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Hydrothermal Carbonization of Municipal Woody and Herbaceous Prunings: Hydrochar Valorisation as Soil Amendment and Growth Medium for Horticulture

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Abstract: In this study, we investigate the suitability of hydrochar, produced at industrial scale by hydrothermal carbonization of municipal woody and herbaceous prunings, to be used as soil amendment and peat substitute in organic growth medium for horticulture. Fresh hydrochar and the products of two different hydrochar post-treatments (i.e., washing and aging) were compared in terms of potential phytotoxicity throughout physicochemical characterization and germination tests, performed with a sensitive species (*Lactuca sativa*). The results showed that the fresh hydrochar obtained from municipal green wastes complies with the Italian regulated parameters for the use as soil amendment. Moreover, hydrochar exhibits biological activity and a high content in organic C, Ca, and other micronutrients (Mg, Zn, Cu, Na, Cl). On the other hand, post-treatments are needed before application of hydrochar as peat substitute in potting mix, since appreciable phytotoxic effects on lettuce seed germination and radicle length of plantlets were observed (e.g., germination percentage of 56% and 54%, with 5 and 10 wt % of hydrochar in the blend, respectively). The inhibition of germination could be mainly attributed to the presence of polyphenols (tannins) and volatile fatty acids, which were most effectively removed through the aging post-treatment.

Keywords: hydrothermal carbonization; hydrochar; soil amendment; phytotoxicity; germination test

1. Introduction

Hydrothermal carbonization (HTC) of wet biomass is receiving increasing attention as a sustainable thermochemical process for the conversion of renewable resources or organic wastes into a solid peat-like product known as hydrochar [1,2]. During HTC, biomass is converted in subcritical water as reaction medium at mild temperature (180–250 °C) and self-generated pressure (up to 2 MPa) to give three products: the solid carbonaceous product (hydrochar), a water-soluble organic fraction (sugars, acetic acid, and other organic acids), and a gas fraction (mainly CO_2). The main transformations are based on hydrolysis and dehydration of the fundamental macromolecules (lignin, cellulose, hemicellulose) to monosaccharides and disaccharides that, in turn, are dehydrated, hydrolyzed, and decarboxylated to give intermediate fragments whose re-condensation leads to hydrochar formation [3]. Different feedstocks, process conditions, and production technologies may result in differences in hydrochar properties [4].

Apart from the expected use as fuel [5–8], hydrochar is a carbonaceous matrix which could be employed in several fields, such as agriculture, horticulture, manufacturing of materials (activated carbons, electrodes, or composites) [9–13].

Besides the worldwide increasing research activity at lab scale, only few industrial applications of HTC process, based on different reactor technologies, have been developed in the last years [2], and at the end, HTC process scale-up from lab to industrial scale has been successfully achieved [14]. To date, the research objectives focus on the development of sustainable and efficient process treatments, in order to conform the product quality to the standards required by the specific commercial applications, as well as to conform to legislation constraints.

Among the potential applications of hydrochar, there is a growing interest on its use as organic growth medium for vegetable seedlings in horticulture and gardening (as peat substitute in potting mix) or soil amendment. In both cases, hydrochar application may contribute to reducing greenhouse gas emissions, increasing the carbon sequestration potential of arable soil [1,4].

In view of potential agronomic applications, recent studies have shown that fresh hydrochar applied to the soil, whatever the raw material source, may display a negative effect on plant germination and growth. A decreased inhibition effect has been observed after hydrochar washing or aging [15–19]. From these studies, it is also evident that, due to the complex chemical routes of the process and the numerous reactions involved, further research is needed to identify the chemical compounds responsible for the undesired effects, and the most effective and cost-efficient process to remove them.

In a recent work [20], characterization and upgrading treatment data of hydrochar, produced in the industrial-scale HTC demonstrative plant of Ingelia (Valencia, Spain), have been reported with specific reference to the use of hydrochar as fuel.

In this work, we extend the characterization and upgrading treatments of hydrochar produced in the same plant with the objective to assess (i) the suitability of fresh hydrochar to be used as soil amendment in compliance with the current Italian legislation; (ii) the feasibility of its application as organic growth medium for horticulture (as peat substitute in potting mix for seedling and plantlet production). For this purpose, fresh hydrochar and the products of two different hydrochar post-treatments (i.e., washing and aging) are compared in terms of potential phytotoxicity throughout chemical characterization and germination tests, performed with a sensitive species (*Lactuca sativa* var. *Capitata* (L.) Janchen). The effects of the treatments on the seed germination percentage and radicle length of lettuce are investigated, with the aim to identify the most effective treatment in terms of improved harmlessness for plants and to speculate the most likely causes of phytotoxicity.

2. Materials and Methods

2.1. Materials

The hydrochar used in this work was produced in the industrial-scale HTC demonstrative plant of Ingelia (Valencia, Spain). The plant and associated post-treatment facilities have been described in detail in a previous work [14]. The reactor, designed for a continuous regime at a rate of 3000–5000 kg biomass per day, is a vertical pressure vessel operating at temperatures between 180 °C and 230 °C, and related autogenous water vapor pressure. After a residence time of 4–16 h, the water–hydrochar slurry exits from the reactor and is filtered (hydrochar humidity after filtration about 50 wt %); the hydrochar can be further dried thermally and pelletized.

Municipal green waste, consisting of woody and herbaceous material from tree pruning and regular maintenance of gardens, was collected in the municipality district of Ingelia plant (from an average distance of 20 km), and used as HTC biomass feedstock (BF). The biomass feedstock is classified according to ISO 17225-1 standard (Table 1 subgroups 1.1.4 prunings, 1.1.7 wood from garden and parks, and 2.1.7 herbaceous biomass from garden and parks). After 6 h of processing at 210 °C, the resulting fresh hydrochar (FH) was filtered, dried at 110 °C, and pelletized.

A representative sample of FH, ground and sieved to a particle size ≤75 µm, was used in the characterization analysis and experimental tests reported here. A commercial potting mix substrate, commonly used for horticultural applications (Humin substrat N3, Neuhaus, Germany), was used as control in the germination tests.

2.2. Hydrochar Post-Treatments and Characterization

FH was subjected to two different post-treatments, i.e., washing and aging. Washing was conducted by suspension of pulverized (≤75 µm)= FH under agitation in distilled water at weight ratio 1:5 (hydrochar/water) for 1 h followed by filtration (using a Whatman filter paper, grade 41). Washing procedure was repeated twice; the pH of the filtered liquid resulted close to neutrality (6.7). The aging treatment was conducted by leaving a pelletized FH batch under free air exchange storage at room conditions for four months. After aging, a sample of pellets was ground and sieved to a particle size ≤75 µm.

The hydrochar samples were oven dried at 105 °C until constant weight, according to the EN-14774-1 standard method, and the moisture content was determined by the weight difference.

For ash content, previously dried and milled samples were calcined at 600 °C for 5 h according to the ASTM D 1102 standard method. The residue obtained is the ash (mineral matter) of the sample; ash content is expressed as weight percent on the dry sample mass.

For approximate analysis, the volatile matter content was determined by thermogravimetric analysis (TGA) (Q500, TA Instruments; New Castle, DE, USA); the previously dried and milled samples, heated and kept at 105 °C for 10 min, were heated from 105 to 900 °C at a heating rate of 20 °C min^{-1}, and kept at 900 °C for 10 min under a nitrogen flux of 100 mL min^{-1}. The weight loss of the sample from 105 to 900 °C is attributed to the volatile matter, and expressed as weight percent on the dry sample mass. The fixed carbon, as weight percent on the dry sample mass, is obtained as the difference up to 100 with respect to the sum of ash and volatile matter content.

Elemental analysis (CHN) was determined according to the ASTM D5373 standard method on the previously dried and milled samples by using a LECO CHN628 analyzer.

Element content (Cd, Cr, Hg, Ni, Pb, Cu, Zn, B, Ca, Fe, Mg, Mn, Mo, Na) was determined, according to the UNI EN 13657:2004 + UNI EN ISO 11885:2009 + UNI EN ISO 17852:2008 standard methods, after microwave acid mineralization in aqua regia by inductively coupled plasma optical emission spectroscopy (ICP-OES) on a Perkin Elmer 5300 analyzer. The Cr(VI) content was determined according to the CNR IRSA 16 Q64 Vol 3 2005 standard method, by hot alkaline digestion and reaction with diphenylcarbazide, followed by spectrophotometric analysis on a Thermo Scientific Helios Zeta analyzer.

Polycyclic aromatic hydrocarbon (PAH) content and speciation were conducted, according to EPA 3445A 2007 + EPA3620C 2007 + EPA8270D 2007 standard methods, after accelerated solvent extraction (ASE) with a mixture acetone/dichloromethane (1:1 v/v) and purification with Florisil, by gas chromatography-mass spectrometry (GC-MS) on a single quadrupole Electronic Impact GC 7890A/MS 5975C Agilent. The operation condition was set as follows: column temperature held at 40 °C for 4 min and temperature programmed to 320 °C at 10 °C/min (nitrogen carrier gas).

Volatile fatty acid (VFA) content was determined after ultrasonic extraction with a mixture of acetone/water (1:1 v/v) by GC on a triple quadrupole TQ8030 Shimadzu analyzer.

Humic and fulvic acid content was determined according to the Italian standards methods for organic fertilizers and soil amendment (D.M. 21 December 2000, published in Gazzetta Ufficiale 26 January 2001, n. 21).

Organic carbon content, pH, cation exchange capability, electrical conductivity, anion and cation content, and soil respiration of hydrochar samples were determined according to the soil analysis standard methods [21]. pH was determined before filtration in a 1:2.5 (w/v) hydrochar/water suspension (10 g of dry sample sieved at 2 mm in 25 mL deionized water) with a Crison GLP 21 pH meter. The filtrate of the suspension was used to determine the available (water soluble) anions (Cl^-,

SO_4^{2-}, NO_3^-) and ammoniac nitrogen with a DX120 dedicated ion chromatograph, equipped with a AS40 automated sampler and a Peaknet chromatography workstation. CG 12A pre-column and CS 12A column were used for cation measurements, while AG4-SC pre-column and AS4A column were used for anion measurements.

Cation exchange capacity determination was performed according to the barium chloride–triethanolamine method [22].

Electrical conductivity was measured in a 1:5 (w/v) hydrochar/water suspension (10 g of dry sample sieved at 2 mm in 50 mL deionized water) after 30 min stirring with GLP 31 Crison conductivity meter.

Hydrochar respiration was evaluated in closed jars according to the method described for soils by Isermeyer [23]. This method evaluates the CO_2 evolved during 7 day incubation of the samples in a closed system at 20 °C; during the incubation period, the CO_2 is trapped in an NaOH solution, which is finally titrated with HCl [24].

Total phenolic content of ethanolic extracts was spectrophotometrically determined using the Folin–Ciocalteu reagent [25]. Tannic acid was used as external standard, and the results were expressed as milligrams of tannic acid equivalents (TAE) per kg of sample dry weight. Total tannin content was determined using the Folin–Denis reagent, according to the method of Schanderl [26] and Rasineni et al. [27], using tannic acid as standard (concentrations ranging from 0.1 to 1.0 mg mL^{-1}). Values were expressed as mg tannic acid equivalent per kg of sample dry weight.

2.3. Lettuce Germination Tests

The potential phytotoxicity of hydrochar on seed germination was determined using the lettuce (*L. sativa* var. *Capitata*) germination bioassay, according to International Seed Test Association guidelines. The seeds of lettuce used for the experiment (cv. Di Kagran 2) have a labeled germination rate of 78%. Germination tests were conducted in plastic container (18 × 18 × 4 cm) by using 170 g total substrate (hydrochar plus potting mix substrate). A commercial potting mix substrate, commonly used for horticultural applications (Humin substrat N3, Neuhaus, Germany), was used as control. Hydrochar was mixed homogeneously with the potting mix substrate at different weight percentages: 25%, 50%, and 75% (wt %) in the preliminary tests and 5%, 10%, 15%, and 20% (wt %) in the following tests. A 100% potting mix substrate was used as control treatment. Each treatment was repeated four times. After wetting the substrate with water, lettuce seeds (100 seeds per container) were placed slightly below the substrate surface. Afterwards, containers were introduced into a climatic chamber and kept at constant temperature of 20 °C, and artificially lighted with cool white-light fluorescent lamps (Osram L18 W/20, 10 µmol photons s^{-1} m^{-2}) for 7 days. The containers were enveloped in transparent plastic bags to avoid moisture loss, and opened in such a way to guarantee free gas exchange. After 7 days, a final germination count was made by assessing the percentage of germinated seeds, as well as by measuring seedlings radicle lengths for each treatment.

Data of seed germination tests were subjected to the analysis of variance (ANOVA) using the statistical software Costat Cohort V6.201 (2002). A factorial design with treatment (T) and hydrochar percentage in the peat-based substrate (D) was used. The effects of T, D, and their reciprocal interactions were analyzed by two-way completely randomized ANOVA. Means were separated on the basis of least significance difference (LSD) test only when the ANOVA *F*-test per treatment was significant at the 0.05 probability level [28]. The Bartlett's test and the Shapiro–Wilk test were performed to assess the homogeneity of error variances and the normality of residual distribution, respectively [28]. Both tests did not give significant results for each analyzed parameter; therefore, no data transformation was needed.

Table 1. Elemental analysis (C, H, N, O) and proximate analysis of fresh hydrochar (FH), washed hydrochar (WH) and aged hydrochar (AH), compared to that of biomass feedstock (BF), peat, and lignite.

Sample	C (wt %) *	H (wt %) *	N (wt %) *	O (wt %) *	Moisture (wt %)	Ash (wt %) **	Volatiles (wt %) *	Fixed C (wt %) *
FH	62.0	6.50	1.40	30.1	8.2	12.3	73.3	73.3
WH	60.9	6.35	1.65	31.1	2.7	10.7	73.1	73.1
AH	60.8	6.10	1.40	31.7	6.9	18.2	72.2	72.2
BF	54.7	6.56	1.74	37.0	46.5	25.9	87.2	87.2
Peat	49–60	5–8	1–4	28–45			70–80	70–80
Lignite	65–73	5–8	0.5–1.5	16–30			47–60	47–60

* Dry and ash-free basis; ** dry basis.

3. Results

Table 1 reports the results of the elemental and proximate analysis of the fresh hydrochar (FH), washed hydrochar (WH), and aged hydrochar (AH) in comparison with the biomass feedstock (BF), and the typical range of composition of peat and lignite [29].

For evaluating the use of FH as soil amendment, typical FH chemical characteristics have been compared with the limitations reported by the current Italian law (D. Lgs. 75/2010) on no-composted and composted green waste used as soil amendment (Table 2).

Table 3 reports additional chemical parameters that may influence the agronomic performance of FH when used as soil amendment, as well as parameters that may result harmful when hydrochar is used as organic growth medium for vegetable nursery production.

Table 2. Regulated FH parameters for the use as soil amendment.

Parameter	Unit		Limitation [a]	Limitation [b]
pH		5.8	6–8.5	6–8.5
Moisture	wt %	8.2	<50	<50
Organic C	wt % d.b.	52.2	>40	>20
Organic N	% of T.N. [†]	>99.8%	>80%	>80%
C/N		44	-	<50
Humic/Fulvic C	wt % d.b.	17.3	-	>2.5
Pb	mg kg^{-1} d.b.	<60	<140	<140
Cd	mg kg^{-1} d.b.	0.22	<1.5	<1.5
Ni	mg kg^{-1} d.b.	<15	<100	<100
Zn	mg kg^{-1} d.b.	84	<500	<500
Cu	mg kg^{-1} d.b.	<60	<230	<230
Hg	mg kg^{-1} d.b.	0.06	<1.5	<1.5
Cr(VI)	mg kg^{-1} d.b.	<0.3	<0.5	<0.5

[a] Limitations on no-composted green waste use as soil amendment according to the Italian law (D. Lgs. 75/2010); [b] Limitations on composted green waste use as soil amendment according to the Italian law (D. Lgs. 75/2010); [†] T.N.: Total Nitrogen; d.b.: dry basis.

Regarding the primary macronutrients, total N content is similar to the average N content of many productive arable soils, while exchangeable K concentration is very low [30]. As far as the secondary macronutrients are concerned (Ca, Mg, sulfates), FH shows a medium content of Ca and a very high concentration of magnesium, and sulfur as sulfates [31]. The micronutrients are satisfactorily represented into FH [32]. Fresh hydrochar shows a very low cation exchange capacity (C.E.C.) [33]. Electrical conductivity (EC) of FH, close to the USDA A class of soil salinity (<2 mS cm^{-1}), determined the level of FH salinity to be very low [34]. On the other hand, sodium concentration in FH is quite high, and may negatively affect crop productivity [27]. Regarding polyphenols and tannins, FH is characterized by a quite high content of these substances, while the VFA content is

quite low. Biomass respiration value indicates a high FH biological activity according to the USDA classification [35].

Table 3. Additional FH chemical parameters.

Parameter		Unit
Total N	0.12	wt % d.b.
Exchangeable K	46.0	mg kg^{-1} d.b.
Calcium	26,640	mg kg^{-1} d.b.
Magnesium	1859	mg kg^{-1} d.b.
SO_4^{2-}	1312	mg kg^{-1} d.b.
Cl^-	675	mg kg^{-1} d.b.
Iron	1414	mg kg^{-1} d.b.
Boron	<25	mg kg^{-1} d.b.
Manganese	<55	mg kg^{-1} d.b.
Zn	84	mg kg^{-1} d.b.
Cu	<60	mg kg^{-1} d.b.
C.E.C. (cation exchange capacity)	3.8	meq 100 g^{-1}
$EC_{1:5}$	2.07	mS cm^{-1}
Sodium	873	mg kg^{-1} d.b.
Total polyphenols (as tannic acid)	27,204	mg kg^{-1} d.b.
Total tannins (as tannic acid)	22,822	mg kg^{-1} d.b.
VFAs	390	mg kg^{-1} d.b.
Soil respiration	4247	mg C CO_2 kg^{-1}

d.b.: dry biomass.

Table 4 reports FH total polycyclic aromatic hydrocarbon (PAH) concentration and the contribution of individual PAHs. A predominance of the low molecular weight naphthalene species may be observed. Table 5 shows the effect of washing (WH) and aging (AH) treatment on selected characteristics of FH in view of its use as organic growth substrate. In comparison with fresh hydrochar, pH increased markedly with washing and aging treatments. Also, EC was affected by the treatments, with a reduction of 68% and 47% in WH and in AH, respectively, compared to FH. Sulfate concentration decreased similarly under washing and aging (59% and 57% lower than FH, respectively). Post-treatment effect on FH chlorine concentration was more evident with washing (−90% in comparison with FH) than aging (−35%). Similarly, washing considerably reduced FH sodium concentration with respect to aging (−85% vs. −72%). FH washing did not affect significantly the organic carbon content, whilst the aging treatment caused a slight decrease. A remarkable decrease (−98%) of VFA content was observed after aging FH, while a lower reduction (−26%) was observed after washing FH. The treatments produced the same effects on FH phenolic compounds concentration, by reducing them both after aging and washing (−41% vs. −15% for total polyphenols, and −37% vs. −15% for total tannins, respectively, for aging and washing compared to FH). Hydrochar respiration decreased only after aging. Table 6 reports the characterization of the potting mix substrate (Humin substrat N3) as far as volatile fatty acid, total polyphenols, and total tannins as concerned. In comparison with the hydrochars, the potting mix substrate showed a remarkably lower content of polyphenols and tannins.

Table 4. FH polycyclic aromatic hydrocarbon (PAH) content.

Parameter	Unit	FH	Rings Number
Benzo(a) anthracene	mg kg^{-1} d.b.	<0.1	4
Benzo(a) pyrene	mg kg^{-1} d.b.	<0.1	5
Benzo(b)fluoranthene	mg kg^{-1} d.b.	<0.1	5
Benzo(k)fluoranthene	mg kg^{-1} d.b.	<0.1	5
Chrysene	mg kg^{-1} d.b.	<0.1	4
Dibenzo(a,h) anthracene	mg kg^{-1} d.b.	<0.1	5

Table 4. *Cont.*

Benzo(e) pyrene	mg kg^{-1} d.b.	<0.1	5
Benzo(j) fluoranthene	mg kg^{-1} d.b.	<0.1	5
Naphthalene	mg kg^{-1} d.b.	0.6	2
Acenaphthene	mg kg^{-1} d.b.	<0.1	3
Acenaphthylene	mg kg^{-1} d.b.	<0.1	3
Anthracene	mg kg^{-1} d.b.	<0.1	3
Benzo(g,h,i) perylene	mg kg^{-1} d.b.	<0.1	6
Phenanthrene	mg kg^{-1} d.b.	<0.1	3
Fluorene	mg kg^{-1} d.b.	<0.1	3
Indeno(1,2,3-c,d) pyrene	mg kg^{-1} d.b.	<0.1	6
Pyrene	mg kg^{-1} d.b.	<0.1	4
$\sum$ PAHs	mg kg^{-1} d.b.	<2.2	

d.b.: dry biomass.

Table 5. Effects of post-treatments on hydrochar chemical properties.

Parameter	Unit	FH †	WH †	AH †
pH		5.82 c	6.31 b	6.90 a
$EC_{1:5}$	mS cm^{-1}	2.07 a	0.66 c	1.10 b
SO_4^{2-}	mg kg^{-1} d.b.	1312 a	538 b	565 b
Cl^-	mg kg^{-1} d.b.	675 a	68 c	436 b
Na	mg kg^{-1} d.b.	873 a	128 c	245 b
C_{org}	wt % d.b.	52.2 a	50.8 a	48.7 b
N_{org}	% of T.N.	>99.8	>99.6	>99.6
Humic/Fulvic C	wt % d.b.	17.3 a	12.9 b	13.8 b
VFAs	mg kg^{-1} d.b.	390 a	288 b	8 c
Total polyphenols (as tannic acid)	mg kg^{-1} d.b.	27,204 a	23,140 b	16,128 c
Total tannins (as tannic acid)	mg kg^{-1} d.b.	22,822 a	19,404 b	14,320 c
Biomass respiration	mg C CO_2 kg^{-1}	4247 a	4124 a	3731 b

d.b.: dry biomass; † within each row, treatment means followed by different letters are different according to the Fisher's least significance difference (LSD) protected test ($p \leq 0.05$).

Table 6. Characterization of the potting mix substrate (Humin substrat N3).

Parameter	Unit	
VFAs	mg kg^{-1} d.b.	n.d.
Total polyphenols (as tannic acid)	mg kg^{-1} d.b.	1450
Total tannins (as tannic acid)	mg kg^{-1} d.b.	209

d.b.: dry biomass; n.d.: not detectable.

The results of the preliminary lettuce germination tests with 25%, 50%, and 75% (wt %) of fresh, washed, and aged hydrochar are reported in Figure 1a. The obtained results show that treatment (fresh, washed, and aged), hydrochar dose and their interaction significantly affected *L. sativa* seed germination. A remarkable reduction of germination percentage has been observed going from the control (0% hydrochar) to each higher concentration of hydrochar (either as FH, WH, or AH) in the substrate, even at the minimum examined dose (i.e., 25%). Averaged over treatments, mean germination percentages of 9.7%, 7.3%, and 3.7% have been observed respectively for 25%, 50%, and 75% of hydrochar in the blend. In particular, the fresh hydrochar was the most phytotoxic growing media for lettuce seed germination. At 25% of FH, the germination percentage decreased from 87% of the control to 1%; at higher FH doses (50% and 75%), no seed germination was observed at all.

The washing treatment generally improved lettuce seed germination, in comparison with FH. In this case, the highest percentage of germination has been observed with the lowest amount of WH (25%) in the substrate blend, showing an inverse correlation between its presence in the potting mix

and seed germination at higher concentration rates (Figure 1a). For the aging treatment, a different trend was observed. Overall, a remarkable increase in the germination percentage has been detected in comparison with the FH and WH, for all the tested doses. The reduction in seed germination was noteworthy only when increasing the presence of AH in the blend from 0 to 25%, and from 50 to 75%, while no significant differences were detected between the two intermediate concentrations (Figure 1a).

These results have been confirmed also by the data of *L. sativa* radicle length (Figure 1b). Averaged over treatments, increased concentrations of hydrochar in the blend considerably reduce lettuce radicle length. Whatever the concentration of hydrochar in the blend, lettuce radicle length was remarkably increased by using washed hydrochar and, even more, aged hydrochar. Fresh hydrochar has reset the growth of the radicle from 50% of concentration in the blend, whilst for treated hydrochar, we never observed values below 1 cm, even at the highest concentration rates. For washed hydrochar, a considerable reduction in radicle length occurred only between 0% and 25%, and between 50% and 75% of concentration. Interestingly, in the case of aged hydrochar, we did not observe a significant reduction of radicle length from 50 to 75%, with values still very close to that shown at 25% of concentration in the blend (Figure 1b).

These results highlighted that FH was an inadequate organic growth medium for nursery production of *L. sativa* (a very sensitive species) and that the post-treatments of hydrochar (WH and AH) all improved the germination of lettuce seeds, with best performances obtained with AH substrate. Nevertheless, any combination between dose and treatment was able to ensure an acceptable seed germination percentage.

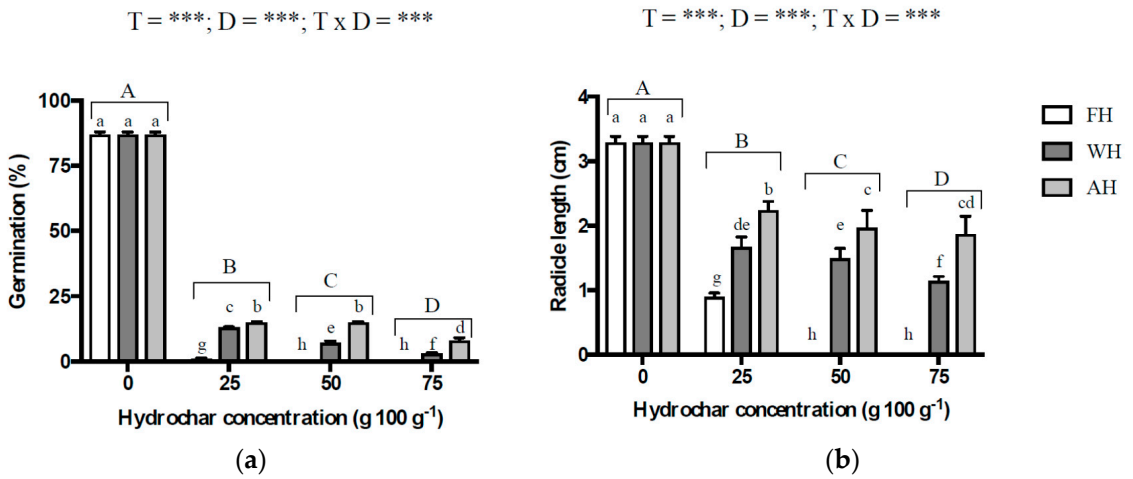

Figure 1. Effect of treatment (fresh, FH, washing, WH, and aging, AH), hydrochar doses (0%, 25%, 50%, and 75%, wt %) and their interaction on seed lettuce germination (**a**,**b**) radicle length. Each bar is the mean ± SE of the results from four replications. Means followed by different letters are different according to the Fisher's LSD protected test. The mean effect of hydrochar treatment and concentration are represented, respectively, by lower- and uppercase letters. T = treatment; D = hydrochar dose; T × D = treatment × hydrochar dose interaction; *** Significant at $p < 0.001$ level.

According to these preliminary results, further tests at lower doses of WH and AH have been carried out in order to reduce the FH phytotoxic effects using, respectively, 5%, 10%, 15%, and 20% (wt %) of the treated hydrochar (WH and AH) in the potting mix (Figure 2).

Overall, phytotoxicity, both in terms of reduction in seed germination and radicle length, considerably decreased, again, with decreasing hydrochar concentration in the potting mix. Aging allowed again for lower reduction in germination percentage and radicle length, compared to washing. The highest values of germination percentage of *L. sativa* seeds have been reached by using 5% and 10% of AH added to the substrate (56% and 54%, respectively; Figure 2a). At the same time,

the aging treatment remarkably increased radicle length of lettuce seedlings, with higher values at 5%, 10%, and 15% doses, in comparison to the washing treatment (Figure 2b).

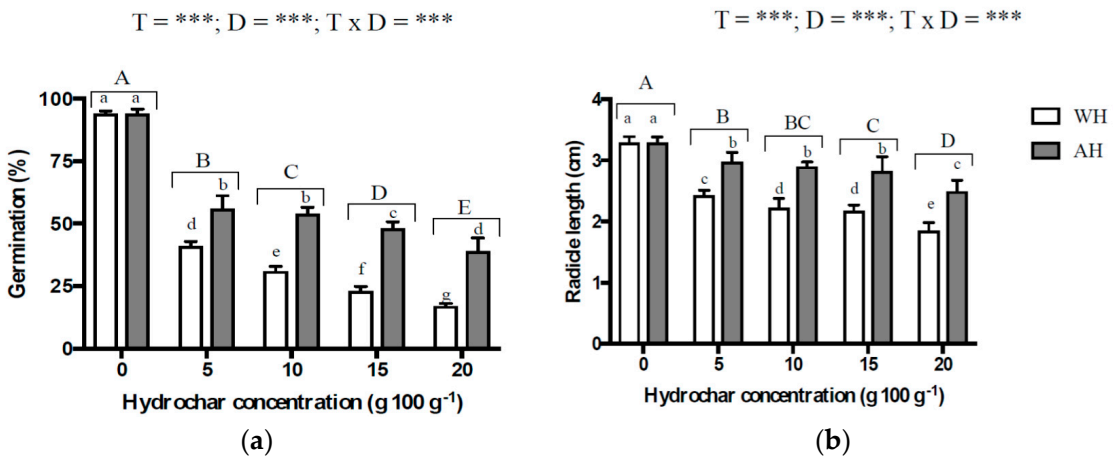

Figure 2. Effect of treatment (washing, WH, and aging, AH), hydrochar doses (0%, 5%, 10%, 15%, and 20%, wt %) and their interaction on lettuce seeds: (**a**) seed germination; (**b**) radicle length. Each bar is the mean ± SE of the germination results from four replications. Treatment means followed by different letters are different according to the Fisher's LSD protected test. T = treatment; D = hydrochar dose; T × D = treatment × hydrochar dose interaction; *** Significant at $p < 0.001$ level.

4. Discussion

4.1. Fresh Hydrochar (FH) Chemical Properties

As expected [1], through the carbonization process, the biomass was converted into a carbonaceous matrix enriched in total and fixed carbon with a lower content of ashes, volatile organic matter and oxygen (Table 1), and characterized by lower O/C and H/C ratios compared to the initial feedstock. According to the elemental and proximate analyses, as well as the van Krevelen diagram, FH composition fits in the overlapping region of composition of peat and low-ranking lignite. According to previous studies on pyrochars [36,37], a H/C ratio ≤0.3 indicates a highly condensed aromatic ring system, whereas a H/C ratio ≥0.7 represents a non-condensed aromatic ring system. Desirable O/C and H/C ratios of biochars suitable for C sequestration in soils are ≤0.4 and ≤0.6, respectively [38], typical of low-polar and highly aromatized carbonaceous matrices that are generally highly refractory to microbial degradation. In our study, O/C ratios of fresh and treated hydrochars were close to 0.4, whereas H/C ratios were by far higher than 0.6. When compared to the initial feedstock, the hydrochar seems to have experienced a transformation that has produced a carbonaceous matrix relatively more stable to microbial degradation in comparison with the initial feedstock, but still susceptible to biological activity and exchange capacity in combination with soil, due to the residual polarity and to the non-condensed aromatic ring system [39].

As reported in Table 2, FH practically complies with the regulated parameters for the use as soil amendment with the exception of pH, which is slightly lower than the minimum value. The results highlight FH positive properties as soil amendment, due to its optimal organic C and C/N values and the very low presence of heavy metals and other contaminants. In compliance with the Italian law, D. Lgs. 75/2010, the biochar produced by residual biomasses through hydrothermal carbonization can be used as soil amendment according to the characteristics and qualities indicated in the above regulation. It could be foreseen that the permission to use as soil amendment will also be extended to hydrochar. The pH is fully consistent with the reported pH values of hydrochars, generally acidic [15,16,39]. Although important, hydrochar pH was found to be not critical, because it was very close to the regulated value, and may be corrected if necessary. Furthermore, materials often used as potting

mix, like composts from agricultural or agro-industrial wastes, tend to be alkaline, and some authors recommend acidifying amendments before using them as growth media [40].

In addition to the compliance with the limits imposed by the Italian regulations, FH showed additional interesting properties in view of its use as soil amendment (Table 3). With respect to cattle fresh manure, the most common soil amendment used for improving arable soil fertility, FH displayed higher content in organic C, Ca, and other micronutrients, such as Mg, Zn, Cu, Na, Cl. In particular, Na and Cl concentrations were higher than cattle manure or peat. Nevertheless, these values are within the typical range reported for different feedstocks [15,37,41], even if Na and Cl could increase FH electrical conductivity (EC). From the agronomical point of view, the EC level measured for FH does not represent a barrier to its utilization, because it could only reduce the yield of the most sensitive crop species [34]. The chloride concentration, close to 600 mg kg^{-1}, although not excessive, can be considered as a critical threshold. In fact, chloride tends to accumulate at toxic levels in plant tissues, particularly in the leaves, causing growth damage and yield reduction, due to water absorption limitation and tissue necrosis [42]. On the contrary, C.E.C. and macronutrient concentrations were low with respect to cattle manure [43,44]. According to the soil chemical evaluation guidelines [45], substrates with C.E.C. lower than 10 meq 100 g^{-1} are unable to supply appreciable amount of nutrients.

Regarding polyphenols and tannins, their potential inhibition effect on seed germination and radicle elongation and development is known [46,47]. Moreover, seed germination and plant growth studies conducted in soils wetted with water extracts with known concentration of VFAs reported the toxicities of VFAs as significant, above 3000 mg L^{-1} [48–50]. In our case, even if the content of VFAs in the raw material (i.e., FH) was relatively low (390 mg kg^{-1}), it could not be excluded that their solubilization in the wetted germination substrate could generate higher concentrations of VFAs in water solutions, and thus, a potential phytotoxic effect, due to the adherence of the substrate to seeds and roots. This may also occur in case of low rate (e.g., 1 t ha^{-1} $year^{-1}$) field application of FH as soil amendment incorporated in the topsoil, with the production and release of VFAs being likely extremely enhanced in the rhizosphere, depending on several soil parameters, such as microbial activity, soil nutrient status, and other physical conditions (moisture, temperature, and pH). Therefore, in particular environmental conditions, we cannot exclude that the phytotoxicity of VFAs generated from hydrochar after its application to the soil could be enhanced. On the other hand, soil biological (e.g., microbial composition and activity) and physical conditions may also play an opposing role by decreasing the production or buffering the phytotoxic effect of VFAs in agricultural soils, as demonstrated by several incubation studies [51].

The polyphenol content of FH was very high (27,204 mg kg^{-1}), considering that these compounds are usually absent in slurry and animal manure, and may reach concentrations of 4.2–39.0 mg kg^{-1} d.b. in peat of different origins [52,53].

Table 4 reports FH total PAHs concentration and the contribution of individual PAHs. The FH total PAH content lower than 2.2 is fully consistent with total mean PAH concentration of hydrochars from different biomass feedstocks (polar wood chips, solid olive residues, wheat straw), which is reported to increase from 1.8 to 5.0 mg kg^{-1} with increasing carbonization temperature from 180 to 230 °C [39]. This result is also consistent with the quite high value of H/C ratio previously discussed. Considering that the standard limits for PAHs in biosolids in the EU range from 3 to 6 mg kg^{-1} [54], FH total PAH content was well below this limit in our study. Among the individual PAHs, the contribution of naphthalene, the lowest molecular weight molecule (two-rings), dominated the FH speciation. This result agrees with Wiedner et al. [39] who showed, for different feedstocks and temperatures, high occurrence of the lighter two- and four-ring molecules, compared to negligibly low percentages of the six-ring ones, even if reporting a heterogenic distribution of PAHs patterns with regard to feedstocks and carbonization temperature. The molecular weight of PAHs plays an important role for the behavior of PAHs in the environment, as well as in determining their carcinogenic and mutagenic properties. PAHs with high molecular weight have a high sorption tendency, and therefore, high potential of bioaccumulation. Besides, the low heavy metals and PAH content, coupled with the individual PAH

pattern and contribution, do not raise specific reasons for concern, either from an environmental or from a phytotoxic point of view, as also reported by other authors [41].

The low C.E.C. and the relatively high concentrations of Na and Cl, associated with the high content of potentially phytotoxic substances (VFAs and polyphenols), suggest that it would be safer to use FH as soil amendment, rather than as a substitute for peat in plant growth substrates. Evidence from previous studies suggested that mixing hydrochar with the soil would be an effective way to definitively reduce its phytotoxicity. Voroney et al. [55], for instance, found that VFAs and polyphenol compounds, accumulated in the soil to phytotoxic concentrations during the decomposition of particular crop residues in laboratory studies, were not detected in field studies when applying the same treatments [55]. Especially in the case of polyphenols, according to Siqueira et al. [56], fresh biomass may be more phytotoxic than soil-retained phytotoxins, due to the complex pathway that phenolic compounds go through in soil.

4.2. Effects of Post-Treatments on Hydrochar Properties

Washing and aging treatment were applied and tested for making FH more suitable to be used as a component of potting compost for seedling production. Both treatments increased hydrochar pH values close to neutrality (Table 5). This positive result may be related to the washing out of chlorides, sulfates, and sodium, as indicated also by the EC reduction. The chloride and sodium reduction was higher with the washing treatment, as well as EC, indicating a strict correlation among these parameters. The pH increase was higher after the aging treatment than with washing; similarly, a more appreciable reduction of polyphenols, tannins, and VFAs was detected in AH samples. These results may be related to the occurrence of biological activity of hydrochar during aging also observed by other authors [16]. This hypothesis is supported by the noteworthy FH organic C decrease after aging (Table 5), by the reduction of the humic/fulvic C ratio in AH samples with respect to FH (13.7% on dry basis compared to 17.3%) and by the values of substrate respiration. The latter indicated 4247 mg C CO_2 kg^{-1} soil $week^{-1}$ for FH and 3731 mg C CO_2 kg^{-1} soil wk^{-1} for AH. In both cases, the hydrochar biological activity was high according to the USDA classification [35]. The remarkable decrease observed in polyphenols, tannins, and VFAs after treatment might have been related to this biological activity, indeed. These findings indicate that hydrochar is therefore capable of stimulating microbial colonies' growth and activity [42].

The results obtained suggest that through the two different post-treatments, as single process or in combination, it is possible to upgrade the FH to get the desired properties according to the specific end use.

4.3. Effect of Hydrochar Application on Lettuce Germination

The results obtained in this study highlighted that hydrochar, independently of treatments (none, washing, and aging) or concentrations, inhibited germination of *L. sativa* seeds. In fact, a progressive reduction of germination percentage has been observed going from the control (0% hydrochar) to progressively higher hydrochar concentration in the substrate, with differences depending on the type of hydrochar (FH, WH, and AH). The use of FH and the highest hydrochar concentrations (from 50 to 75%) drastically reduced lettuce seeds germination and radicle length (Figure 2), while AH and the lowest hydrochar concentration (i.e., 25%) showed appreciable germination percentage, as well as radicle length.

The most likely causes of phytotoxicity reported in literature are related to the presence of germination-inhibiting substances and stress factors, such as in the hydrochar matrix, as volatile organic acids (VFAs), phenols, aldehydes, PAHs, as well as salinity, alkalinity, or acidity [15–19,41,57], whose presence have been observed in the hydrochar tested in this study, with the highest content in the FH samples. According to the germination test results, the inhibition of germination could be mainly attributed to the presence of polyphenols (tannins) and VFAs, since the higher germination percentage, as well as the higher radicle length, were obtained with AH, which showed the lowest

concentration of these substances (Table 5). On the other hand, these hypotheses are corroborated by the remarkably low content of such substances in the potting mix substrate used as control in the germination tests (Table 6) that did not show phytotoxic effects.

Buss and Mašek [58] reported that the phytotoxic effects of the different types of biochar examined in their study, could be partly attributed to a reduction in pH caused by volatiles and dissolved compounds, and they concluded that other compounds, such as polyphenols, could be most likely involved in the adverse effects on germination, due to char application. Also, salinity might have played a stress role, since WH, characterized by the lowest salt concentration, displayed a less severe germination inhibition with respect to FH, whilst, despite a partial washout of polyphenols, tannins, and VFAs, the residual content of these phytotoxic species were still too high after the treatment. The result was a more severe germination and radical length inhibition with respect to AH sample. These findings confirmed the results reported by Bargmann et al. [15], who observed that washing, followed by drying, tends to further improve the germination rate of plants. These authors demonstrated that, even if the toxic substances present in the fresh hydrochar cannot be totally eliminated or evaporated by simple drying, a considerable amount could be washed out by using water. Additionally, Busch et al. [16] demonstrated remarkable improvement of germination performance when cress seeds were exposed to vapors from hydrochar that had been treated by drying and storing (in a closed container). Negative effects on plant germination and growth have also been reported for *Taraxacum* in greenhouse experiments with hydrochar mixed with soil [59], and for *Lolium perenne* L. in field experiments where hydrochar was mixed with pig slurry added as top dressing [60]. Also in these studies, hydrochar pre-washing or pre-incubation with slurry were suggested as possible attempts to alleviate the initial detrimental impact on germination and growth, which is, however, supposed to naturally decline after long-term aging of hydrochar in the soil.

Definitively, according to the results obtained in the present study, the type of treatment, as well as the hydrochar doses, play a key role in affecting the germination parameters (germination percentage and radicle length). In particular, our data highlight that aging was the most effective treatment in reducing hydrochar phytotoxicity, whatever the tested doses, even if the highest values of germination percentage registered (56% and 54%, with 5% and 10% of AH in the blend, respectively), are relatively low for practical application, and far below the threshold of crop economical sustainability.

However, according to Bargmann et al. [15], this phytotoxicity could be transient in the soil; these authors, in fact, showed that, after only 9 weeks from soil incorporation, hydrochar could be altered by physicochemical and/or biological soil processes, with a considerable reduction in the germination-inhibiting effect of hydrochar. A positive side effect of adding phenols and their functional groups through soil amendments, such as hydrochar, has been also demonstrated in terms of improved soil fertility and soil-borne disease control [61].

5. Conclusions

In this study, the chemical characterization of fresh hydrochar from municipal woody and herbaceous prunings allowed us to assess the compliance with Italian regulated parameters for its use as soil amendment at field scale. The results of germination tests performed with a sensitive species (*Lactuca sativa* var. *Capitata* (L.) Janchen) also suggest the use of fresh hydrochar as amendment at low application rate.

Regarding the use of hydrochar as substitute for peat in plant growth substrates, the concentration of potentially phytotoxic substances (VFAs, polyphenols, Na, and Cl) demands the upgrade of the fresh hydrochar by washing or aging. The greater reduction of chlorides and sodium content was obtained through the washing treatment, while a more remarkable reduction of polyphenols, tannins, and VFAs was detected in the aged samples. The lettuce germination tests of upgraded hydrochar showed the highest values of germination percentage applying aged hydrochar in potting mix (56% and 54%, with 5% and 10% in the blend, respectively), in comparison with fresh and washed hydrochar. According to these results of lettuce germination tests, the inhibition of germination could be mainly

attributed to the presence of polyphenols and VFAs, since the higher germination percentage, as well as the higher radicle length, were obtained with aged hydrochar, which showed the lowest concentration of these substances. Therefore, aged hydrochar showed promising properties as organic growth medium in horticulture and gardening. Further research is needed at field scale to highlight the interactions between hydrochar and the soil biological community.

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References

1. Libra, J.; Ro, K.S.; Kammann, C.; Funke, A.; Berge, N.D.; Neubauer, Y.; Titirici, M.-M.; Fühner, C.; Bens, O.; Kern, J.; et al. Hydrothermal carbonization of biomass residuals: A comparative review of the chemistry, processes and applications of wet and dry pyrolysis. *Biofuels* **2011**, *2*, 71–106. [CrossRef]
2. Pavlovič, I.; Knez, Ž.; Škerget, M. Hydrothermal reactions of agricultural and food processing wastes in sub- and supercritical water: A review of fundamentals, mechanisms, and state of research. *J. Agric. Food Chem.* **2013**, *61*, 8003–8025. [CrossRef] [PubMed]
3. Funke, A.; Ziegler, F. Hydrothermal carbonization of biomass: A summary and discussion of chemical mechanisms for process engineering. *Biofuels Bioprod. Biorefin.* **2010**, *4*, 160–177. [CrossRef]
4. Kambo, H.S.; Dutta, A. A comparative review of biochar and hydrochar in terms of production, physico-chemical properties and applications. *Renew. Sustain. Energy Rev.* **2015**, *45*, 359–378. [CrossRef]
5. Liu, Z.; Quek, A.; Balasubramanian, R. Preparation and characterization of fuel pellets from woody biomass, agro-residues and their corresponding hydrochars. *Appl. Energy* **2014**, *113*, 1315–1322. [CrossRef]
6. Liu, Z.; Quek, A.; Kent Hoekman, S.; Balasubramanian, R. Production of solid biochar fuel from waste biomass by hydrothermal carbonization. *Fuel* **2013**, *103*, 943–949. [CrossRef]
7. Lu, L.; Namioka, T.; Yoshikawa, K. Effects of hydrothermal treatment on characteristics and combustion behaviors of municipal solid wastes. *Appl. Energy* **2011**, *88*, 3659–3664. [CrossRef]
8. Reza, M.T.; Uddin, M.H.; Lynam, J.G.; Coronella, C.J. Engineered pellets from dry torrefied and HTC biochar blends. *Biomass Bioenergy* **2014**, *63*, 229–238. [CrossRef]
9. Titirici, M.-M.; White, R.J.; Falco, C.; Sevilla, M. Black perspectives for a green future: Hydrothermal carbons for environment protection and energy storage. *Energy Environ. Sci.* **2012**, *5*, 6796–6822. [CrossRef]
10. Igalavithana, A.D.; Ok, Y.S.; Niazi, N.K.; Rizwan, M.; Al-Wabel, M.I.; Usman, A.R.A.; Moon, D.H.; Lee, S.S. Effect of corn residue biochar on the hydraulic properties of sandy loam soil. *Sustainability* **2017**, *9*, 266. [CrossRef]
11. Sethupathi, S.; Zhang, M.; Rajapaksha, A.U.; Lee, S.R.; Nor, N.M.; Mohamed, A.R.; Al-Wabel, M.; Lee, S.S.; Ok, Y.S. Biochars as potential adsorbers of CH_4, CO_2 and H_2S. *Sustainability* **2017**, *9*, 121. [CrossRef]
12. Puccini, M.; Stefanelli, E.; Hiltz, M.; Seggiani, M. Activated Carbon from Hydrochar Produced by Hydrothermal Carbonization of Wastes. *Chem. Eng. Trans.* **2017**, *57*, 169–174. [CrossRef]
13. Nguyen, M.V.; Lee, B.K. Removal of dimethyl sulfide from aqueous solution using cost-effective modified chicken manure biochar produced from slow pyrolysis. *Sustainability* **2015**, *7*, 15057–15072. [CrossRef]
14. Hitzl, M.; Corma, A.; Pomares, F.; Renz, M. The hydrothermal carbonization (HTC) plant as a decentral biorefinery for wet biomass. *Catal. Today* **2015**, *257*, 154–159. [CrossRef]
15. Bargmann, I.; Rillig, M.C.; Buss, W.; Kruse, A.; Kuecke, M. Hydrochar and Biochar Effects on Germination of Spring Barley. *J. Agron. Crop Sci.* **2013**, *199*, 360–373. [CrossRef]
16. Busch, D.; Kammann, C.; Grünhage, L.; Müller, C. Simple Biotoxicity Tests for Evaluation of Carbonaceous Soil Additives: Establishment and Reproducibility of Four Test Procedures. *J. Environ. Qual.* **2012**, *41*, 1023–1032. [CrossRef] [PubMed]

17. Fornes, F.; Belda, R.M.; Lidón, A. Analysis of two biochars and one hydrochar from different feedstock: Focus set on environmental, nutritional and horticultural considerations. *J. Clean. Prod.* **2015**, *86*, 40–48. [CrossRef]
18. Jandl, G.; Eckhardt, K.-U.; Bargmann, I.; Kücke, M.; Greef, J.-M.; Knicker, H.; Leinweber, P. Hydrothermal Carbonization of Biomass Residues: Mass Spectrometric Characterization for Ecological Effects in the Soil–Plant System. *J. Environ. Qual.* **2013**, *42*, 199–207. [CrossRef] [PubMed]
19. Sun, Y.; Gao, B.; Yao, Y.; Fang, J.; Zhang, M.; Zhou, Y.; Chen, H.; Yang, L. Effects of feedstock type, production method, and pyrolysis temperature on biochar and hydrochar properties. *Chem. Eng. J.* **2014**, *240*, 574–578. [CrossRef]
20. Burguete, P.; Corma, A.; Hitzl, M.; Modrego, R.; Ponce, E.; Renz, M. Fuel and chemicals from wet lignocellulosic biomass waste streams by hydrothermal carbonization. *Green Chem.* **2016**, *18*, 1051–1060. [CrossRef]
21. Thomas, G.W. Exchangeable Cations. In *Agronomy Monograph, Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*; American Society of Agronomy, Soil Science Society of America: Madison, WI, USA, 1982; pp. 159–165. [CrossRef]
22. Mehlich, A. Determination of Cation- and Anion-Exchange Properties of Soils. *Soil Sci.* **1948**, *66*, 429–446. [CrossRef]
23. Isermeyer, H. Estimation of soil Respiration in Closed Jars. In *Methods in Applied Soil Microbiology and Biochemistry*; Alef, K., Nannipieri, P., Eds.; Academic Press: London, UK, 1952; ISBN 9780125138406.
24. Alef, K.; Nannipieri, P. *Methods in Applied Soil Microbiology and Biochemistry*; Academic Press: London, UK, 1995; ISBN 9780125138406.
25. Dewanto, V.; Wu, X.; Adom, K.K.; Liu, R.H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014. [CrossRef] [PubMed]
26. Schanderl, S.H. Methods in Food Analysis. In *Physical, Chemical and Instrumental Methods of Analysis*; Academic Press Inc.: New York, NY, USA, 1970.
27. Peacock, W.; Christensen, L. Interpretation of soil and water analysis. In *Raisin Production Manual*, Publication 3393 ed.; University of California Division of Agricultural and Natural Resources: Oakland, CA, USA, 2000.
28. Gomez, A.A.; Gomez, K.A. Statistical procedures for agricultural research. In *Statistical Procedures for Agricultural Research*; John Wiley & Sons: Hoboken, NJ, USA, 1984; Volume 6, p. 680.
29. Jess, A.; Wasserscheid, P. *Chemical Technology: An Integral Textbook*; Wiley-VCH: Weinheim, Germany, 2013; ISBN 3527304460.
30. Giandon, P. *L'Interpretazione delle Analisi del Terreno*; ARPAV: Vicenza, Italy, 2007; ISBN 8875041156.
31. Horneck, D.A.; Sullivan, D.M.; Owen, J.S.; Hart, J.M. *Soil Test Interpretation Guide*; Oregon State University Extension Service: Corvallis, OR, USA, 2011; pp. 1–12. [CrossRef]
32. Bonner, J.; Varner, J.E. *Mineral Metabolism, Plant Biochemistry*; Academic Press: London, UK, 1976; ISBN 0121148602.
33. Sonon, L.S.; Kissel, D.E.; Saha, U.K. *Cation Exchange Capacity and Base Saturation*; Cicular 1040 ed.; University of Georgia Extension: Athens, GA, USA, 2014.
34. Richards, L.A. Diagnosis and Improvement of Saline and Alkali Soils. In *USDA Ag. Handbook 60*; United States Department of Agriculture: Washington, DC, USA, 1954.
35. Natural Resources Conservation Service Soil. Available online: www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/health/assessment/?cid=nrcs142p2_053870 (accessed on 11 January 2017).
36. Hammes, K.; Smernik, R.J.; Skjemstad, J.O.; Herzog, A.; Vogt, U.F.; Schmidt, M.W.I. Synthesis and characterisation of laboratory-charred grass straw (*Oryza sativa*) and chestnut wood (*Castanea sativa*) as reference materials for black carbon quantification. *Org. Geochem.* **2006**, *37*, 1629–1633. [CrossRef]
37. Kuhlbusch, T.A.J.; Crutzen, P.J. Toward a global estimate of black carbon in residues of vegetation fires representing a sink of atmospheric CO_2 and a source of O_2. *Glob. Biogeochem. Cycles* **1995**, *9*, 491–501. [CrossRef]
38. Schimmelpfennig, S.; Glaser, B. One Step Forward toward Characterization: Some Important Material Properties to Distinguish Biochars. *J. Environ. Qual.* **2012**, *41*, 1001–1013. [CrossRef] [PubMed]
39. Wiedner, K.; Naisse, C.; Rumpel, C.; Pozzi, A.; Wieczorek, P.; Glaser, B. Chemical modification of biomass residues during hydrothermal carbonization—What makes the difference, temperature or feedstock? *Org. Geochem.* **2013**, *54*, 91–100. [CrossRef]

40. Carrion, C.; Garcia-de-la-Fuente, R.; Fornes, F.; Puchades, R.; Abad, M. Acidifying Composts from Vegetable Crop Wastes to Prepare Growing Media for Containerized Crops. *Compost Sci. Util.* **2008**, *16*, 20–29. [CrossRef]
41. Busch, D.; Stark, A.; Kammann, C.I.; Glaser, B. Genotoxic and phytotoxic risk assessment of fresh and treated hydrochar from hydrothermal carbonization compared to biochar from pyrolysis. *Ecotoxicol. Environ. Saf.* **2013**, *97*, 59–66. [CrossRef] [PubMed]
42. Dang, Y.P.; Dalal, R.C.; Mayer, D.G.; McDonald, M.; Routley, R.; Schwenke, G.D.; Buck, S.R.; Daniells, I.G.; Singh, D.K.; Manning, W.; et al. High subsoil chloride concentrations reduce soil water extraction and crop yield on Vertosols in north-eastern Australia. *Aust. J. Agric. Res.* **2008**, *59*, 321–330. [CrossRef]
43. Saharinen, M.H.; Vuorinen, A.H.; Hostikka, M. Effective cation exchange capacity of manure compost of varying maturity stages determined by the saturation-displacement method. *Commun. Soil Sci. Plant Anal.* **1996**, *27*, 2917–2923. [CrossRef]
44. Miller, J.; Beasley, B.; Drury, C.; Larney, F.; Hao, X. Influence of long-term application of composted or stockpiled feedlot manure with straw or wood chips on soil cation exchange capacity. *Compost Sci. Util.* **2016**, *24*, 54–60. [CrossRef]
45. Costantini, E.A.C. *Metodi di Valutazione dei Suoli e Delle Terre*; Cantagalli: Firenze, Italy, 2006; Volume 7.
46. Cochran, V.L.; Elliott, L.F.; Papendick, R.I. The Production of Phytotoxins from Surface Crop Residues1. *Soil Sci. Soc. Am. J.* **1977**, *41*, 903–908. [CrossRef]
47. Patrick, Z.A. Phytotoxic substances associated with the decomposition in soil of plant residues. *Soil Sci.* **1971**, *111*, 13–18. [CrossRef]
48. Bremner, J.M.; Krogmeier, M.J. Effects of urease inhibitors on germination of seeds in soil. *Commun. Soil Sci. Plant Anal.* **1990**, *21*, 311–321. [CrossRef]
49. Lynch, J.M. Effects of organic acids on the germination of seeds and growth of seedlings. *Plant Cell Environ.* **1980**, *3*, 255–259. [CrossRef]
50. Wallace, J.M.; Elliott, L.F. Phytotoxins from anaerobically decomposing wheat straw. *Soil Biol. Biochem.* **1979**, *11*, 325–330. [CrossRef]
51. Wanniarachchi, S.D.; Voroney, R.P. Phytotoxicity of canola residues: Release of water-soluble phytotoxins. *Can. J. Soil Sci.* **1997**, *77*, 535–541. [CrossRef]
52. Zak, D.; Roth, C.; Gelbrecht, J.; Fenner, N.; Reuter, H. Polyphenols as enzyme inhibitors in different degraded peat soils: Implication for microbial metabolism in rewetted peatlands. In Proceedings of the EGU General Assembly Conference, Vienna, Austria, 12–17 April 2015.
53. Sharma, H.S.S.; McCall, D.; Lyons, G. Chemical changes in Peat as a Result of Neutralizing with Lime during the Preparation of Mushroom Casing. In *Mushroom Biology and Mushroom Products, Proceedings of the 2nd International Conference, University Park, PA, USA, 9–12 June 1996*; Pennsylvania State University: UniversityPark, PA, USA, 1996; pp. 363–372.
54. European Commision, Working Group On Polycyclic Aromatic Hydrocarbons. *Ambient Air Pollution by Polycyclic Aromatic Hydrocarbons (PAH)*; Office for Official Publications of the European Communities: Luxembourg, 2001; ISBN 928942057X.
55. Voroney, R.P.; Farquharson, B.J.; Janovicek, K.J.; Bauchamp, E.G.; Vyn, T.J.; Fortin, M. *Technology Evaluation and Development Sub-Program: Yield Reduction Effects of Crop Residues in Conservation Tillage, Agriculture Canada Research Station*; Agriculture Canada, Research Station: Harrow, ON, Canada, 1992.
56. Siqueira, J.O.; Nair, M.G.; Hammerschmidt, R.; Safir, G.R.; Putnam, A.R. Significance of phenolic compounds in plant-soil-microbial systems. *CRC Crit. Rev. Plant Sci.* **1991**, *10*, 63–121. [CrossRef]
57. Eibisch, N.; Helfrich, M.; Don, A.; Mikutta, R.; Kruse, A.; Ellerbrock, R.; Flessa, H. Properties and Degradability of Hydrothermal Carbonization Products. *J. Environ. Qual.* **2013**, *42*, 1565–1573. [CrossRef] [PubMed]
58. Buss, W.; Mašek, O. Mobile organic compounds in biochar—A potential source of contamination—Phytotoxic effects on cress seed (*Lepidium sativum*) germination. *J. Environ. Manag.* **2014**, *137*, 111–119. [CrossRef] [PubMed]
59. Rillig, M.C.; Wagner, M.; Salem, M.; Antunes, P.M.; George, C.; Ramke, H.-G.; Titirici, M.-M.; Antonietti, M. Material derived from hydrothermal carbonization: Effects on plant growth and arbuscular mycorrhiza. *Appl. Soil Ecol.* **2010**, *45*, 238–242. [CrossRef]
60. Schimmelpfennig, S.; Müller, C.; Grünhage, L.; Koch, C.; Kammann, C. Biochar, hydrochar and uncarbonized feedstock application to permanent grassland—Effects on greenhouse gas emissions and plant growth. *Agric. Ecosyst. Environ.* **2014**, *191*, 39–52. [CrossRef]

61. Makoi, J.; Ndakidemi, P. Biological, ecological and agronomic significance of plant phenolic compounds in rhizosphere of the symbiotic legumes. *Afr. J. Biotechnol.* **2007**, *6*, 1358–1368.