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Hydrochars Derived from Spent Coffee Grounds as Zn Bio-Chelates for Agronomic Biofortification

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Abstract: Previous studies have attributed both phytotoxicity and the capacity to mobilize nutrient elements to the presence of polyphenols and melanoidins in spent coffee grounds (SCG) and SCG-hydrochars obtained through hydrothermal carbonization (HTC). This work aimed to evaluate SCG and two SCG-hydrochars obtained at 160 and 200 °C that were functionalized with Zn salts (bio-chelates), to achieve the in vitro biofortification of lettuce. Two application modes were established: (1) a fixed Zn concentration of 10 mg kg^{-1} of soil and (2) a fixed dose of 0.5% bio-product. Soil alone (control A) and commercial chelates (control B) were used as controls. Outcomes showed that SCG-hydrochars retain the capacity to mobilize Zn compared to SCG. However, the chelating capacity was reduced (Zn: 94%) and the toxicity was significantly increased ($p < 0.05$) with higher temperatures of HTC (200 °C). Both fresh and dry lettuce weights were less affected at doses of 0.5% of bio-product and registered a maximum increase of 136% of Zn in the plant content. The present study approaches the possibility of using these by-products as bioinorganic fertilizers at subtoxic doses, although more research is needed.

Keywords: hydrothermal carbonization; micronutrients; bio-chelates; hidden hunger; agricultural soil; spent coffee grounds; biofortification

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1. Introduction

Dietary micronutrient deficiency of zinc, iodine, vitamin A, or iron is a form of undernutrition known as 'hidden hunger'. It affects more than two billion people worldwide and occurs when the intake or absorption of essential vitamins and minerals is insufficient. This results in extensive health problems in the developing world [1]. Zinc deficiency is related, for instance, to physical growth retardation, impairment of the immune system, and poor reproductive health, etc. [2]. According to Adriano [3], available Zn in soils represents between 1.4 and 8% of total Zn content. Several strategies have been used to tackle micronutrient deficiencies. Commercial chelates (diethylenetriamine pentaacetic acid [DTPA], ethylenediaminetetraacetic acid [EDTA], ethylenediamine-N, N′-bis (2-hydroxyphenylacetic acid) [EDDHA]) and salts are the most commonly used tools to accomplish agronomic Zn biofortification [4,5]. Despite this, all of these strategies are characterized by certain limitations. Commercial chelates, may have negative effects on plants, such as toxicity and impaired nutrient balance [5], whilst salts, when highly soluble, tend to be unavailable in soil [6].

Recent studies have shown the potential of bio-residues to overcome these issues. Over 120 million tons of bio-residues are generated annually in Europe, most of which are deposited in landfills, with small amounts being incinerated [7], causing economic and environmental problems. To combat this process, waste from the agri-food industry is being increasingly revalued as organic amendments in order to promote a circular economy [8].

The aim of a circular economy is to manage bio-residues and keep nutrients inside the economic cycle for as long as possible. In recent years, some sustainable management technologies have been applied to agri-food waste materials, such as anaerobic digestion, incineration, gasification, pyrolysis, and hydrothermal carbonization (HTC) [7]. Due to the rich lignocellulosic nature of agricultural waste, thermochemical conversion is more frequently used for its chemical transformation [9]. The resulting HTC solid by-products are known as hydrochars [10]. Generally, this process leads to an increase in fixed carbon, ashes, and carbon content, with a decrease in volatile matter and oxygen content also occurring [11–13]. The vast majority of studies conducted on hydrochar generation found in existing literature are focused on aspects of bioenergy and chemistry [14,15], with very little information existing on the use of hydrochar in the agronomic field [16,17]. The use of spent coffee grounds (SCG) as an organic soil amendment has recently been studied due to their interesting macronutrient composition, of mainly lignin, cellulose, and hemicellulose, as well as their diverse array of mineral and bioactive compounds [18]. The addition of SCG to soil is reported to improve its physical, chemical, and biological properties [19–21]. However, the high content of toxic compounds, such as caffeine, polyphenols, and tannins [22], has been found to reduce the crop biomass of lettuce [19]. Similar findings have been reported for the application of SCG hydrochars in lettuce [17]. However, SCG have been found to increase plant mineral elements such as vanadium (V), iron (Fe), cobalt (Co), manganese (Mn), and zinc (Zn) [23]. This effect has been attributed to the presence of chelating capacity compounds such as melanoidins [24] and polyphenols [25].

Given that HTC increases the total polyphenol content in SCG hydrochars [17], we hypothesize that the chelating power of these residues will also increase. If confirmed, SCG-hydrochars could be used as bio-chelates when applied in non-phytotoxic quantities. In order to examine this hypothesis, the aim of the present study was to assess the Zn biofortification effect of SCG-hydrochars functionalized at two temperatures (at 160 and 200 °C) in in vitro lettuce assays. Lettuce was taken as a reference vegetable as it is one of the most highly consumed vegetables globally and has been included in some agronomic biofortification studies [4,26,27]. In order to verify the effect of functionalization, non-functionalized bio-products were also examined, since these bio-products, by themselves, have the capacity to mobilize the aforementioned elements. The present study follows the line of research explored by a working group set up by the present researchers to examine the chelating capacity of both SCG and melanoidins functionalized with Zn. The present study is important due to the large amount of SCG generated worldwide and the challenges faced when using it as a soil organic amendment due to its high phytotoxicity.

2. Materials and Methods

2.1. Spent Coffee Grounds, Soil, and Lettuce

SCG were obtained from the cafeteria within the Faculty of Pharmacy (University of Granada, Spain). They were spread into a thin layer and dried at room temperature (22–25 °C) for one week to remove residual moisture. Soil samples were taken from the arable layer (0–20 cm) of Mediterranean agricultural soil. These were collected from an experimental plot that is the property of the Andalusian Government, Spain (IFAPA Center), Camino de Purchil, Granada (37°11′15″ N, 3°36′39″ W). According to the IFAPA Center, the soil has the following properties: sand 37.5%, silt 50%, clay 12.5% (texture loamy–silty), pH 8.7, electrical conductivity (EC_{25}) 0.35, OC 0.91%, carbonates as $CaCO_3$ 13.78%, C/N 13, total N 0.03%, available K 206.15 ppm, available P 11.02 ppm, water content at field capacity 20.7% (*w:w*), and water content at permanent wilting point 6.1% (*w:w*). Soil samples were air-dried applying the same procedure as for SCG and sieved (5 mm). The plant used for the study was 30-day-old lettuce (*Lactuca sativa* var. *longifolia*) of the "Little Duende" variety, acquired from a commercial greenhouse (Saliplant S.L., Granada, Spain).

2.2. Hydrochars Production via Hydrothermal Carbonization

Hydrothermal carbonization (HTC) was carried out in a 1 L reactor (Highpreactor™ BR-300, Berghof Ltd., Königsee, Germany) consisting of a reactor body, heater, and steam condenser. Thirty grams of SCG and deionized water with a solid:water (*w:w*) ratio of 1:10 were loaded into the reactor. The temperature was then set to 160 and 200 °C (heating rate of 15–20 min) to produce H160 and H200 hydrochars, respectively. Reaction time was set at 1 h and agitation speed at 300 rpm. No initial pressure conditions were used. Hydrochars produced by HTC were recovered by vacuum filtration after the reactor reached atmospheric pressure and room temperature. These by-products were dried in an oven at 60 °C overnight.

2.3. Bio-Chelates

SCG and SCG-hydrochars (H160, H200) were functionalized with Zn following a modified procedure described by Morikawa and Saigusa [25]. The reagent employed for Zn chelates was zinc sulfate heptahydrate $ZnSO_4 \bullet 7H_2O$ (MW 287.56). All reagents were obtained from Sigma (St. Louis, MO, USA). In each case, 40 g of bio-product, 10 g of reagent, and 400 mL of deionized water were used. The mixture was prepared in the dark at room temperature and stirred at 120 rpm for 24 h. The mixtures were then centrifuged (2500× *g*, 10 min, 20 °C) and decanted. The precipitate was washed 3 times with 250 mL of deionized water using the same centrifuging conditions and then decanted. The functionalized products were dried in an oven at 50 °C for 24 h and stored at room temperature.

2.4. Experimental Design

In consideration of the previously studied micronutrient concentrations [26] and phytotoxic background of these bio-products, two different assays were carried out to evaluate maximum biofortification potential:

(1) Fixed Zn concentration of 10 mg kg^{-1} soil from the functionalized bio-products (assay Zn-1).
(2) Fixed 0.5% dose of bio-product corresponding to 2 g per pot, regardless of Zn content (assay Zn-2).

For the Zn-1 assay, the quantity of each bio-product added to each pot was calculated from the Zn content of bio-chelates obtained via functionalization (Table 1). Non-functionalized SCG and SCG-hydrochars were used in the same quantities as functionalized hydrochars. Final Zn concentrations, alongside the quantity of bio-product added to the soil in each treatment, are described in Table S1.

Table 1. Chemical and physicochemical properties of SCG and hydrochars.

Bio-Product	pH	EC_{25} (dS m^{-1})	P av. (ppm)	K av. (ppm)	TP (mg GAE g^{-1})	Zn Content (ppm)	
						Non-Func.	Func.
SCG	5.43	9.01	666.15	3706.07	10.23	10.16	6009
H160	4.60	4.34	540.24	684.11	14.08	10.05	1748
H200	3.89	4.17	324.23	506.99	36.42	11.65	332

Bio-product	Elemental analysis (%)				C/N	Proximate analysis (%)			
	C	N	H	O *		Fixed carbon	Volatile matter	Ash content	Moisture
SCG	47.96	2.29	7.58	42.17	20.94	13.97	84.55	1.45	7.53
H160	52.01	2.21	8.08	37.70	23.53	13.45	86.10	0.41	3.58
H200	62.57	2.47	7.62	27.34	25.33	26.02	73.42	0.54	3.46

SCG: spent coffee grounds; H160: hydrochar at 160 °C; H200: hydrochar at 200 °C; EC_{25}: electrical conductivity measured at 25 °C; TP: total polyphenols; GAE: gallic acid equivalent; av: available; Non-Func.: non-functionalized; Func.: functionalized. * Calculated by difference.

For each soil microcosm, a triple-15 fertilizer was added in quantities of 0.675 g kg^{-1} soil to provide 100 mg kg^{-1} of N, 44 mg kg^{-1} of P, and 84 mg kg^{-1} of K. Two controls were set as follows:

- Control A: soil containing NPK alone.
- Control B: a commercial chelate at a concentration of 10 mg kg^{-1} soil.

The commercial chelate used in this study was zinc ethylenediaminetetraacetate (EDTA-Zn, 14% *w:w*) supplied by Trade Corporation International, S.A.U. (Madrid, Spain). Each treatment was run in triplicate. An overall mixture of 400 g of soil containing the bio-products was transferred to 300 mL polyethylene pots (height 8.5 cm, superior diameter 11 cm, base diameter 7.5 cm) that were sealed with a fiberglass double mesh located at the base to avoid the loss of fine particles. One 30-day-old lettuce was transplanted per pot with the same substrate of origin so as not to mistreat the roots. Pots were incubated in a growth chamber under controlled conditions with a relative humidity of 50–60%, temperature of 22/18 °C (day/night) and 12/12 h photoperiod. Soil water content was maintained with distilled water throughout the assay between field capacity and permanent wilting point. The irrigation requirements were calculated by weighing. Plants were harvested after 40 days.

2.5. Analytical Procedures

Bio-products: pH was measured in 1:5 (*w:w*) bio-product–water suspensions. Electrical conductivity at 25 °C (EC_{25}) was measured from extracts of the aforementioned bio-product–water suspension. Total polyphenols (TP) were estimated by using the Folin–Ciocalteu reagent method. Available phosphorus was determined according to Olsen Watanabe's method [28] using a Helios alpha spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Available potassium was extracted with N ammonium acetate (pH = 7) and determined with a PFP7 flame photometer (Jenway, Staffordshire, UK) according to USDA protocols [28]. Elemental analysis (carbon, hydrogen, nitrogen, and oxygen content by difference) was performed using a CNH analyzer (Thermo Scientific™ Flash 2000). Proximate analysis (volatile matter [at 950 °C], ash content [at 750 °C], moisture content [at 105 °C], and fixed carbon [calculated from the above parameters]) was conducted using the ASTM D1762-84 method. Mineral content in bio-chelates was extracted by acid attack with H_2SO_4 at 350 °C catalyzed with Se. Total Zn content of extracts was determined by atomic absorption spectrometry (AAS) (Varian SpectrAA 140, Mulgrave Victoria, Australia). Physical parameters (BET specific surface area, total volume in pores, and total area in pores) of the bio-products were investigated on the basis of nitrogen adsorption/desorption measurements at 77 K, employing a nitrogen adsorption analyzer ASAP™ 2420—Micromeritics (USA). Scanning electron microscopy (SEM) images were captured from SCG and hydrochars fixed with double-sided adhesive carbon tape, metalized with carbon, and analyzed with variable pressure and high-resolution scanning electron microscope (VPESEMFESEM) SUPRA40VP (ZEISS, Oberkochen, Germany).

Soil: Methods of soil analysis from the American Society of Agronomy and Soil Science Society of America [28] were followed. Soil pH was measured in 1:2.5 (*w:w*) soil–water suspension and EC_{25} was measured in 1:5 (*w:w*) soil–water suspension. Organic carbon (OC) was determined by wet digestion with $K_2Cr_2O_7$ and H_2SO_4 at 155 °C for 5 min—from ebullition—and further determination by titration with Mohr's salt (Tyurin's method). Soil-available Zn was extracted with DTPA (pH 7.3) via the method described by Lindsay and Norvell (1978).

Plants: After cultivation, the edible part of the lettuce (leaves) was harvested and weighed (total fresh weight, TFW). Subsequently, leaves were washed with distilled water, dried at 60 °C overnight, and weighed again (total dry weight, TDW). Lettuce samples were labeled and stored at −80 °C for later analyses. Mineralization of plant material was carried out in a microwave digester (Anton Paar, Muliwave 5000), with HNO_3, HCl, and HF at 180 °C. Total Zn content from the extracts was determined following the same approach used for the bio-products.

2.6. Efficiency Evaluation

Zn utilization efficiency (UE) in lettuce with respect to the total amount of Zn added to soil was calculated according to Zhao et al. [29]:

$$\text{UE } (\%) = \frac{\text{Uptake in treatment} - \text{Uptake in control}}{\text{Micronutrient added}} \times 100 \quad (1)$$

In the same way, Zn available reserve efficiency (ARE) in soil was calculated as follows:

$$\text{ARE } (\%) = \frac{\text{Extractable in treatment} - \text{Extractable in control}}{\text{Micronutrient added}} \times 100 \quad (2)$$

Finally, the transfer factor (TF) relating the parameters determined in the soil and plant was estimated as follows [30]:

$$\text{TF} = \frac{\text{Total concentration in plant}}{\text{Concentration extracted from soil}} \quad (3)$$

2.7. Statistical Analysis

Analysis of variance (ANOVA) combined with the Tukey test for treatment effects was conducted. Significance level was set at 95% ($p < 0.05$) for all tests. We created a multifactorial ANOVA to identify the interaction between the two factors (Zn and type of bio-product). SPSS 26.0 for Windows (IBM SPSS Inc., New York, NY, USA) was used. Principal component analysis (PCA) was used for clustering samples and their relationships with plant and soil parameters. This was conducted using Origin b9.5.5409 (OriginLab Corp., Northampton, MA, USA).

3. Results and Discussion

3.1. Characteristics of Assayed By-Products

The two hydrochars obtained in the present work exhibited some differences compared to SCG. The temperature applied during HTC has been identified as the main cause of physical and physicochemical changes in hydrochar properties [14], due to chemical dehydration and decarboxylation reactions [31]. Because of the carbonization process, both the carbon content and the C/N ratio increased. Similar results were reported by Cervera-Mata et al. [17] when applying HTC temperatures of 175 and 185 °C. With regard to oxygen content and moisture, both values decreased, whilst no pattern was identified for fixed carbon, volatile matter, and ash content as H160 produced lower values for these parameters than SCG.

pH, EC_{25}, and available K and P decreased with increasing HTC temperature (Table 1). Despite SCG being acidic, HTC produced more acidic by-products with lower mineral sorption capacity [12]. These changes in the particles led directly to the easy release of nutrients in water during this process. Another parameter that is positively affected by HTC temperature is carbon content. Some authors have reported SCG increases of up to 30.7% at a working temperature of 220 °C [14]. Total polyphenols also increase as these are products of the hydrolysis of coffee melanoidins [32].

With regard to chelation capacity, SCG particles were found to be the best at retaining mineral elements at the surface (Table 1). In the same way, Cervera-Mata et al. [26] reported a 18,000-fold increase in Zn content in SCG particles after functionalization. Hydrochars showed significant decrease in chelation capacity of Zn by 94.5%. HTC itself as well as the higher temperature used in this process also appear to affect this capacity.

The study of some physical properties of the tested bio-products (Table 2) raises an interesting question regarding Zn retention. An increase in the specific surface area of the hydrochars is observed compared to the SCG; however, this increase is contradictory to the Zn retention capacity (Table 1). However, the scanning electron microscope (SEM) images provide more information. The surface of the SCG particles shows limited porosity in which no depth is appreciated (Figure 1a). During the HTC process, the surface of these

pores is defined, and greater depth is evident, but as the temperature increases, between 160 and 200 °C (Figure 1b,c), they begin to clog with material from the HTC transformation. Similarly, the SEM image of an H200 particle reported by Cervera-Mata et al. [33] revealed that HTC leads to greater pore obstruction when compared to SCG. The morphological changes observed in this study are contradictory to the structural values collected in Table 2. This may suggest that the increase in the specific surface area of these hydrochars may not be caused by the improvement in the definition of the pores of the particles themselves, but by the reduction in the particle size produced by the effect of the temperature applied during HTC [17].

Table 2. Physical properties of SCG and hydrochars.

Bio-Product	Specific Surface Area (m^2 g^{-1})	Total Vol in Pores (cm^3 g^{-1})	Total Area in Pores (m^2 g^{-1})
SCG	0.503	0.00084	0.248
H160	1.041	0.00162	0.512
H200	15.942	0.06485	9.348

SCG: spent coffee grounds; H160: hydrochar at 160 °C; H200: hydrochar at 200 °C.

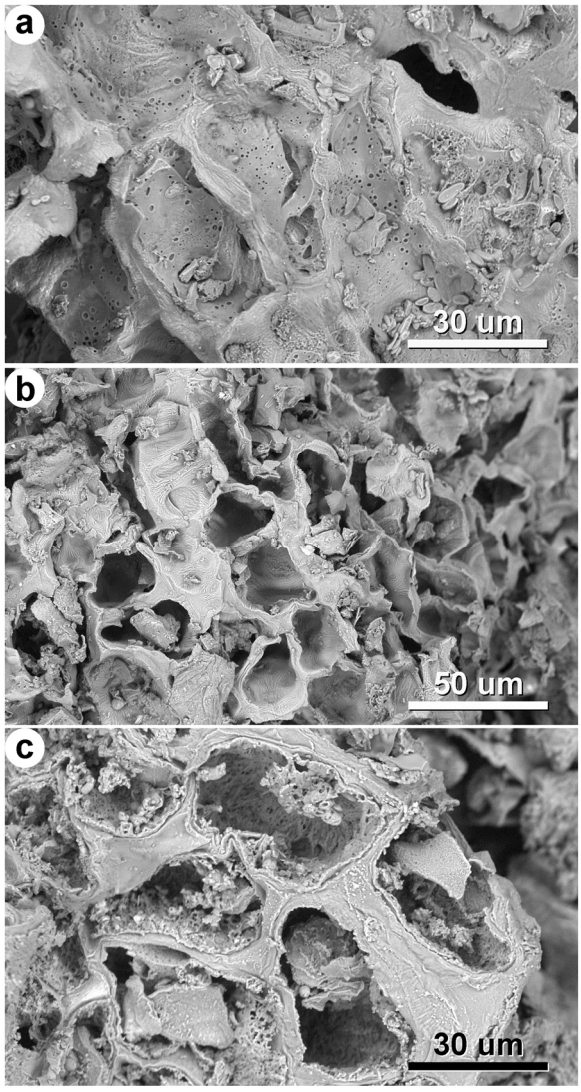

Figure 1. SEM images of the bio-products used for the preparation of the bio-chelates. (**a**) SCG, (**b**) H160, (**c**) H200.

One hypothesis to explain the loss of chelating capacity pertains to hydrochar O/C ratio (0.72 and 0.44 for H160 and H200, respectively). According to Law et al. [34], despite HTC products having a greater surface area (Table 2 and Figure 1) and micropore volume, low O/C ratios lead to greater hydrophobicity. Furthermore, the use of higher temperatures may destroy important functional groups, such as =OH and =CH, promoting a reduction in the efficiency of adsorption of inorganic compounds such as soluble minerals, including Zn. The present findings, in which H200 exhibits a more greatly reduced chelating capacity than H160 (Table 1), also support this conclusion.

From a chemical point of view, another hypothesis that would explain the difference in Zn retention is related to the content of melanoidins and polyphenols of the bio-products. These compounds have been identified as responsible for the chelating capacity of SCG [35], with this being due to their anionic behavior in the case of the former [24]. Despite the increase seen in total polyphenols, functionalization was affected drastically. This could indicate that melanoidins chelate more than polyphenols. The melanoidins contained in SCG are broken down into polyphenols during the HTC process, thus generating by-products with a higher total polyphenol content but poorer mineral absorption capacity (Table 1). In support of this finding, the chelating capacity of isolated coffee melanoidins has been previously demonstrated by Cervera-Mata et al. [26].

The resolution of this contradiction would require a greater number of tests to determine if the retention of Zn is more a process of chemisorption than of physisorption. In any case, a physical or chemical activation treatment applied to the SCG-hydrochars prior to functionalization could offset the loss of chelating capacity by improving some key particle properties (e.g., surface chemistry, available functional agents) [34].

3.2. Effects on Soil Fertility Properties

The addition of SCG and SCG-hydrochars to soil as organic amendments has been found to affect its physical, chemical, and physicochemical properties [17]. Three parameters were measured to evaluate these changes after cultivation: pH, EC_{25}, and OC content. Table 3 shows only slight variations between soils with bio-products versus controls. This may be related to the small quantity of residue added to every pot (<0.57%), with the only exceptions pertaining to H200-1 and H200-Zn-1. The amounts of H200 and H200-Zn added in the fixed mineral concentration assay (3.01%) (Table S1) support the significant variations in these properties due to the low Zn content found following functionalization (Table 1). Emerging trends point to proportional increases in EC_{25} and OC and a decrease in pH with the increasing addition of bio-product. Nevertheless, findings from the present work pertain specifically to calcareous soil as this was used to perform the assays.

The present outcomes correlate well with previous reports pertaining to SCG [19] and hydrochars [17]. Cervera-Mata et al. [19] observed a subsequent stabilization of these properties linked to: (1) soil type as this may influence buffering capacity depending on the presence of carbonates, and (2) incubation/cultivation length. Both aspects should be considered in further research. These observations are also in accordance with other literature [21,25].

Hydrochars are highly carbonaceous products and their application to soil can directly lead to an increase in OC content. This is in accordance with trends identified in the present study. Nevertheless, this trend was not statistically significant due to the small amount of bio-product added. Significant changes in OC content have been reported when adding up to 1% of SCG hydrochars (175 and 185 °C) to soil [17]. According to Sun et al. [36], the addition of 0.5 and 1.5% hydrochars (260 °C) derived from wood and grass shifts the OC composition of soil towards carbon molecules with higher molecular weight, thermal stability, and more aromatic compounds. Still, most degradable compounds of hydrochars have a dominant labile character that may be conducive to initial biological activity [33].

Table 3. Effects of SCG hydrochars and bio-chelates on soil properties after 40 days of cultivation.

Sample	pH	EC_{25} (dS/m)	OC (%)
Control A	8.37 ± 0.05 fg	0.79 ± 0.05 ab	1.10 ± 0.07 a
Control B	8.31 ± 0.02 efg	0.88 ± 0.07 ab	1.02 ± 0.08 a
SCG-1	8.26 ± 0.12 cdef	0.82 ± 0.13 ab	1.25 ± 0.25 a
H160-1	8.18 ± 0.03 cd	0.90 ± 0.07 ab	1.32 ± 0.10 a
H200-1	7.75 ± 0.03 a	1.39 ± 0.15 d	2.96 ± 0.43 b
SCG-Zn-1	8.19 ± 0.02 cd	0.90 ± 0.09 ab	1.14 ± 0.04 a
H160-Zn-1	8.15 ± 0.02 c	0.92 ± 0.07 ab	1.33 ± 0.13 a
H200-Zn-1	7.85 ± 0.05 b	1.15 ± 0.06 c	3.02 ± 0.19 b
SCG-2	8.41 ± 0.02 g	0.70 ± 0.03 a	1.31 ± 0.08 a
H160-2	8.33 ± 0.02 efg	0.83 ± 0.02 ab	1.34 ± 0.13 a
H200-2	8.28 ± 0.02 def	0.87 ± 0.03 ab	1.31 ± 0.04 a
SCG-Zn-2	8.31 ± 0.03 efg	0.83 ± 0.10 ab	1.22 ± 0.03 a
H160-Zn-2	8.25 ± 0.02 cdef	0.94 ± 0.06 ab	1.28 ± 0.06 a
H200-Zn-2	8.22 ± 0.02 cde	0.95 ± 0.10 b	1.38 ± 0.06 a

Control A: only NPK; Control B: commercial chelate + NPK; SCG: spent coffee grounds; H160: hydrochar at 160 °C; H200: hydrochar at 200 °C; SCG-Zn: SCG bio-chelate; H160-Zn: H160 bio-chelate; H200-Zn: H200 bio-chelate; EC_{25}: electrical conductivity measures at 25 °C; OC: organic carbon. Different numbers in the samples indicate the assay: (1) bio-chelates at 10 mg kg^{-1} soil; (2) 0.5% of bio-product. Different letters in the same assay column indicate statistically significant differences ($p < 0.05$).

3.3. Effects on Plant Growth

To evaluate the effects on plant growth, total fresh weight (TFW) and total dry weight (TDW) were analyzed. Figure 2a,b present outcomes for the two Zn assays. The addition of bio-products significantly ($p < 0.05$) limited plant growth, except when SCG and SCG-chelates at a fixed mineral concentration (SCG-Zn-1) were examined. The negative effect caused by SCG-hydrochars was more evident in assays with a fixed mineral concentration than a fixed quantity of bio-product. At the same rate of application (0.5%), all products inhibited growth equally when compared with controls. However, outcomes for the fixed mineral concentration assay suggest that the greater side effects seen could be attributed to the greater amount of bio-product added. For instance, H200 (assay Zn-1) (3.01%) resulted in substantially less growth than other treatments. This fact corroborates the trend discussed above in which a greater quantity of product leads to a greater negative effect.

Limited plant growth may mainly be attributed to the SCG composition of caffeine, polyphenols, and tannins [16,22], which have been identified to be not only phytotoxic but also ecotoxic [37]. Several authors have reported toxicity effects with different doses of SCG in lettuce (2.5, 10%) [19] and stone pine seedlings, which are also rich in polyphenols and tannins, at 10, 20, 30, and 100% [38]. In contrast, some authors describe enhanced dry weight when plants are found in sandy low mineral soil [39,40]. Different treatments may suppress phytotoxic effects (vermicompost and 400 °C pyrolysis), whilst other treatments (ethanol: water washing, hydrothermal carbonization) may have the opposite outcome given that polyphenol content also increased. Thus, given the increase seen in the total polyphenol content of SCG-hydrochars following HTC (Table 1), the present findings are in accordance with the aforementioned conclusion around SCG toxicity and outcomes reported by Rillig et al. [41] for beetroot chip hydrochar.

Overall, the addition of small quantities of SCG-chelates (approx. 0.17%) seems to be the best bio-product of those tested for plant growth, since TFW was not meaningfully affected and TDW was found to be the same as in controls ($p > 0.05$). Commercial chelates did not have any effect on TFW or TDW in lettuce.

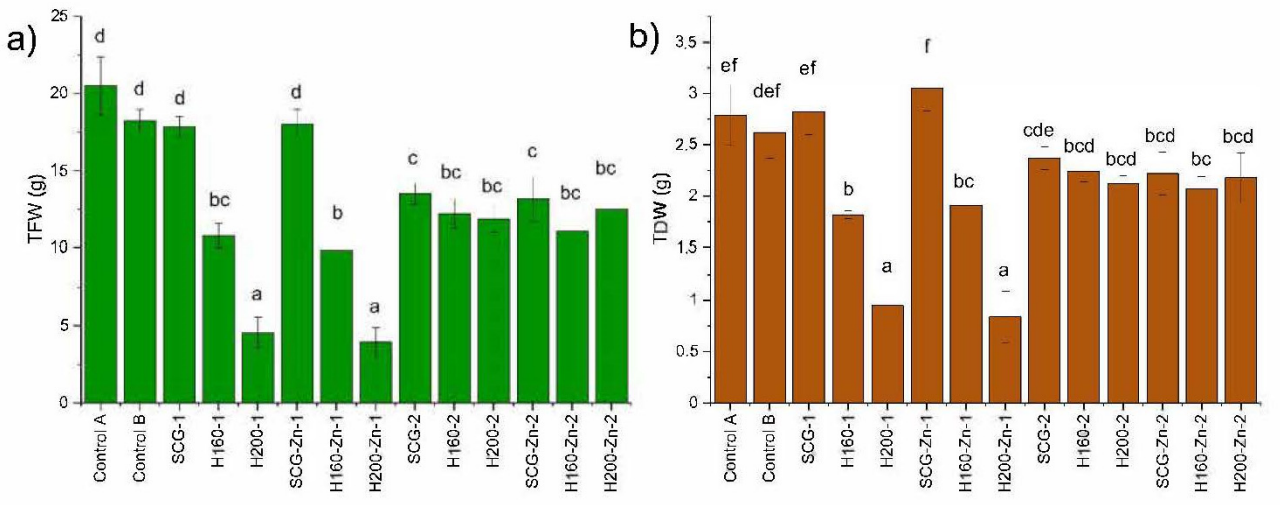

Figure 2. (**a**) Total fresh weight (TFW) and (**b**) total dry weight (TDW) of lettuces after 40 days of cultivation. Different numbers in the samples indicate the assay: (1) bio-chelates at 10 mg kg^{-1} soil; (2) 0.5% of bio-product. Control A: only NPK; Control B: commercial chelate + NPK; SCG: spent coffee grounds; H160: hydrochar at 160 °C; H200: hydrochar at 200 °C; SCG-Zn: SCG bio-chelate; H160-Zn: H160 bio-chelate; H200-Zn: H200 bio-chelate. Different letters indicate statistically significant differences ($p < 0.05$).

3.4. Agronomic Biofortification of Zn. Evaluation Parameters

Only SCG-Zn-2 showed a significant increase in Zn concentration, specifically by 91% ($p < 0.05$), compared to control A (Table 4). Moreover, no bio-product achieved the Zn concentration of the commercial chelate examined (control B). This was also reflected in the low utilization efficiency values recorded, with the vast majority being negative. In contrast, Cervera-Mata et al. [26] reported a significant increase in the concentration of this micronutrient in lettuce leaves when using SCG and melanoidin bio-chelates (SCG-Zn: 416%, Mel-Zn: 177%). The greater Zn content of the functionalized SCG used in the aforementioned study (17,924 mg kg^{-1}), relative to the lower Zn concentration of the SCG (6009 mg kg^{-1}) used in the present study, may explain these differences. Soil composition also plays an important role in the mobilization of this mineral. Zn exchangeable forms are usually found in fine-textured soil fractions [3], meaning that heavy clay soil facilitates the mobilization and subsequent plant uptake of this mineral. The clay composition of the soil used by Cervera-Mata et al. [26] was 58% compared to 12.5% in the present research.

Following application of the method described by Lindsay and Norvell to determine available Zn in the soil, in which DTPA is fixed at pH 7.3, the available Zn content of soil can be seen to be positively affected by the examined bio-chelates. All bio-chelates led to a more than five-fold increase in Zn concentration, whereas only a four-fold increase was seen with the commercial chelate (control B) (Table 4). The examination of assay Zn-2 reveals that the SCG-chelate creates the biggest Zn reserve in soil. However, the H160-chelate produced the best available reserve efficiency (ARE) in both assays.

TF is an indicator of the availability of metals in plants. A higher TF indicates greater mobility of metal between the soil and the plant. In contrast, when TF is low, nutrient deficiency can be suspected [30]. TF is much lower in bio-chelates than in SCG-hydrochars because of the greater reserve created in soil rather than in the plant itself. From this, it could be hypothesized that plant Zn biofortification, in the case of bio-chelates, may occur gradually over subsequent crop cycles, acting as a slow-release chelate. Cervera-Mata et al. [26] reported a TF close to 0.01 for 89.61 mg Zn kg^{-1} soil of SCG-Zn chelate. This was even lower than that obtained in the present work.

Table 4. Plant and soil mineral content and efficiency evaluation parameters.

Treatment	Plant Mineral Content (mg/100 g)	UE (%)	Soil Available Content (ppm)	ARE (%)	TF
Control A	0.11 ± 0.01 a	-	1.19 ± 0.12 a	-	-
Control B	0.65 ± 0.10 c	0.34	4.71 ± 0.11 b	4.92	0.06
SCG-1	0.11 ± 0.01 a	−0.09	1.15 ± 0.02 a	−0.40	0.09
H160-1	0.13 ± 0.02 a	−0.23	1.28 ± 0.08 a	0.85	0.10
H200-1	0.19 ± 0.01 ab	−0.36	1.52 ± 0.24 a	3.29	0.12
SCG-Zn-1	0.18± 0.02 ab	0.22	5.95 ± 0.25 cd	47.54	0.03
H160-Zn-1	0.18 ± 0.03 ab	−0.12	6.37 ± 0.31 d	51.82	0.03
H200-Zn-1	0.20 ± 0.01 ab	−0.38	5.70 ± 016 cd	45.11	0.03
SCG-2	0.14 ± 0.03 a	−0.04	1.21 ± 0.07 a	0.05	0.11
H160-2	0.14 ± 0.04 a	−0.18	1.16 ± 0.05 a	−0.38	0.12
H200-2	0.14 ± 0.02 a	−0.88	1.20 ± 0.13 a	0.51	0.12
SCG-Zn-2	0.26 ± 0.03 b	0.10	14.71 ± 0.76 e	45.00	0.02
H160-Zn-2	0.21 ± 0.05 ab	0.00	5.31 ± 0.38 bc	47.17	0.04
H200-Zn-2	0.15 ± 0.01 a	−0.52	1.89 ± 0.13 a	42.31	0.08

Control A: only NPK; Control B: commercial chelate + NPK; SCG: spent coffee grounds; H160: hydrochar at 160 °C; H200: hydrochar at 200 °C; SCG-Zn: SCG bio-chelate; H160-Zn: H160 bio-chelate; H200-Zn: H200 bio-chelate; UE: Utilization efficiency calculated with Equation (1); ARE: Available reserve efficiency calculated with Equation (2); TF: Transfer factor calculated with Equation (3). Different numbers in the samples indicate the assay: (1) bio-chelates at 10 mg kg^{-1} soil; (2) 0.5% of bio-product. Different letters in the same assay column indicate statistically significant differences ($p < 0.05$).

It should be noted that all the bio-chelates, which come from organic waste, have managed to improve the Zn content in lettuce despite not reaching statistical significance. The products assayed promise to be accessible and low cost due to the significant volume collected of the residue; however, further studies regarding waste transformation and functionalization as well as suitable doses of application, would be necessary to prove their industrial scalability.

3.5. Relationship between Examined Variables

A principal component analysis was performed to analyze the comprehensive response of plant and soil parameters to the addition of SCG and SCG-hydrochars functionalized (Figure 3). A matrix containing the values of plant parameters (Zn in plant, TFW and TDW) and soil parameters (Zn in soil, EC_{25}, OC, and pH) were selected. Two PCAs were performed, one for trial 1 (Figure 3a) and one for trial 2 (Figure 3b). Figure 3a shows the space defined by PC1 and PC2 for the trial 1 (which captures 83.17% of variance) with the score values of the samples. Figure 3b shows the space defined by PC1 and PC2 (which captures 72.73%). The second component of trial 1 explained the 19.20% of the variance. The lower values in this PC correspond to samples without functionalization with Zn. On the contrary, the higher values correspond to samples with the highest content of Zn in the soil and plant. It is observed how the addition of H200 increases the OC content in the soil to a greater extent. Similar results are found for trial 2 in Figure 3b. As a result of the multifactorial analysis, it has been shown that there is an interaction between the two factors: Zn dose and type of bio-product.

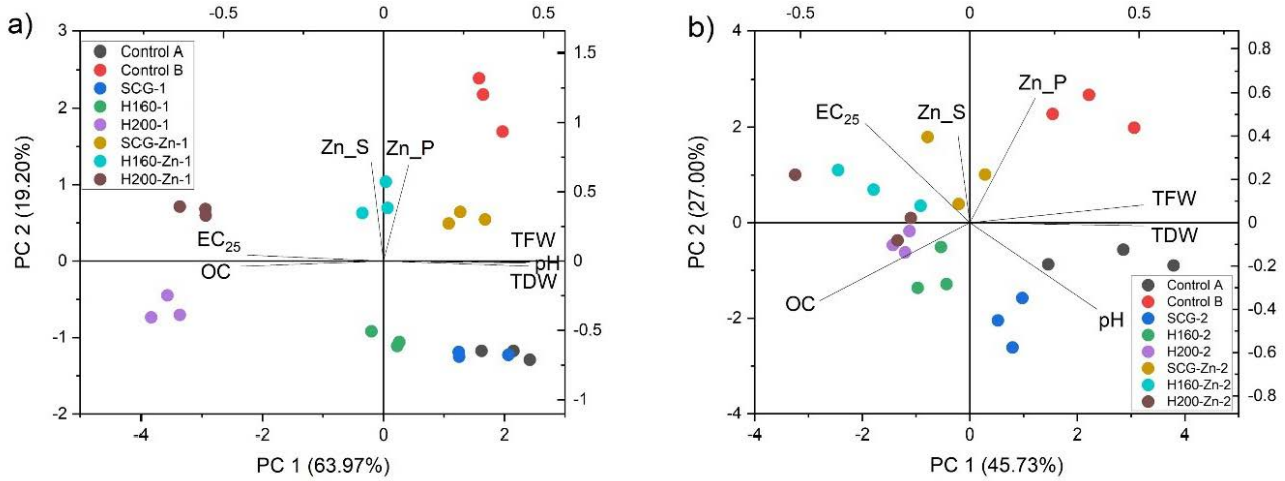

Figure 3. Superimposed graphs of PCA scores obtained for samples (PC2 vs. PC1) and loadings of soil and plant parameters. (**a**) Zn assay with bio-chelates at 10 mg kg^{-1} soil; (**b**) Zn assay at 0.5% of bio-product. Control A: only NPK; Control B: commercial chelate + NPK; SCG: spent coffee grounds; H160: hydrochar at 160 °C; H200: hydrochar at 200 °C; SCG-Zn: SCG bio-chelate; H160-Zn: H160 bio-chelate; H200-Zn: H200 bio-chelate; OC: organic carbon; X_P: plant micronutrient content; X_S: soil available micronutrient; TFW: total fresh weight; TDW: total dry weight; EC_{25}: electrical conductivity at 25 °C.

4. Conclusions

SCG-derived hydrochars exhibit compositional changes that differentiate them from non-transformed SCG. Differences increase concomitantly with HTC temperature. No direct relationship has been found between some properties of the tested bio-products and their ability to retain Zn as bio-chelates; clarification of this discrepancy requires further investigation. Except for H200, the addition of these bio-products did not lead to drastic changes in the properties of the soil. Likewise, a significant but not dramatic decrease was found in lettuce growth, with the exception, again, of H200, when bio-products were added in doses greater than 0.5%. Lettuce grown with Zn bio-chelates contained more Zn (biofortification), although differences were only significant in some cases when compared with a non-bio-product control. In no case did the biofortification generated by the bio-chelates exceed that of the commercial chelate. However, when considering the efficiency parameters calculated under the conditions used in the present research, it can be hypothesized that, after the addition of Zn bio-chelates, soil Zn reserves are increased for use by subsequent crops. All of these conclusions can only be applied to calcareous Mediterranean agricultural soil and more research is therefore needed to generalize findings to other soils. Given all the discussed outcomes, approaches that serve to recycle SCG-hydrochars as micronutrient fertilizers may constitute a paradigm shift since traditional methods lean towards increasing organic carbon in soil; however, further investigation is required.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151310700/s1, Table S1: Micronutrient concentration and quantity of bio-product in each sample.

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References

1. Global Hunger Index, The Challenge of Hidden Hunger. Available online: https://www.ifpri.org/sites/default/files/ghi/2014/index.html (accessed on 3 November 2021).
2. Levenson, C.W.; Morris, D. Zinc and Neurogenesis: Making new neurons from development to adulthood. *Adv. Nutr.* **2011**, *2*, 96–100. [CrossRef]
3. Adriano, D.C. *Trace Elements in the Terrestrial Environment*, 2nd ed.; Springer: New York, NY, USA, 2001; Volume 1, pp. 61–86.
4. Kozik, E.; Tyksiński, W.; Bosiacki, M. A comparison of the efficiency of organic and mineral iron compounds in the greenhouse cultivation of lettuce. *J. Elem.* **2011**, *16*, 59–68. [CrossRef]
5. Mohammadi, P.; Khoshgoftarmanesh, A.H. The effectiveness of synthetic zinc (Zn)-amino chelates in supplying Zn and alleviating salt-induced damages on hydroponically grown lettuce. *Sci. Hortic.* **2014**, *172*, 117–123. [CrossRef]
6. Gregory, P.J.; Wahbi, A.; Adu-Gyamfi, J.; Heiling, M.; Gruber, R.; Joy, E.J.; Broadley, M.R. Approaches to reduce zinc and iron deficits in food systems. *J. Glob. Food Sec.* **2017**, *15*, 1–10. [CrossRef]
7. Chojnacka, K.; Moustakas, K.; Witek-Krowiak, A. Bio-based fertilizers: A practical approach towards circular economy. *Bioresour. Technol.* **2020**, *29*, 122223. [CrossRef]
8. Velenturf, A.P.M.; Archer, S.A.; Gomes, H.I.; Christgen, B.; Lag-Brotons, A.J.; Purnell, P. Circular economy and the matter of integrated resources. *Sci. Total Environ.* **2019**, *689*, 963–969. [CrossRef]
9. Cao, L.; Zhang, C.; Chen, H.; Tsang, D.C.W.; Luo, G.; Zhang, S.; Chen, J. Hydrothermal liquefaction of agricultural and forestry wastes: State-of-the-art review and future prospects. *Bioresour. Technol.* **2017**, *245*, 1184–1193. [CrossRef]
10. 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]
11. Azzaz, A.A.; Khiari, B.; Jellali, S.; Ghimbeu, C.M.; Jeguirim, M. Hydrochars production, characterization and application for wastewater treatment: A review. *Renew. Sustain. Energy. Rev.* **2020**, *127*, 109882. [CrossRef]
12. Libra, J.A.; 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]
13. Yu, S.; Park, J.; Kim, M.; Ryu, C.; Park, J. Characterization of biochar and byproducts from slow pyrolysis of hinoki cypress. *Bioresour. Technol. Rep.* **2019**, *6*, 217–222. [CrossRef]
14. Afolabi, O.O.D.; Sohail, M.; Cheng, Y.L. Optimisation and characterisation of hydrochar production from spent coffee grounds by hydrothermal carbonisation. *Renew. Energy* **2020**, *147*, 1380–1391. [CrossRef]
15. García-Hernández, P.N.; Baas-López, J.M.; Toledano-Thompson, T.; Valdez-Ojeda, R.; Pacheco-Catalán, D. Revalorization of pleurotus djamor fungus culture: Fungus-derived carbons for supercapacitor application. *Sustainability* **2021**, *13*, 10765. [CrossRef]
16. Cervera-Mata, A.; Navarro-Alarcón, M.; Rufián-Henares, J.Á.; Pastoriza, S.; Montilla-Gómez, J.; Delgado, G. Phytotoxicity and chelating capacity of spent coffee grounds: Two contrasting faces in its use as soil organic amendment. *Sci. Total Envi.* **2020**, *717*, 137247. [CrossRef] [PubMed]
17. Cervera-Mata, A.; Lara, L.; Fernández-Arteaga, A.; Ángel Rufián-Henares, J.; Delgado, G. Washed hydrochar from spent coffee grounds: A second generation of coffee residues. Evaluation as organic amendment. *Waste Manag.* **2021**, *120*, 322–329. [CrossRef]
18. Gemechu, F.G. Embracing nutritional qualities, biological activities and technological properties of coffee byproducts in functional food formulation. *Trends Food Sci. Technol.* **2020**, *104*, 235–261. [CrossRef]
19. Cervera-Mata, A.; Pastoriza, S.; Rufián-Henares, J.Á.; Párraga, J.; Martín-García, J.M.; Delgado, G. Impact of spent coffee grounds as organic amendment on soil fertility and lettuce growth in two Mediterranean agricultural soils. *Arch. Agron. Soil Sci.* **2018**, *64*, 790–804. [CrossRef]
20. Comino, F.; Cervera-Mata, A.; Aranda, V.; Martín-García, J.M.; Delgado, G. Short-term impact of spent coffee grounds over soil organic matter composition and stability in two contrasted Mediterranean agricultural soils. *J. Soils Sediments* **2020**, *20*, 1182–1198. [CrossRef]
21. Vela-Cano, M.; Cervera-Mata, A.; Purswani, J.; Pozo, C.; Delgado, G.; González-López, J. Bacterial community structure of two Mediterranean agricultural soils amended with spent coffee grounds. *Appl. Soil Ecol.* **2019**, *137*, 12–20. [CrossRef]
22. Leifa, F.; Pandey, A.; Soccol, C.R. Solid state cultivation-an efficient method to use toxic agro-industrial residues. *J. Basic Microbiol.* **2000**, *40*, 187–197. [CrossRef] [PubMed]

23. Cervera-Mata, A.; Navarro-Alarcón, M.; Delgado, G.; Pastoriza, S.; Montilla-Gómez, J.; Llopis, J.; Sánchez-González, C.; Rufián-Henares, J.Á. Spent coffee grounds improve the nutritional value in elements of lettuce (*Lactuca sativa* L.) and are an ecological alternative to inorganic fertilizers. *Food Chem.* **2019**, *282*, 1–8. [CrossRef]
24. Rufián-Henares, J.A.; de La Cueva, S.P. Antimicrobial activity of coffee melanoidins—A study of their metal-chelating properties. *J. Agric. Food Chem.* **2009**, *57*, 432–438. [CrossRef]
25. Morikawa, C.K.; Saigusa, M. Recycling coffee and tea wastes to increase plant available Fe in alkaline soils. *Plant Soil* **2008**, *304*, 249–255. [CrossRef]
26. Cervera-Mata, A.; Fernández-Arteaga, A.; Navarro-Alarcón, M.; Hinojosa, D.; Pastoriza, S.; Delgado, G.; Rufián-Henares, J.Á. Spent coffee grounds as a source of smart biochelates to increase Fe and Zn levels in lettuces. *J. Clean Prod.* **2021**, *328*, 129548. [CrossRef]
27. Giordano, M.; El-Nakhel, C.; Pannico, A.; Kyriacou, M.C.; Stazi, S.R.; de Pascale, S.; Rouphael, Y. Iron biofortification of red and green pigmented lettuce in closed soilless cultivation impacts crop performance and modulates mineral and bioactive composition. *Agronomy* **2019**, *9*, 290. [CrossRef]
28. Unated States Department of Agriculture. *Soil Survey Field and Laboratory Methods Manual—Soil Survey Investigations Report No. 51*; Unated States Department of Agriculture: Washington, DC, USA, 2014; Volume 2.
29. Zhao, A.; Yang, S.; Wang, B.; Tian, X. Effects of ZnSO4 and Zn-EDTA applied by broadcasting or by banding on soil Zn fractions and Zn uptake by wheat (*Triticum aestivum* L.) under greenhouse conditions. *J. Plant Nutr. Soil Sci.* **2019**, *182*, 307–317. [CrossRef]
30. Almendros, P.; Gonzalez, D.; Alvarez, J.M. Long-term bioavailability effects of synthesized zinc chelates fertilizers on the yield and quality of a flax (*Linum usitatissimum* L.) crop. *Plant. Soil.* **2013**, *368*, 251–265. [CrossRef]
31. Kim, D.; Lee, K.; Bae, D.; Park, K.Y. Characterizations of biochar from hydrothermal carbonization of exhausted coffee residue. *J. Mater. Cycles Waste Manag.* **2017**, *19*, 1036–1043. [CrossRef]
32. Wang, Z.; Zhang, Z.; Li, S.; Zhang, X.; Xia, M.; Xia, T.; Wang, M. Formation mechanisms and characterisation of the typical polymers in melanoidins from vinegar, coffee and model experiments. *Food Chem.* **2021**, *355*, 129444. [CrossRef]
33. Cervera-Mata, A.; Delgado, G.; Fernández-Arteaga, A.; Fornasier, F.; Mondini, C. Spent coffee grounds by-products and their influence on soil C–N dynamics. *J. Environ. Manage.* **2022**, *302*, 114075. [CrossRef]
34. Law, X.N.; Cheah, W.Y.; Chew, K.W.; Ibrahim, M.F.; Park, Y.-K.; Ho, S.-H.; Show, P.L. Microalgal-based biochar in wastewater remediation: Its synthesis, characterization and applications. *Environ. Res.* **2022**, *204*, 111966. [CrossRef] [PubMed]
35. Takenaka, M.; Sato, N.; Asakawa, H.; Wen, X.; Murata, M.; Homma, S. Characterization of a Metal-Chelating Substance in Coffee. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 26–30. [CrossRef] [PubMed]
36. Sun, K.; Han, L.; Yang, Y.; Xia, X.; Yang, Z.; Wu, F.; Li, F.; Feng, Y.; Xing, B. Application of Hydrochar Altered Soil Microbial Community Composition and the Molecular Structure of Native Soil Organic Carbon in a Paddy Soil. *Environ. Sci. Technol.* **2020**, *54*, 2715–2725. [CrossRef]
37. Janissen, B.; Huynh, T. Chemical composition and value-adding applications of coffee industry by-products: A review. *Resour. Conserv. Recycl.* **2018**, *128*, 110–117. [CrossRef]
38. Caliskan, S.; Ozok, N.; Makineci, E. Utilization of Spent Coffee Grounds as Media for Stone Pine (*Pinus pinea*) Seedlings. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 2014–2024. [CrossRef]
39. Gomes, T.; Pereira, J.A.; Ramalhosa, E.; Casal, S.; Baptista, P. Effect of fresh and composted spent coffee grounds on lettuce growth, photosynthetic pigments and mineral composition. In Proceedings of the Congreso Ibérico de Agroingeniería y Ciencias Hortícolas, Madrid, Spain, 26–29 August 2013; pp. 1–5.
40. Kasongo, R.K.; Verdoodt, A.; Kanyankogote, P.; Baert, G.; Van Ranst, E. Response of Italian ryegrass (*Lolium multiflorum* Lam.) to coffee waste application on a humid tropical sandy soil. *Soil Use Manag.* **2013**, *29*, 22–29. [CrossRef]
41. 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]