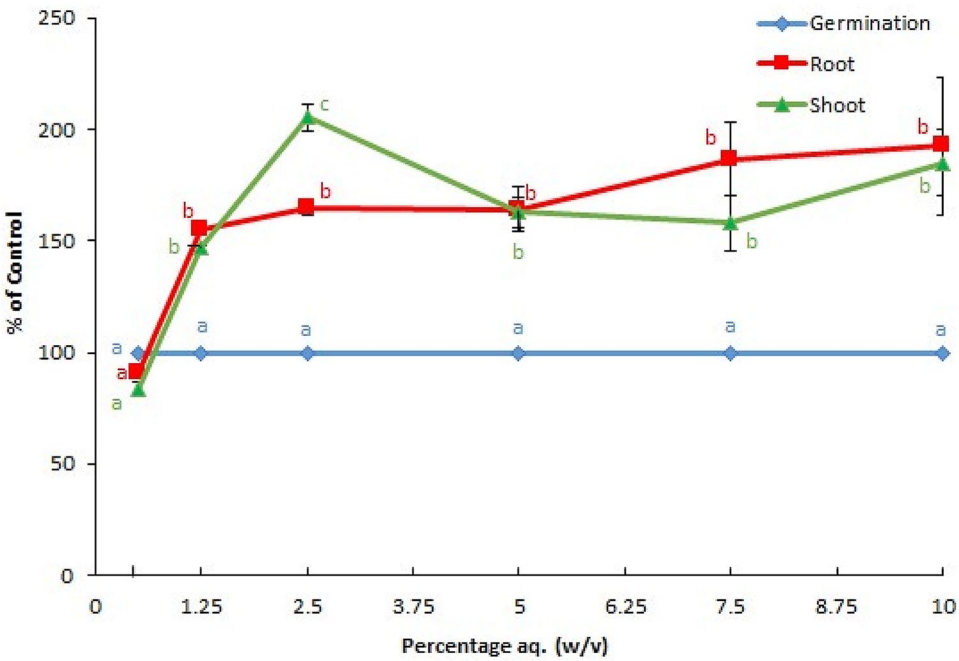

Fig. 1 Effects of biochar water-extractable substances (BWES) from the B-PS on germination and root and shoot growth of *L. sativa*, computed at the end of the experiment (day 3). Data are expressed as means of three independent bioassays (three replicates for each concentration (aqueous extracts) per bioassay) ± SE. Different letters (a–b) indicate significant differences between treatment effects when compared to the control (ANOVA, REML and DGC test, $p < 0.01$)

vibration mode of hydroxyl functional groups had a lower intensity than in pure cellulose. Other significative difference was the increment of bands between 1680 and 1520 cm^{-1} that can be assigned to C = C vibrations in aromatic and olefinic region. The XRD patterns of biochars from PS and SSS showed two broad diffraction peaks corresponding to the typical peaks of carbon (Fig. 1 ia, ib, SI). The strongest peak located at $2\Theta = 20–30°$ is attributed to the amorphous carbon structure, while the weaker diffraction peak at around $2\Theta = 40–50°$ is assigned to the reflection from the incipient graphite component.

To the best of our knowledge, the characterisation of biochars from fast pyrolysis of SSS, PS and Sp at low temperatures is reported here for the first time, precluding any direct comparisons.

Reported data for biochars derived from sunflower husks have been obtained using diverse pyrolysis methods and temperatures [67, 72]. These authors found relatively high C contents, which increased with temperature (≤ 60% at ≤ 300 °C, ca. 72% at 400–500 °C), and a decrease in H/C (0.11 to 0.06) and O/C (0.75–0.34) as temperature increased (240–500 °C). Compared to our results, these authors obtained similar percentages of O (21.8–24%), H (3.7–4.5%), N (0.91–2.64%) and ashes (1.55–9.5%). BET values were only reported by Saleh et al. [67] which found an extremely low surface area of 3.85 m^2g^{-1} compared to the 176.55 m^2g^{-1} found in our study, and higher total pore volume (cf. Table 3, 0.124 vs. 0.038 cm^3g^{-1}). These results may be at least partially explained by the depleting effect that *Ganoderma lucidum* had on SSS lignin content (Table 1).

Peanut hulls-derived biochars have been characterised with different equipment and running conditions. Using a fluidised bed catalytic steam reformer (475–481 °C), Lee et al. [43] found higher C (> 70%), much lower O (15–16.6%), but more similar H (2.9%) and N (ca. 2%) values compared to our results. A similar trend was observed in slow pyrolysis biochars produced using furnaces at temperatures between 300 and 700 °C [26, 85], particularly at the lower temperatures. These authors also found an increase in C, and a decrease in O, H and N as temperature increased. BET values reported by Yao et al. [85] were significantly lower compared with our results, even at the higher temperatures tested (0.8–27.1 m^2g^{-1}, 300–600 °C).

In *Spirulina*, Chaiwong and Kiatsiriroat [27] reported the results of biochars derived from slow and fast pyrolysis at 500 °C. Their results showed similar C values (45% and 39%, respectively) to that found in this study, higher O (51%, 53%) and lower percentages of N (2.65%, 5.85%) and H (1.24%, 1.37%). Reported ash contents were 1.5 to 1.8 × higher than the value obtained in our experiments.

3.4 Bioassay of biochar water-extractable substances

The biochar water extracts of the two agronomic wastes (B-SSS and B-PS) at 280 °C did not affect the germination of *Lactuca sativa* seeds at any of the concentrations tested; and all the germinated seeds produced seedlings with normal morphology. However, a different pattern of response was observed between feedstocks regarding growth. B-PS water extracts exhibited a potent stimulatory

effect on root and shoot growth (Fig. 1). Roots and shoots were 50 to 105% longer than the control at all concentrations (1.25–10% w/v). In contrast, B-SSS water extracts only promoted shoot growth at the lower range of concentrations (1.25–2.5% w/v) and to a lesser extent (up to 20%) (Fig. 2). At these concentrations, root growth was unaffected, but it was substantially reduced (up to 40%) at concentrations ≥ 5% (w/v) relative to controls (Fig. 2).

BWES obtained from pyrolysis of peanut husks at 350 °C (named as B-PS350) showed a totally different response compared to the same material pyrolysed at 280 °C, particularly regarding shoot and root growth (cf. Figs. 1 and 3. Although seed germination remained mostly unaffected, a reduction of ca. 40% was observed at the highest concentration as shown in Fig. 3. In contrast, root and shoot growth exhibited a V-shape response, in which ca. 75% reduction was observed at 5% (w/v). While shoot growth was unaffected at the remainder concentrations, a significant reduction in

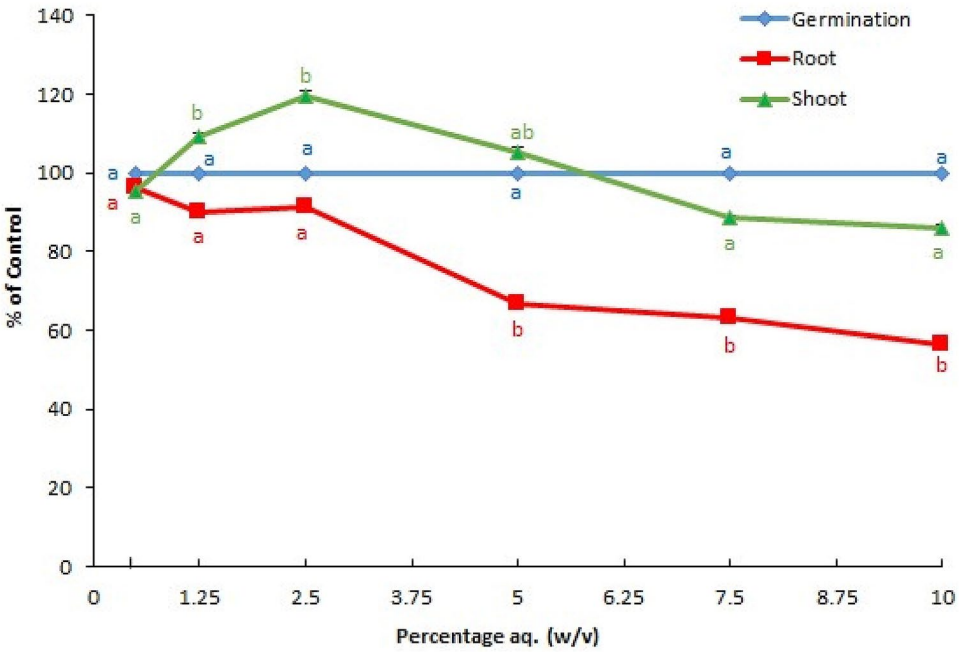

Fig. 2 Effects of biochar water-extractable substances (BWES) from the B-SSS on germination and root and shoot growth of *L. sativa*, computed at the end of the experiment (day 3). Data are expressed as means of three independent bioassays (three replicates for each concentration (aqueous extracts) per bioassay) ± SE. Different letters (a–b) indicate significant differences between treatment effects when compared to the control (ANOVA, REML and DGC test, $p < 0.01$)

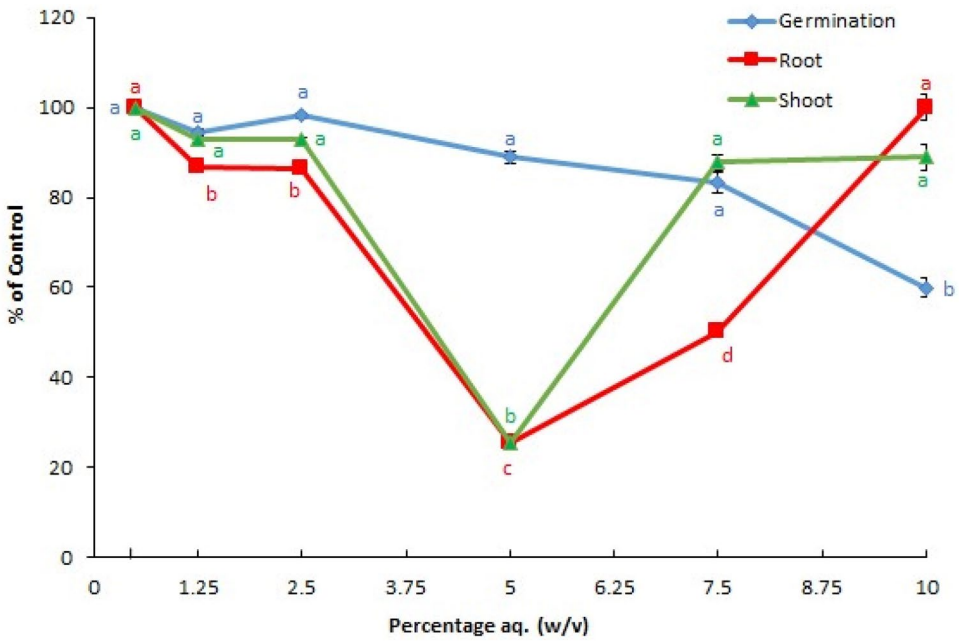

Fig. 3 Effects of biochar water-extractable substances (BWES) from B-PS350 on germination and root and shoot growth of *L. sativa*, computed at the end of the experiment (day 3). Data are expressed as means of three independent bioassays (three replicates for each concentration (aqueous extracts) per bioassay) ± SE. Different letters (a–d) indicate significant differences between treatment effects when compared to the control (ANOVA, REML and DGC test, $p < 0.01$)

root growth (10–75%) was observed in four of the five concentrations tested (1.25–7.5% w/v) (Fig. 3).

The BWES from B-Sp, in turn, produced a drastic inhibition of *L. sativa* seed germination. At 1.25% (w/v), germination was reduced by ca. 40%; it was almost nil at 2.5 and 5% (w/v) and completely inhibited at ≥ 7.5% (w/v) (Fig. 4).

Regarding growth, it was surprising that at the lowest concentration (1.25% w/v) shoot and root growth was promoted up to 20% relative to the controls. However, for the remainder concentrations root growth inhibition mimicked the response of seed germination, being severely affected at concentrations ≥ 2.5%, and reaching 100% inhibition at ≥ 7.5% (w/v). In contrast, shoot growth inhibition was visible at a higher concentration (5% w/v, 65% inhibition) and 100% inhibition occurred at concentrations ≥ 7.5% (w/v) (Fig. 4). The mean effective concentrations of the biochar water extract that inhibited germination (Ecg50), root (Ecr50) and shoot (Ecs50) growth were 2.6, 3.35 and 3.8%, respectively.

The few reports in which germination bioassays have been performed with biochar water extracts have yielded variable results, depending on feedstocks, thermo-conversion processes involved and running conditions [3, 20, 51]. Using the same experimental protocol described here, we found that the biochar water extracts from pyrolysed leaves of *Flourensia oolepis* (at 280 °C) (B-FO) did not affect the viability of *L. sativa* seeds and only produced a transient arrest of germination at the higher concentrations (7.5 and 10% w/v), which was overcome once the seeds were transferred to water [68]. Regarding seedling growth, B-FO water extracts showed a hormetic type of response, in which growth was enhanced at lower concentrations and inhibited at higher doses. In fact, a dramatic 225 to ca. 160% stimulatory effect was observed on root and shoot growth at concentrations between 1.5 and 5% (w/v), while inhibition occurred at higher concentrations (≥ 7.5% w/v) [68].

In experiments performed with biochar water extracts from waste products, Alburquerque et al. [4] found increased germination of sunflower seeds when five different lignocellulosic agricultural and forest wastes (olive stone, almond shell, wheat straw, pine woodchips and olive tree pruning) were tested at 10% w/v. Rogovska et al. [63] reported no effect on corn seed germination but a decrease in seedling growth, particularly shoot length, in three out of six biochar extracts derived from different feedstocks at the highest temperature treatments. The use of pyrolysed biochar leachates of coconut shells and wicker (350–650 °C) did not affect seed germination of *Lepidium sativum* and exerted a stimulatory effect (12 to 41%) on root growth that was independent of the biochar concentration [57].

The presence of volatile organic compounds (VOCs) and water-soluble organic compounds (WSOCs) in biochars has been associated with both positive and negative biological effects (including plants, microorganisms and aquatic organisms) [9, 14, 64, 69]. For instance, Deenik et al. [20] reported reduced germination of radish and corn seeds when subjected to high volatile matter charcoal extracts of macadamia nut shell (430 °C). Similarly, the leachates derived from softwood pellets biochar (550 °C) contaminated with high levels of VOCs caused phytotoxic effects on seed germination of *L. sativum*, while no phytotoxicity was observed in low-VOCs biochars [14]. It has also

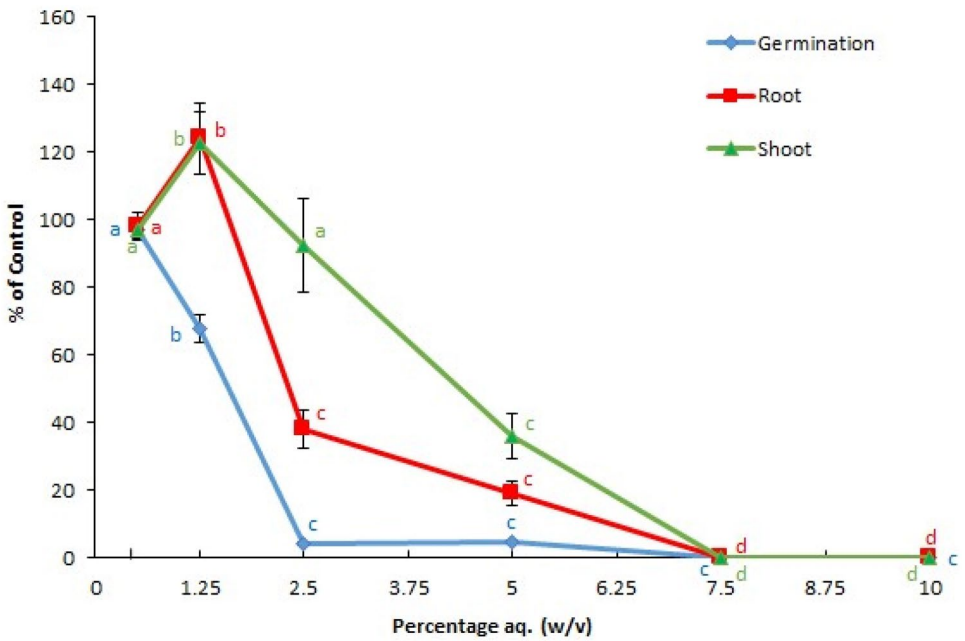

Fig. 4 Effects of biochar water-extractable substances (BWES) from B-Sp on germination and root and shoot growth of *L. sativa*, computed at the end of the experiment (day 3). Data are expressed as means of three independent bioassays (three replicates for each concentration (aqueous extracts) per bioassay) ± SE. Different letters (a–d) indicate significant differences between treatment effects when compared to the control (ANOVA, REML and DGC test, $p < 0.01$)

been shown that the detrimental effects on germination may be significantly alleviated by different methods that reduce the phytotoxicity and number of VOCs [13, 63, 64].

On the other hand, it was also found that the germination rate of *L. sativum* was unaffected by biochar extracts from pelletised corn stalks at 350–650 °C [28]. Similar results were obtained by Rombolà et al. [64] and were also consistent with those reported in a previous study in which corn stalk biochars with high contents of VOCs and WSOCs did not present inhibiting effects on seed germination [29]. Moreover, shoot growth was promoted in all treatments, and biochars with greater WSOCs content (350–500 °C) exhibited longer shoots than those obtained at higher temperatures (550–650 °C), suggesting that WSOCs could be involved in the enhancement of plant growth. Backer et al. [9] also showed that VOCs and WSOCs from biosolids (270 °C—10 min, 320 °C—20 min) and softwood chips (500 °C, slow pyrolysis) did not affect maize seed germination and shoot and root lengths after a 4-d incubation period, and even a stimulatory effect on shoot length was observed by the VOCs emitted by one of the biosolids (320 °C).

In investigations in which the growth of blue-green algae was tested against BWES, no phytotoxicity was found for pyrolysed peanut seed hulls and chicken litter, while negative effects were detected for pinewood-derived biochar [69]. Moreover, their results showed that peanut shell water extracts could even promote growth of the cyanobacterial culture (up to 60%) when incubated at 1.13 g L^{-1}. Authors determined that neither the differences in DOC (dissolved organic matter) nor pH values found in the water extracts of pinewood and peanut seed husks (DOC: 56.2% vs. 10.7%, pH: 3.94 vs. 8.87, respectively) were responsible for the observed responses, and proposed that other specific component(s) present in the water extracts would be contributing to the phytotoxicity in pinewood. In a subsequent in-depth study, Smith et al. [70] performed a molecular characterisation of the water-soluble components of this species and compared it to that found in the non-phytotoxic peanut-shell-derived biochar. Results showed important differences related to the O/C and H/C ratios, where pinewood water extracts were characterised by high ratios—more typical of carbohydrate-like compounds, whereas more formulas with lower ratios and an aromatic nature were found in peanut shells. The same authors proposed that phytotoxicity in pinewood biochar water extractables would be most probably related to degrade lignin-like species rich in oxygenated functionalities, like carboxyl, hydroxyl and methoxyl groups. In contrast, the non-phytotoxic peanut seed husks biochar water extracts seem not to contain these easily charged species. However, variable negative impacts on aquatic species of alga, bacteria, protozoa and crustaceans have also been documented for biochar extracts from different feedstocks (Miscanthus, coconut shell, wicker and wheat straw), where toxicity was closely related to the number of certain PAHs [57].

3.5 Analysis and characterisation of BWES

GC/MS and NMR analysis of the BWES of all residues confirmed the presence of a mixture of organic compounds, which can be classified in aromatics (phenols and polycyclic aromatic hydrocarbons) and non-aromatics (carbonyl and nitrogen derivatives). Some of these compounds were also detected in the pyrolytic oils of the investigated biomasses [54], indicating that the removal of these chemicals from the carbonaceous residue in the course of the pyrolysis process was not complete. All specific compounds were identified based on their mass spectra and the proton and carbon signals in the NMR experiments of the mixture, and their relative amount was calculated from the ^{1}H NMR spectrum.

In the case of the water extract from B-PS obtained at 280 °C, non-aromatic compounds were prevalent, with cyclic ketones and aldehydes as main contributors, while phenols and one polycyclic aromatic hydrocarbon (an anthracene derivative) were minor components (Table 4). Among the group of carbonyl compounds, 2-hydroxy-3-methyl-cyclopenten-1-one and 3-methyl-imidazolidine-2,4-dione were the major contributors. The abundance of the heterocyclic and the cyclic ketone compounds found in this extract may possibly be due to the specific pyrolytic process that favours the formation of such structures. The cyclopentenone derivative can be formed by degradation of carbohydrates during the early stages of the PS pyrolysis. The formation of imidazolidinedione, instead, seems complex; however, Sun et al. [75] have also detected similar heterocyclic compounds in the aqueous extracts from wheat and maize biochars. In turn, hydroxylated aromatics and PAHs are mainly formed from the degradation of the lignin component of PS, and it has been shown that these compounds can leach out from biochars into water extracts [14, 64].

B-PS350 water extracts showed a significant increase in aromatics relative to B-PS at 280 °C, with a preponderant contribution of phenolic compounds as guaiacol, creosol and eugenol, which have also been identified in the liquid fraction of pyrolysis [54].

The analysis of water-soluble organic compounds from B-SSS showed a high concentration of aromatics, as phenols and polycyclic hydrocarbons, while ketones and other carbonyl derivatives were detected in smaller quantities (Table 4).

It is important to stress that although the compounds found in the water extracts from B-PS and B-SSS were very

Table 4 Composition of organic compounds in water extracts from biochars

Extract	Non-aromatics[a]	Aromatics[a]	Ratio Non-aromatics/Aromatics[b]
B-PS[c]	2-Hydroxy-3-methyl-cyclopenten-1-one; 3-Methyl-imidazolidine-2,4-dione Aliphatic aldehydes[g]	Guaiacol, Eugenol, PAHs[k]	3.2
B-PS350[d]	2-Hydroxy-3-methyl-cyclopenten-1-one; Aliphatic aldehydes[g]	Guaiacol, Eugenol, Creosol, PAHs	1.1
B-SSS[e]	2-Hydroxy-3-methyl-cyclopenten-1-one; 3-Methyl-2-cyclopenten-1-one; Aliphatic aldehydes[g] and acids[h]	Guaiacol, Ethyl-guaiacol; Syringol; 4-Hidroxy-2-methyl-acetophenone PAHs	2.0
B-Sp[f]	Cyclic and acyclic amines[i]; Long-chain aliphatic nitriles[j]	Phenol; PHAs	3.4

[a]Compounds characterised by GC/MS and NMR spectroscopy. [b]Ratio of compounds determined by peak area integration method in ^{1}H NMR. [c]Biochar derived from pyrolysis of peanut shells at 280 °C. [d]Biochar derived from pyrolysis of peanut shells at 350 °C. [e]Biochar derived from pyrolysis of sunflower seed shells at 280 °C. [f]Biochar derived from pyrolysis of *Spirulina* at 280 °C. [g]Butanal, octanal and octadecenal. [h]Palmitic acid, octadecenoic acid. [i]Piperazine, 4-amino-4-methyl-2-pentanone and aliphatic amines. [j]Hexanedecanenitrile and pentadecanenitrile. [k]Polycyclic aromatic hydrocarbons (anthracene derivatives)

similar, the concentration of these derivatives changed in each case. The calculated ratios between non-aromatics/aromatics were 3.2, 1.1 and 2 for B-PS, B-PS280 and BSSS, respectively (Table 4). The differential contribution of these categories may explain the different bioactivities exhibited by these BWES.

In contrast, the water extract obtained from B-Sp showed a totally different composition (Table 4). As expressed by the non-aromatics/aromatics ratio (i.e. 3.4), there was a clear preponderance of non-aromatic nitrogenated compounds, mainly represented by cyclic and acyclic amines and long-chain nitriles, while aromatics were minoritary (only phenol and one polycyclic hydrocarbon).

A van Krevelen diagram (Fig. 5) was used to visualise the possible differences among the various biochar's water extracts based on the relative H/C and O/C atomic ratios of the molecular formulas of the detected organic compounds (Table 4). According to the stoichiometric ranges used to establish boundaries of the chemical classification space for the components found in natural organic materials, regions can be considered as: proteins (H/C = 1.5–2.2 and O/C = 0.3–0.67), lipids (H/C = 1.5–2.0, O/C = 0–0.3), lignins (H/C = 0.7–1.5 and O/C = 0.1–0.67), carbohydrates

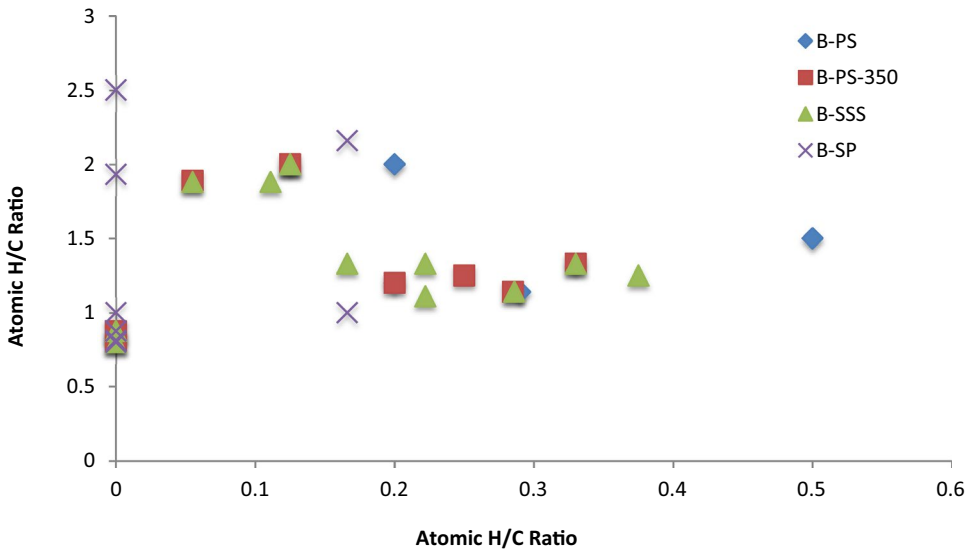

Fig. 5 Van Krevelen diagram of detected compounds in BWES from B-PS, B-PS350, B-SSS and B-Sp

(H/C = 1.5–2.4 and O/C = 0.67–1.2), unsaturated hydrocarbons (H/C = 0.8–1.5 and O/C = 0–0.1) and polycyclic aromatics (H/C = 0.2–0.8 and O/C = 0–0.67). The plot derived from the compounds determined in Table 4 shows a distribution pattern that is strongly dependent on biochar origin, with the majority of the components corresponding to lignin, protein, lipids and condensed aromatics (Fig. 5).

The diagram also shows proximity of the molecules detected in B-PS350 and B-SSS, which is consistent with the fact that both water extracts share 50% of the total molecules detected. Less overlap is observed for B-PS, where compounds are more dispersed along the two axes and where two distinct molecules (3-methyl-imidazolidine-2,4-dione and butanal) exclusively found in this extract are visualised.

In contrast, in the case of the B-Sp, the mixture of compounds is mainly clustered at the far left side of the diagram. This distribution clearly obeys to the particular composition found in this extract, in which 75% of the total compounds (nitrogenated and PAH) do not have oxygen atoms.

It is also worthy to note that B-SS and B-Sp showed a larger number of exclusive compounds that were not present in B-SPs extracts, which represented as much as 50% and 75% of the total for each extract, respectively.

Regarding the chemical nature of the BWES and correlating them with their effects on germination and growth of root/shoot of *L. sativa*, it can be strongly suggested that the increase in the concentration of aromatic compounds for B-PS and B-SSS may be responsible for the observed reduction in germination and growth. These results are in line with the toxicity of biochar water-soluble organic compounds described previously in the literature [25]. It is known that negative effects of BWES on seed germination, soil microbes and aquatic microorganisms, among others, can be associated with the presence of PAHs [63], dioxins [33], phenols and organic acids [14, 64]. In our study, the water extracts of B-PS and B-SSS showed a far greater contribution of phenol derivatives relative to PAHs. However, without specific experiments, it is difficult to assure that only one of these classes is completely responsible for the observed toxicity.

In the case of BWES derived from B-Sp and taking into account the predominance of nitrogen organic (non-aromatic) compounds over aromatics, amines and nitriles could lead to the detected detrimental effects on germination and growth of *L. Sativa*.

The stimulatory growth effect on roots and stems of *L. sativa* produced by B-PS water extracts could be attributed to the presence of imidazolidinedione and/or cyclopentenone derivatives, present in large amounts in the leachate. In this regard, it is worth mentioning that heterocyclic nitrogen compounds identified in the water extract from maize biochar have been indicated as responsible for the enhanced germination of maize seed and seedling growth [75]. The fact that the water extract from B-PS at 280 °C was the only one exhibiting promoting growth activity at all concentrations tested, and that the imidazolidinedione derivative was solely found in this extract, strongly suggests the involvement of this compound in the growth-promoting activity; however, a direct implication of this carbonyl compound (or other) in the observed response would deserve further testing.

4 Conclusions

To the best of our knowledge, this is the first report in which the bioactivity of the biochar water extracts derived from sunflower seed shells, peanut shells and *Spirulina* have been tested against the germination and growth of *Lactuca sativa* as a test system, and the first to document the bioactivity, specifically the phytotoxicity of *Spirulina*.

The experimental approach using BWES is a good proxy when trying to mimic the fate of the biochar incorporated into the soil matrix relative to the leaching of water-extractable substances. Within the soil, biochars will be exposed to wet–dry cycles under rain and/or irrigation, where leaching will be favoured during soil wetting. In this sense, our results show that B-PS at 280 °C would be the most suitable soil amendment, since its BWES not only did not affect germination but exhibited an outstanding stimulatory growth effect on both roots and stems of *L. sativa*. The inhibitory effects of B-PS350, B-SSS and B-Sp, even present at the lower range of concentrations tested, would indicate that their direct incorporation into the soil would not be advisable without further, in-depth studies.

In addition, the promoting growth effect of BWES of peanut shells strongly suggests that these extracts could be used as growth enhancers or biostimulants, providing a high-valued by-product that could be obtained prior to its incorporation into the soil. Carbonyl compounds (heterocyclic and/or carbocyclic) could be responsible for these stimulatory effects with hormone-like activity. On the other hand, the remarkable germination inhibition detected in BWES of *Spirulina* allows speculating on its potential application as natural herbicide, where the nitrogen organic compounds would be responsible for the inhibitory effects. However, the possible influence of the mineral fraction present in the ashes, particularly in B-SSS and B-Sp, on the observed responses cannot be ruled out.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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