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# Assessing Seed Germination and Plant Growth of *Eruca vesicaria* (L.) Cav. Cultivated in Biochar-Enriched Substrates

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**Abstract:** This study evaluates the use of biochar as a sustainable substitute to peat in the soilless cultivation of rocket salad (*Eruca vesicaria* (L.) Cav.). Biochar was added to a peat-based substrate at concentrations of 0% (control), 5%, 10%, 20%, 40%, and 70% *v*/*v* to assess its effects on seed germination, plant growth, mineral content, and nitrate accumulation. The results show that biochar concentrations up to 40% *v*/*v* maintained germination rates above 80%, similar to the control, while higher concentrations (70% *v*/*v*) drastically reduced germination to 29% and entirely compromised plant development and growth. A moderate biochar concentration (20%) had a positive effect on fresh weight and leaf area, while maintaining comparable levels of nutrient uptake, chlorophyll, and flavonols. In addition, biochar-enriched substrates (≥20% *v*/*v*) reduced nitrate accumulation in leaves by 26–30%, addressing a critical quality and safety concern. A high biochar content (≥40% *v*/*v*) altered the substrate's physicochemical properties, including pH, porosity, and electrical conductivity, negatively affecting plant growth (a 38% reduction in plant growth and 42% in leaf area) and increasing heavy metal concentrations, such as that of zinc (~30%). These findings suggest that incorporating up to 20% *v*/*v* biochar in soilless substrates offers a sustainable alternative to peat, supporting rocket salad performance and improving leaf nitrate quality, without compromising yield or safety.

**Keywords:** rocket salad; biochar; soilless cultivation; growing media; mineral content; heavy metals; nitrates



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## 1. Introduction

Rocket salads, part of the Brassicaceae family, are a popular group of leafy vegetables, with *Eruca vesicaria* (L.) Cav. and *Diplotaxis tenuifolia* (L.) D.C. accounting for most of global production [1]. Harvested as whole single leaves to reduce processing and increase post-harvest life, these species are often used in mixed salads [2]. Rocket salads are recognized for their distinct, pungent flavors and aromas. Presently, an increase in popularity and market has been observed in the baby leaf category due to their sensorial, nutritional, and antioxidant characteristics [3]. In addition, rocket salad tissues contain an interesting fatty acid profile which mitigates some human diseases [4]. On the other hand, they are well known to accumulate high amounts of nitrates, which are a negative quality factor for leafy vegetables [5], possibly causing detrimental effects on human health, mainly in

newborns [6,7]. The EU Regulation No. 1258/2011 establishes the maximum nitrate levels allowed for the marketing of rocket at 6000 or 7000 mg $NO_3$ $kg^{-1}$ of fresh weight depending on harvest time [8].

Soilless cultivation may help to control nitrate accumulation in leafy vegetables [9]. Furthermore, such systems prevent diseases transmitted through the soil and provide complete control over input factors, increase yield, and allow for a better standardization of quality [9]. Many different soilless techniques can be applied such as the utilization of growing media to replace soil or systems that do not need a substrate (hydroponics or aeroponics) [10].

Substrate cultivation consists of the utilization of a growing medium replacing the soil for the whole plant cycle, from the nursery to final harvesting. Growing media must exhibit a well-balanced combination of physical and chemical properties. These include factors such as pH, electrical conductivity (EC), bulk density, porosity, organic matter content, cation exchange capacity (CEC), and the ability to retain both nutrients and water [11]. Growing media are known to significantly influence plant growth by regulating nutrient availability, water retention, and aeration [12]. A well-balanced substrate ensures the adequate supply of elements necessary for plant health [13,14]. Conversely, imbalances or deficiencies in the substrate can lead to nutrient disorders, affecting overall plant vigor [15].

Due to its excellent physical and chemical characteristics for plant cultivation, peat is a major component of horticultural growing media [16]. About 40% of the peat consumed in the European Union is employed as a substrate for plant growth and, of this, approximately one-third is intended for vegetable production [17] with projections indicating further increases from 2020 to 2050 [18]. However, the intensive usage of peat in agriculture has a strong impact on peatlands, which represent ecologically important but fragile ecosystems [19]. Furthermore, the process of extracting peat from peatlands releases $CO_2$ into the atmosphere [20], posing further environmental concerns. Therefore, over the last years, the scientific community has focused research on substrates possibly capable of completely or partially replacing peat without negative effects on yield and quality of crops [21]. Studies on the use of biochar in soilless cultivation fit into this context. Biochar is obtained by the pyrolysis or gasification of vegetal/biosolid wastes (e.g., herbaceous/woody wastes and sludge) in an almost oxygen-free environment [22], which leads to the production of a porous material made of an organic fraction, rich in carbon structures, and an inorganic part, composed of minerals [23]. In agronomic applications, biochar physicochemical properties may have positive effects leading to the increase in cationic exchange capacity (CEC), pH, nutrient content/availability, and water-holding capacity [24]. Moreover, its characteristics have been reported to support the growth of diverse microbial communities, which are essential for nitrogen fixation [25]. Additionally, biochar's high carbon content and microporous structure contribute to increase carbon sequestration in soils, which in turn helps reduce greenhouse gas emissions [26].

The application of biochar as a growing medium component has shown different results depending on its characteristics and the crops involved [27]. Many studies have been conducted on the addition of biochar in soilless substrates in various vegetable crops and baby leaves [27–31]. Moderate biochar concentration (~20% $v/v$) from forestry waste improved vegetative growth in tomatoes without affecting yield or quality [29,30]. Biochar from hardwood and sugarcane bagasse successfully replaced bark-based substrates for tomato and basil [32]. In addition, an increase in yield was observed in leafy vegetables using a perlite and rice husk biochar mixture [33].

Regarding rocket salad (*Eruca vesicaria*), some studies have been conducted on pot cultivation, showing varying results [27,28,34–37]. In these studies, biochar was added in the growing medium in a very low concentration (less than 2.5% $v/v$) to a maximum

of 15% $v/v$ and different parameters, such as seed germination, plant production, and physiological performance, were analyzed.

In our previous work, a forestry biochar mixed at concentrations up to 40% $v/v$ with a peat-based medium resulted in an increase in the plant biomass of tomato plants, and tomato fruit yield in biochar 10% $v/v$ was comparable with that of the control [38]. Based on these previous results, we supposed that the same biochar mixtures could be suitable for rocket cultivation. Thus, the current study aimed to evaluate the effects of biochar at five different concentrations (5%, 10%, 20%, 40%, and 70% $v/v$) on rocket seed germination, plant growth, and nutrients and nitrates in the leaves. To the best of our knowledge, this is the first work that tests rocket germination and growth in media with high biochar contents.

## 2. Materials and Methods

### *2.1. Experimental Setup and Substrate Characterization*

Agricultural biochar was derived from woody biomass sourced from coppice waste. The production process was conducted by BioEsperia SME using a patented full-scale gasification plant. In more detail, the woody biomass underwent to a gasification process at 800–900 °C with a 10 min contact time. Representative samples from various production lots were gathered for thorough physicochemical characterization, along with the analysis of heavy metals and organic micropollutants, following the protocol reported by Del Bubba et al. [22]. Biochar characterization and properties have already been reported by Bini et al. [38].

The biochar was incorporated and thoroughly mixed into pots to a commercial substrate consisting of peat and lapillus 10/14 mm (1:1 $v/v$), resulting in six treatments: BC0 (100% commercial substrate) as the control, BC5 (5% $v/v$ biochar content), BC10 (10% $v/v$ biochar content), BC20 (20% $v/v$ biochar content), BC40 (40% $v/v$ biochar content), and BC70 (70% $v/v$ biochar content). Substrates BC0, BC5, BC10, BC20, and BC40 were employed as growing media for tomato in a previous study, and their physicochemical properties as well as the analytical procedures to determine them have already been reported by Bini et al. [38]. The substrate BC70, not used for tomato [38], was analyzed in this study with the same procedures.

The experiment took place in a greenhouse at the DAGRI Department in Sesto Fiorentino, Italy (DMS coordinates: 43°81′68″ N, 11°19′99″ E), from September to December 2022. The average temperature during this period was recorded at 20 °C, accompanied by a humidity level of 65%. Commercial rocket seeds (Franchi Sementi Spa, Grassobbio, Bergamo, Italy) were sown soon after purchase in plastic pots (40 L, 72 × 28 × 40 cm, AgriVivai, Pistoia, Italy) filled with the different substrates (150 seeds per pot), obtaining a plant density of 750 plants $m^{-2}$. Five pots per substrate were arranged in a randomized block design. Irrigation was automated using an irrigation controller, with three drippers per pot. Fertilization was applied every seven days post sowing using Compo Expert Hakaphos Base NPK 7-12-40 (COMPO EXPERT GmbH, Münster, Germany) with a dosage of 1 g per L of water in each pot.

### *2.2. Germination Assessment*

Rocket seed germination was monitored every day for each pot and treatment. Seeds were considered germinated when two cotyledons had fully opened. The seeds were counted daily until new seedlings were not detected for two consecutive days, leading to a total count of 12 days.

The four-parameter Hill function (4-PHF) was used to compute the cumulative germination of each rocket pot as previously described by El-Kassaby et al. [39] with the following equation:

$$y = y_0 + \frac{ax^b}{c^b + x^b}$$

where $y$ represents the percentage of cumulative germination at time $x$; $y_0$ is the intercept on the $y$-axis ($\leq 0$); a is the asymptote and represents the maximum cumulative germination percentage, which is considered as the maximum germination capacity; $b$ is a mathematical parameter related to the shape and steepness of the germination curve; and c represents the number of days required for 50% of the seeds to germinate (D50). By calculating the four parameters $a$, $b$, $c$, and $y_0$, the time of germination onset (LAG) and the final germination percentage (GP) can be obtained using the following equations:

$$\text{LAG} = b\sqrt{\frac{-y_0 c^b}{a + y_0}}$$

$$\text{GP} = y_0 + a$$

LAG was used to calculate Dlag50, which is the duration between the time at germination onset and that at 50% germination. Moreover, the Mean Germination Time (MGT) was calculated using the formula of Ellis and Roberts [40]:

$$\text{MGT} = \sum(n \times D)/\sum n$$

where $n$ represents the number of seeds germinated at time $D$, which considered to be the number of days from sowing, and $\sum n$ is the final germination.

In addition, other parameters were computed: t50total, which is the time (d) to reach 50% of germination; the last germination time (LastGermTime), which is the day on which the last germination occurs; and time spread germination (TimeSpreadGerm), which is the difference between the last and first day of germination. Moreover, the speed of germination was computed as previously reported [41] using the following formula:

$$S = \sum_{i=1}^{k} \frac{N_i}{T_i}$$

where $T_i$ represents the time elapsed from the beginning of the experiment to the $i$-th time interval, $N_i$ denotes the number of seeds that germinated during the $i$-th interval (not the cumulative count, but the specific number for that interval), and $k$ indicates the total number of time intervals.

Lastly, the germination index (GI) reported by Melville et al. [42] was obtained with the subsequent formula:

$$\text{GI} = \sum_{i=1}^{k} \frac{|(T_k - T_i)N_i|}{N_t}$$

where $T_i$ represents the time elapsed from the beginning of the experiment to the $i$-th time interval, $N_i$ denotes the number of seeds that germinated during the $i$-th interval (not the cumulative count, but the specific number for that interval), $N_t$ is the total number of seeds used in the test, and $k$ indicates the total number of time intervals. The reported parameters were calculated by using the package germinationmetrics [43] in R-project.

### 2.3. Plant Growth and Biomass Analysis

Fifty days after sowing, 15 representative random plants per pot were selected and used to carry out biometric analyses (i.e., the number of leaves, blade and petiole lengths, and leaf area). Leaf area was determined using the software Tomato Analyzer 3.0.3 [44]. Moreover, leaf color was measured using an electronic colorimeter (Minolta Chromameter CR200, Konica Minolta, Osaka, Japan). Subsequently, each leaf was weighed, dried at 65 °C for 48 h, and the fresh and dry weights of the total aerial part were calculated by summing up each leaf's weight. Specific leaf area (SLA) was calculated by dividing the leaf area by its dry mass.

### 2.4. Chlorophyll and Flavonols Assessment

Chlorophyll content (Chl) and epidermal flavonols (Flavs) were determined using a non-destructive method based on Dualex Scientific sensor (Force-A, Montpellier, France). In more detail, 10 representative plants per pot were measured (3 different leaves per plant), for a total of 150 leaves per treatment. For each leaf, the measurement was conducted for both the upper and lower leaf blade, avoiding the veins. Tests were performed on healthy leaves (without clearly visible symptoms) in the morning (9:00–13:00) during a sunny day. The upper and lower leaf blade values were averaged for Chl content, and summed for Flav content.

The estimation of Chl (μg $cm^{-2}$) relies on the difference in transmission between two specific wavelengths: visible light at 650 nm and near-infrared at 710 nm. In contrast, the content of epidermal Flavs (μg $cm^{-2}$) is assessed by comparing the absorbance at ultraviolet A (UVA) at 375 nm with that at 650 nm.

### 2.5. Nitrates

Nitrate concentration was determined using the salicylsulphuric acid method reported by Cataldo et al. [45]. In more detail, rocket leaves (5 independent replicates per treatment) were freeze-dried for 48 h and 100 mg was ground with a mortar and pestle. Then, the plant material was suspended in 30 mL of distilled water and agitated for 2 h at room temperature. Subsequently, 200 μL of the sample was combined with 800 μL of 5% salicylic acid in sulfuric acid and 30 mL of 1.5 N NaOH. The samples were then allowed to cool to room temperature before measuring absorbance at 410 nm with a Hitachi U-2000 spectrophotometer (Hitachi High Tech Corporation, Tokyo, Japan). Nitrate levels were computed using a calibration curve of $KNO_3$ standards, and the results were expressed on a fresh-weight (f.w.) basis by accounting for the fresh-to-dry weight ratio.

### 2.6. Mineral Content

The concentrations of minerals and heavy metals (P, Ca, Mg, Na, K, Fe, Cu, Zn, Mn, Ni, Cr, Pb, Cd, and V) were determined using inductively coupled plasma–optical emission spectrometry (ICP-OES) with the iCAP 7000 Plus system (Thermo Fisher Scientific, Waltham, MA, USA), following microwave-assisted acidic digestion (Mars Microwave Digestor, CEM, Matthews, NC, USA), as reported by Bini et al. [38]. Specifically, 300 mg of pre-dried, crushed leaves (3 replicates per treatment) was weighed and placed into the microwave reaction vessel, followed by the addition of 2 mL of nitric acid. The vessel was then sealed, and the digestion process was carried out by heating to 190 °C for 15 min, followed by 15 min at 190 °C. After cooling, the vessels were opened, and the digestion solution was transferred to a 50 mL volumetric tube, topped up with deionized water, and analyzed by ICP-MS, following ISO 11466 standards [46]. The data are expressed on a dry-weight basis (d.w.).

### *2.7. Statistical Analysis*

Statistical analyses were conducted using the Kruskal–Wallis non-parametric test, followed by Dunn's post hoc multiple comparisons, with Benjamini–Hochberg adjustment for $p$-values to control the false discovery rate at a 95% confidence level ($p < 0.05$), utilizing the dunn.test [47] package in R (version 4.3.1). Variations in chlorophyll, flavonols, leaf color, and nitrate content were further assessed using the Games–Howell test, implemented via the rstatix package [48], which enables the comparison of mean values and their standard deviations while accommodating unequal variances across variables.

In addition, a multivariate analysis was carried out to evaluate all plant-related variables for each substrate. Initially, a correlation matrix was constructed using the R package corrplot 0.92 [49] based on the Pearson correlation coefficient to streamline the variables for further analysis. In particular, plant variables were selected by removing traits with a correlation coefficient >0.95. Subsequently, the selected plant traits were considered to perform a principal component analysis (PCA) biplot, using the FactoMineR 2.7 [50] and factoextra 1.0.7 [51] packages in R.

## 3. Results

### *3.1. Physicochemical Properties of Substrates*

Table S1 reports the physicochemical properties of the substrates used in this study. Data on BC0, BC5, BC10, BC20, and BC40 have been already published in Bini et al. [38], and are reported here again due to the necessity of comparing the whole set of substrates. The incorporation of biochar into the media resulted in modifications in the physicochemical properties of the substrates. Specifically, increasing the biochar content led to a decrease in bulk density, with BC0 and BC70 showing the highest and lowest densities ($0.804 \pm 0.06$ g cm$^{-3}$ and $0.401 \pm 0.01$ g cm$^{-3}$, respectively). Conversely, as biochar concentration increased, a rise in medium porosity and water-holding capacity was revealed, with the lowest values ($68.4 \pm 0.3\%$ $v/v$, and $39.3 \pm 0.1\%$ $v/v$, respectively) found in BC0, and the highest detected in BC70 ($80.6 \pm 1.6\%$ $v/v$, and $55.63 \pm 1.3\%$ $v/v$, respectively). No substantial changes were observed in easily available water, for which only BC5 showed a significantly lower value. Furthermore, biochar affected both pH and EC levels, causing a significant and consistent increase in these parameters with higher biochar concentrations in the substrate. Specifically, all biochar-based mixtures exhibited a high pH, with values ranging between 8 and 10.8, whereas the control had a slightly acidic pH (6.2). It is important to note that the pH exceeded the threshold established by L.D. 75/2010 for mixed media (pH = 8.5) in all biochar substrates except BC5. As for EC, a biochar content above 20% $v/v$ led to a notable increase in EC levels within the media. Additionally, biochar influenced CEC levels, which were higher in all substrates with biochar compared to the control (BC0). However, no significant differences were found among the biochar-enriched substrates, except for BC10, which showed lower CEC values than BC70 ($23.1 \pm 0.85$ and $28.6 \pm 0.71$ meq 100 g$^{-1}$, respectively). Biochar-enriched substrates also exhibited higher total organic carbon (TOC) concentrations in comparison with the control, while no significant changes were noted in chloride levels. Conversely, higher biochar contents (>20% $v/v$) led to increased ammonia and nitrate, while a significant rise in total nitrogen was observed only in BC70 ($0.22 \pm 0.02\%$). In addition, the incorporation of biochar to peat-based substrates affected the levels of macro- and microelements, as well as heavy metals. Although no statistically significant differences were observed in calcium (Ca), its concentration increased with the increment of biochar content in the substrates. Biochar also led to increases in magnesium (Mg) and manganese (Mn) concentrations. There were no changes in iron (Fe) and zinc (Zn) levels, but a decrease in phosphorus (P) and sodium (Na) was noted in the biochar-enriched media. Although nickel (Ni) levels were influenced

by biochar and increased with higher biochar concentrations, they remained within the limits established by L.D. 75/2010.

### *3.2. Germination Assessment*

Table 1 presents the mean values and standard deviations of germination parameters recorded for 12 days after rocket sowing. No significant differences in germination rate were observed among the BC0, BC5, BC10, BC20, and BC40 treatments, all of which met the minimum commercial standard for rocket seed germination (GP > 80%). In contrast, the germination percentage dropped sharply in the BC70 substrate, where only 29.17 ± 6.34% of the seeds germinated successfully. A similar pattern was observed for germination speed (S), with substrates BC0 to BC40 showing comparable values (range: 34.06–40.13 n $d^{-1}$), while BC70 exhibited a drastic reduction of approximately 79% (7.21 ± 1.75 n $d^{-1}$).

**Table 1.** The germination parameters of rocket seeds grown in biochar-enriched substrates. The average and standard deviation (in brackets) are reported. Different letters in the same row represent significant variations among treatments ($p < 0.05$), using Games–Howell non-parametric test.

| | **BC0** | **BC5** | **BC10** | **BC20** | **BC40** | **BC70** |
|---|---|---|---|---|---|---|
| GP (%) | 84.33 (7.65) a | 85.17 (2.95) a | 88.33 (3.98) a | 86.33 (3.33) a | 86.00 (2.83) a | 29.17 (6.34) b |
| LastGermTime (d) | 8.25 (1.5) a | 7.50 (1) a | 7.25 (1.71) a | 7.00 (1.63) a | 9.00 (0.82) a | 9.50 (1.29) a |
| S (n $d^{-1}$) | 35.67 (9.11) a | 35.43 (7.81) a | 40.13 (5.31) a | 35.26 (6.30) a | 34.06 (5.91) a | 7.21 (1.75) b |
| Dlag50 (d) | 3.20 (1) b | 3.27 (0.96) b | 2.85 (0.34) b | 2.98 (0.57) b | 3.50 (1) ab | 5.89 (0.41) a |
| TimeSpreadGerm (d) | 4.75 (0.96) a | 4.00 (1.63) a | 4.25 (1.71) a | 4.00 (1.63) a | 6.00 (0.82) a | 5.50 (1.29) a |
| GI (d) | 8.15 (0.81) a | 8.09 (0.88) a | 8.45 (0.47) a | 8.26 (0.6) a | 7.7 (0.98) ab | 5.47 (0.35) b |
| t50total (d) | 3.26 (0.99) a | 3.35 (0.96) a | 2.96 (0.51) a | 3.13 (0.72) a | 3.75 (1.12) a | 0 (0) b |
| MGT (d) | 3.88 (0.91) ab | 3.82 (0.95) ab | 3.42 (0.46) b | 3.56 (0.66) b | 4.11 (1.01) ab | 6.93 (1.28) a |

GP = germination percentage; LastGermTime = last germination time; S = germination speed; Dlag50 = duration between the time at germination onset and that at 50% germination; TimeSpreadGerm = time-spread germination; GI = germination index; t50total = time to reach 50% of germination; MGT = Mean Germination Time.

No significant differences were found among the six substrates on the final day of germination (LastGermTime) and the time between the last and first day of germination (TimeSpreadGerm). However, germination timing displayed an opposite trend compared to the previous parameters. Specifically, the highest Dlag50 value was recorded in BC70 (5.89 ± 0.41 d), while the lowest was observed in BC10 (2.85 ± 0.34 d). There was no substantial variation between BC5, BC10, BC20, BC40, and the control (BC0), and all were significantly lower than BC70. A similar trend was noted for t50total and Mean Germination Time (MGT), where substrates with up to 40% biochar showed no statistical difference compared to the control and were significantly lower than BC70.

Moreover, biochar concentration significantly affected the germination index (GI). Higher biochar concentrations (≥40% $v/v$) led to a decrease in the GI compared to the control and lower biochar concentrations.

### *3.3. Rocket Yield*

The germination rate in BC70 was extremely low and a not-standardized and commercial production was obtained; therefore, BC70 samples were not considered for further analyses. The biometric parameters of rocket grown in the biochar-enriched substrates are reported in Table 2. The greatest aerial part fresh weight (FW) was recorded in BC20 (0.68 ± 0.12 g $plant^{-1}$), followed in order by BC0, BC10, BC5, and BC40 (0.59 ± 0.25, 0.49 ± 0.16, 0.45 ± 0.12, and 0.33 ± 0.1 g $plant^{-1}$ respectively). The latter (BC40) turned out to be statistically different and lower than BC20 (−47%) and the control BC0 (−38.98%). Although variations were observed in leaf FW, no substantial divergences were detected among the five substrates regarding aerial part dry weight (DW). An analogous trend

was noticed for the number of leaves (NL), blade length (BL), and petiole length (PL), for which all substrates were statically similar. Meaningful variations were observed in leaf area (LA) and specific leaf area (SLA). In more detail, the trend was the same in both parameters: BC20 > BC0 > BC10 > BC5 > BC40. The substrate BC20 showed the highest values (24.58 ± 2.51 $cm^2$ $plant^{-1}$ and 711.76 $cm^2$ $g^{-1}$, respectively) and BC40 the lowest ones (12.43 ± 2.87 $cm^2$ $plant^{-1}$ and 588.69 ± 112.11 $cm^2$ $g^{-1}$, respectively). No differences were observed for BC20, BC10, and BC5 compared to the control (BC0).

**Table 2.** The biometric parameters of rocket plants grown in biochar-enriched substrates. The average and standard deviation (in brackets) are reported. Different letters in the same row represent significant variations among treatments ($p < 0.05$), using Kruskall–Wallis and non-parametric Dunn test.

| | BC0 | BC5 | BC10 | BC20 | BC40 |
|---|---|---|---|---|---|
| Fresh Weight (g $plant^{-1}$) | 0.59 (0.09) a | 0.49 (0.1) ab | 0.54 (0.07) ab | 0.68 (0.12) a | 0.36 (0.03) b |
| Dry Weight (g $plant^{-1}$) | 0.033 (0.005) a | 0.025 (0.013) a | 0.025 (0.006) a | 0.035 (0.006) a | 0.023 (0.005) a |
| Number of Leaves (n $plant^{-1}$) | 5.25 (0.48) a | 4.63 (0.53) a | 4.97 (0.54) a | 5.3 (0.18) a | 4.7 (0.57) a |
| Blade Lenght (cm) | 4.96 (1.01) a | 4.28 (0.81) a | 4.48 (0.56) a | 5.23 (1.26) a | 3.87 (0.42) a |
| Petiole Lenght (cm) | 12.19 (2.05) a | 10.55 (0.86) a | 10.99 (1.30) a | 12.38 (1.20) a | 9.79 (0.88) a |
| Leaf Area ($cm^2$ $plant^{-1}$) | 21.45 (6.08) ab | 15.88 (3.73) bc | 16.52 (1.32) bc | 24.58 (2.51) a | 12.43 (2.87) c |
| Specific Leaf Area ($cm^2$ $g^{-1}$) | 689.92 (69.75) ab | 645.24 (129.92) bc | 657.89 (110.92) bc | 711.76 (86.90) a | 588.69 (112.11) c |

### *3.4. Mineral and Heavy Metal Contents*

Table 3 reports the macro- and microelements and heavy metal contents in rocket shoots 50 days after sowing. Biochar-enriched substrates influenced the macro- and microelements. Low biochar concentrations in the substrates (10% $v/v$) led to a slight increase in Ca in comparison to the control (+17.36%), while a reduction was observed for biochar content > 20% $v/v$ (−23.75%). Conversely, no substantial variations were noted in the Fe and K contents among the treatments. A substantial increase in Na was detected in biochar-based substrates, with BC20 showing the highest accumulation (12.15 ± 0.43 g $kg^{-1}$ d.w.) and the control (BC0) the lowest one (8.24 ± 0.26 mg/kg d.w.). Low biochar concentrations (BC5) did not show variations compared to the control, whilst higher concentrations (BC10, BC20, and BC40) produced leaves richer in Na. A similar trend was observed in the Mg accumulation in the leaves, for which the content was higher in biochar-enriched substrates in comparison to the control (3.67 ± 0.40 g $kg^{-1}$). Conversely, a slight significant decrease was observed for P content in rocket leaves with increasing biochar amounts. No variations were observed between BC0 and BC5, while a P reduction was detected in BC > 5%. Arsenic, Cd, V, and Cr were not detected in the rocket leaves. No considerable divergences were found in the Cu content. Indeed BC10, BC20, and BC40 exhibited similar values (4.24 ± 0.47, 2.82 ± 0.27, and 3.91 ± 0.92 mg $kg^{-1}$ d.w., respectively) in comparison with the control (4.82 ± 1.1 mg $kg^{-1}$ d.w.); BC5 revealed the lowest value (2.73 ± 0.43 mg $kg^{-1}$ d.w.), but it was statistically similar to the other ones. No variations were observed in the Ni content among the different biochar-enriched media. Conversely, Zn content increased with the increment in biochar concentration, showing its highest value in BC40 (24.35 ± 2.20 mg $kg^{-1}$ d.w.). No considerable variations were detected in Mn content in the rocket leaves.

**Table 3.** The mineral and heavy metal composition of rocket plants grown in biochar-enriched substrates. The average and standard deviation (in brackets) are reported. Different letters in the same row represent significant variations among treatments ($p < 0.05$), using the Kruskall–Wallis and non-parametric Dunn test.

| | BC0 | BC5 | BC10 | BC20 | BC40 |
|---|---|---|---|---|---|
| Elements (g $kg^{-1}$ d.w.) | | | | | |
| Ca | 21.47 (0.66) b | 23.82 (0.89) ab | 25.98 (0.30) a | 22.06 (0.52) b | 16.37 (0.71) c |
| K | 55.79 (0.99) ab | 51.48 (0.81) b | 55.35 (1.10) ab | 53.94 (1.38) ab | 59.93 (2.5) a |
| Mg | 3.67 (0.40) b | 5.69 (0.04) a | 5.41 (0.05) a | 5.03 (0.19) ab | 5.28 (0.20) a |
| Na | 8.24 (0.26) b | 8.94 (0.03) ab | 11.36 (0.74) ab | 12.15 (0.43) a | 11.95 (0.06) a |
| P | 7.25 (0.11) a | 7.11 (0.15) a | 6.50 (0.08) b | 6.17 (0.19) b | 6.50 (0.10) b |
| Heavy metals (mg $kg^{-1}$ d.w.) | | | | | |
| Fe | 420.67 (8.71) a | 415.60 (21.50) a | 456.15 (47.30) a | 418.20 (26.03) a | 396.56 (88.06) a |
| Cu | 4.82 (1.1) a | 2.73 (0.43) a | 4.24 (0.47) a | 2.82 (0.27) a | 3.91 (0.92) a |
| Mn | 173.58 (7.12) a | 174.56 (10.07) a | 192.98 (12.60) a | 150.11 (26.58) a | 165.25 (37.76) a |
| Ni | 2.07 (0.62) a | 1.34 (0.40) a | 1.99 (0.32) a | 1.88 (0.83) a | 1.46 (0.59) a |
| Zn | 16.98 (2.33) b | 18.67 (2.6) ab | 19.73 (1.66) ab | 18.02 (1.50) ab | 24.35 (2.20) a |

### *3.5. Chlorophyll, Flavonols, Nitrate Content, and Leaf Color*

No significant differences were observed in chlorophyll content and flavonols among the biochar-enriched substrates compared to the control (Table 4). The chlorophyll concentration varied from 13.78 ± 1.13 μg $cm^{-2}$ (BC40) to 14.7 ± 0.99 μg $cm^{-2}$ (BC10), and flavonols from 0.17 ± 0.03 μg $cm^{-2}$ (BC0) to 0.23 ± 0.01 μg $cm^{-2}$ (BC40). Conversely, slight differences were revealed in luminosity (L*), with the highest value (52.47 ± 0.77) found in BC40 and the lowest (50.51 ± 00.81) in BC10. No variations were detected for the other color parameters (a* and b*).

**Table 4.** The chlorophyll (Chl) and flavonol (Flav) contents and leaf color parameters (L*, a*, and b*) of rocket plants grown in biochar-enriched substrates. The average and standard deviation (in brackets) are reported. Different letters in the same row represent significant variations among treatments ($p < 0.05$), using Games–Howell non-parametric tests.

| | BC0 | BC5 | BC10 | BC20 | BC40 |
|---|---|---|---|---|---|
| Chl (μg $cm^{-2}$) | 14.03 (1.13) a | 13.5 (0.88) a | 14.7 (0.99) a | 13.78 (0.97) a | 13.08 (1.13) a |
| Flav (μg $cm^{-2}$) | 0.17 (0.03) a | 0.19 (0.04) a | 0.2 (0.03) a | 0.22 (0.03) a | 0.23 (0.01) a |
| L* | 50.31 (1.45) ab | 51.28 (0.64) ab | 50.51 (0.81) b | 51.87 (0.92) ab | 52.47 (0.77) a |
| a* | −5.08 (0.85) a | −3.78 (1.35) a | −4.47 (1) a | −3.77 (0.96) a | −4.59 (0.6) a |
| b* | 34.06 (0.66) a | 33.89 (0.49) a | 33.53 (0.48) a | 32.75 (0.81) a | 33.16 (1.06) a |

Significant differences were observed for nitrate content (Figure 1). In more detail, the increasing biochar content in the substrates (≥20% *v*/*v*) led to a decrement in nitrate concentration in rocket leaves. The highest value was observed in BC5 (2702.61 ± 12.9 mg $kg^{-1}$ f.w.) and the lowest in BC40 (1797.13 ± 18.69 mg $kg^{-1}$ f.w.). The trend observed was the following: BC5 > BC0 > BC10 > BC20 > BC40. In any case, all the values compiled with the threshold established by EC N. 1258/2011 [8] as the maximum levels allowed for the marketing of rocket.

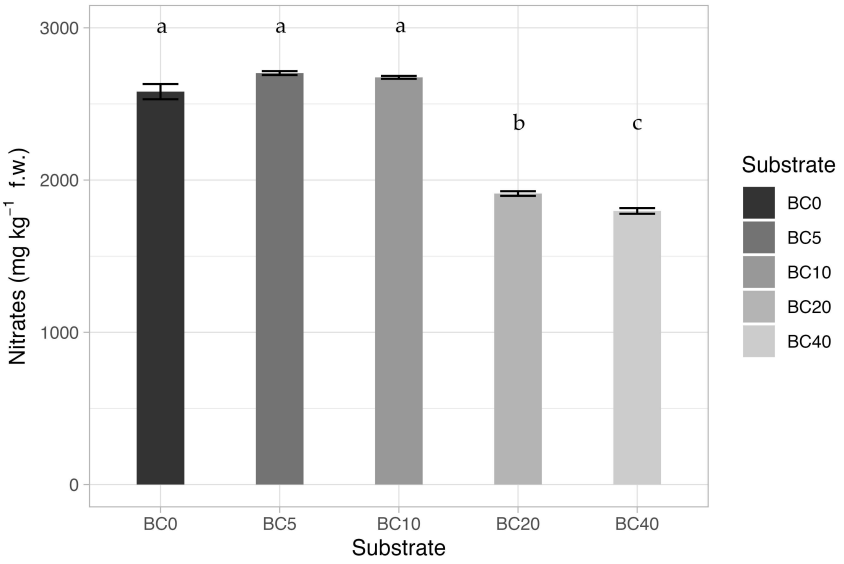


**Figure 1.** Bar plot representing the nitrate concentrations in rocket plants grown in different biochar-enriched substrates. Different letters in the same row represent significant variations among treatments ($p < 0.05$), using Games–Howell non-parametric tests.

### *3.6. Multivariate Analysis*

The multivariate approach was focused on the generation of a principal component analysis (PCA). Plant variables were primarily chosen to determine redundant traits which showed a Pearson correlation coefficient $\geq$0.95 (Figure S1). Specifically, ten variables were removed from a total of 31 traits considered in the correlation. In this context, a high correlation (r > 0.95) was observed among biometric parameters, such as blade length (BL), petiole length (PL), leaf area (LA), and specific leaf area (SLA), which were associated with fresh weight (FW). Furthermore, germination traits were greatly affected by each other. Indeed, a strong correlation (r = 0.95) was detected between the last germination time (LastGermTime) and the time-spread germination (TimeSpreadGerm). Similarly, t50total and Dlag50 were extremely associated with MGT. Finally, a remarkable association was detected between Fe and chlorophyll (r = 0.97) as well as S (r = 0.98), and between the P and b color parameters (r = 0.95).

PCA was carried out by using the mean and standardized values of each selected variable. The majority of the total variation (i.e., 83.87%) was explained by the first three components (PC1, PC2, and PC3), which accounted for 40.85%, 24.40%, and 18.62% variability, respectively. Table 5 reports the PCA loadings of the first two components. PC1 turned out to be positively correlated with FW, metal contents (Fe and Ni) as well as nitrates and the germination index (GI). Conversely, germination parameters related to time (i.e., TimeSpreadGerm and MGT) were negatively associated with PC1 as well as K, Zn, flavonols, and color luminosity (L). PC2 was highly associated with flavonols, Gp, Mg, and Na, while it was negatively correlated to Cu, P, and MGT.

Based on the first two components, the multivariate analysis clearly divided the treatment based on the biochar addition in the substrate as displayed in the PCA-biplot (Figure 2). In more detail, the control group (BC0) was positioned in the bottom-right quadrant of the biplot, indicating a positive association with PC1, influenced by nitrates and FW, and a negative correlation with PC2, primarily driven by P and Cu contents. The BC5 treatment was closest to BC0, showing an influence neither from PC1 nor from PC2, being located near the center of origin of the plot. BC10 was located in the upper-right quadrant, positively linked to both PC1 and PC2, reflecting its correlation with variables such as nitrates, Ca, FW, Fe, and GI. Conversely, BC20 was positively influenced only by

PC2 and mainly correlated with GP and the a* color parameter, and negatively associated with Cu and P. The substrates BC5, BC10, and BC20 turned out to be close to each other, being positioned in the same quadrant. Finally, BC40 was the most distant treatment from the others, situated in the lower-left quadrant, and was highly associated with time-related germination parameters (e.g., TimeSpreadGerm, MGT), Zn content, and color luminosity (L*).

**Table 5.** The PCA component loading of the rocket plant traits for the first two PCs. The percentage of variation per each PC and the cumulative variation are described. * represents the variables with PCA loadings >0.23 or <−0.23.

| | **PC1** | **PC2** |
|---|---|---|
| NL | 0.190 | 0.001 |
| FW | 0.257 * | 0.124 |
| DW | 0.168 | 0.002 |
| Flav | −0.250 * | 0.279 * |
| L* | −0.303 * | 0.165 |
| a* | −0.011 | 0.365 * |
| Ca | 0.294 * | 0.155 |
| Cu | 0.062 | −0.318 * |
| Fe | 0.263 * | 0.117 |
| K | −0.221 | −0.152 |
| Mg | −0.117 | 0.317 * |
| Mn | 0.139 | −0.099 |
| Na | −0.159 | 0.311 * |
| Ni | 0.232 * | −0.061 |
| P | 0.104 | −0.345 * |
| Zn | −0.304 * | 0.022 |
| Nitrates | 0.255 * | −0.115 |
| GP | 0.028 | 0.296 * |
| TimeSpreadGerm | −0.271 * | −0.214 |
| GI | 0.307 * | 0.170 |
| MGT | −0.244 * | −0.282 * |
| Variance percentage | 40.85% | 24.40% |
| Cumulative variance percentage | 40.85% | 65.25% |

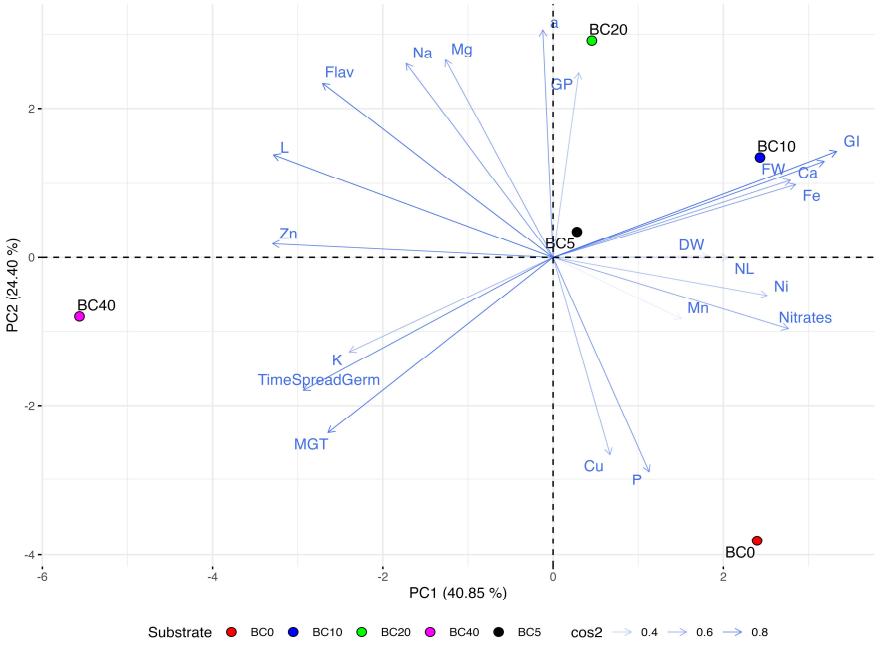


**Figure 2.** PCA biplot of the 5 biochar-enriched substrates considering plant traits.

## 4. Discussion

Many studies are available on the hydroponic growth of *Eruca vesicaria* [9,52,53], but few works have focused their tests on soilless cultivation using biochar as substrate amendment. In particular, to the best of our knowledge, this is the first study that evaluates rocket seed germination and plant performance in substrates with a high content of biochar (>15%). Moreover, little information about the mineral content of rocket grown in biochar-enriched media is found in the literature [54].

Our study demonstrated that a biochar concentration up to 40% as a substrate amendment (BC40) did not affect rocket seed germination. This result does not agree with findings by Milone et al. [35], in which 2.5% and 5% biochar concentrations caused a reduction in seed germination. Conversely, our results were in line with Sanchez et al. [28], who obtained a rocket seed germination ≥80% in substrates made of saline soil and different biochar concentrations (i.e., 5%, 10%, and 15%). The germination percentage observed in our study was >80% and was similar to the control in all biochar-enriched substrates up to 40%. Conversely, higher biochar concentrations (BC = 70% $v/v$) caused a drastic dwindling of GP. These findings are in agreement with those observed by Carril et al. [55] in tomato and basil, where a decrease in germination rates and indices was noted with concentrations of solid biochar fraction ≥50% ($v/v$). Many papers have reported that germination is influenced by EC and biochar can affect this parameter due to its high content of soluble salts (e.g., $Na^+$ and $Cl^-$) [56]. This may reduce the availability of key soluble nutrients (such as $NO_3^-$, $K^+$), resulting in nutrient imbalances and a decline in plant osmotic potential [57,58]. Carrill et al. [55] also stated that preventive washing treatments of biochar may reduce pH and EC amounts with a positive effect on germination. Our results confirmed that biochar up to 40% can replace peat in pot cultivation without affecting seed development.

Regarding plant biometric parameters, rocket yield (g/plant) was in agreement with results obtained by Fontana and Nicola [9] in rocket cultivated in a soilless floating system. Similar results were observed also in other studies reported by Nicola et al. [53]. Our findings revealed that moderate biochar concentrations (up to 20% $v/v$) did not affect rocket growth. Many diverse results are reported in the literature about the effect of biochar on the plants of this species. Zabaleta et al. [27] evidenced an increment in plant height with the increase in almond shell biochar concentration (up to 15%) 60 days after sowing, while Milone et al. [35] observed that the increase in bamboo biochar in the substrate caused a decrease in that parameter. Our results are more in line with Zabaleta et al. [27], with moderate biochar concentrations that did not affect plant performance or even increase it. However, it is worth mentioning that no effect resulted from the use of the same bamboo biochar concentration in the study of Milone et al. [35] in radish. Thus, the effect of a specific biochar may be species-dependent. A slight decrease in rocket salad yield and performance in pots with 40% $v/v$ biochar concentration was noted in our study. No previous studies have been conducted using rocket salad with biochar >15%; however, Shen et al. [59] observed a similar trend in the plant growth of xiao bai cai (*Brassica rapa* cv. Group Pak Choi Green-petioled Form), which belongs to the same Family of *E. vesicaria* (i.e., Brassicaceae). In particular, the authors recorded the best plant performance (fresh weight) of xiao bai cai with ~20–30% of biochar, while the growth was reduced with the increase in biochar (>30%), aligning with our results.

The type of substrate, and its organic matter, have an impact not only on substrate quality and crop productivity but also on the nutritional value of products, influencing their content of minerals, vitamins, antioxidants, and phenolic compounds [60]. Minerals are indispensable for supporting a wide range of biological activities throughout the different stages of plant growth and development [61]. The overall mineral concentrations (macro- and micronutrients) in commercial edible rocket leaves detected in this work are comparable

with those reported in previous works [52,62]. An increase in the biochar concentration of the substrates led to a decrease in P content in the rocket leaves. However, some studies have stated that P availability surged when applying biochar to soil or substrates [63]. Nevertheless, it is noteworthy that the availability of P is influenced by changes in pH and soil composition, mainly calcium content, which may have a negative effect on P uptake [64,65]. These considerations are in line with our results, as an increase in Ca was observed in the biochar-enriched substrates, whilst a decrease in P was detected in the rocket leaves. Moreover, we noticed an increment in Mg concentration (+30–35%) in biochar-enriched substrates. A similar result was observed by Farangi-Abriz and Ghassemi-Golezani [66] who detected an upsurge of Mg content in both safflower and mint leaves (+41% and +63% Mg, respectively). Magnesium availability can be related to substrate pH, with lower pH levels reducing its availability [67]. Thus, as biochar applications generally increase soil pH, magnesium becomes more accessible to plants [68]. The concentrations of heavy metals reported in our study are comparable with the ones observed in previous research on rocket salad [1,52,62]. Although biochar-enriched substrates contained higher Ni levels, no differences were observed in Ni concentrations in rocket leaves. Thus, biochar did not influence Ni uptake, confirming the trend observed in tomato fruits using the same biochar concentrations [38]. Conversely, a significant and slight increase in Zn (+30%) was observed in BC40 in comparison with the control BC0. Anyway, the value was in line with the amount of Zn normally found in rocket commercial leaves [62]. However, this increase in Zn content may be related to the lower yield detected in BC40. Indeed, the correlation plot (Figure S1) showed a strong negative correlation between FW and Zn ($r = -0.86$) which led to a strong influence of Zn in BC40 as further reported by the PCA (Figure 2). These results suggest that rocket salad cultivation and production can be compiled by adding up to 20% of woody biochar in a peat-based substrate without affecting plant growth and mineral content. No substantial changes were observed in the chlorophyll and flavonol contents or leaf color traits, except for a significant increase in color luminosity in BC40.

In baby leaf vegetables, such as lettuce, rocket salad, chicory, and spinach, one critical parameter in terms of food quality and safety is the nitrate concentration in the leaves [69]. These kinds of vegetables are the main contributors to dietary nitrate intake, accounting for approximately 40–92% of daily intake [70]. An excessive nitrate concentration in vegetables is generally considered a negative quality factor [5] and dangerous for human health [6]. Indeed, nitrate itself is not harmful, but its conversion to nitrite in the body poses health risks, including the formation of carcinogenic nitrosamines and methemoglobin. The latter can lead to oxygen deficiency, particularly in newborns, causing the "blue baby syndrome" [6]. Therefore, the World Health Organization (WHO) has set an acceptable daily intake (ADI) for nitrate at 3.7 mg per kg of body weight [71]. Among baby leaf vegetables, rocket is especially known for its ability to accumulate nitrates, with levels influenced by factors like soil nitrate availability, cultivation practices, seasonal timing, and environmental conditions [72]. In this study, decrease in nitrates content with the increment of biochar in the substrates was observed. Particularly, our findings revealed a ~26–30% reduction in nitrate levels in BC20 and BC40 treatments compared to the control. Similarly, a decline in nitrate content was observed in cabbage genotypes with increasing biochar concentrations in the substrate [73]. This effect has been linked to the enhanced substrate properties caused by biochar, which enriches elements such as C, N, O, S, and Ca. Elevated levels of C and N not only improve nutrient use efficiency and crop nutritional quality but also contribute to overall soil fertility [73]. Additionally, biochar's characteristics (i.e., large surface area, high porosity, and cation exchange capacity) make it an effective soil conditioner, altering the substrate's physicochemical and microbial properties to enhancing nutrient use efficiency in soil/plant systems [74,75]. In this specific context, Saffeullah

et al. [73] demonstrated that *Prosopis* wood biochar can improve N-metabolizing enzyme activity, and therefore, enhance nitrogen use efficiency by reducing nitrate leaching and nitrate content in cabbage leaves. Our study also confirmed an increase in CEC and substrate porosity, supporting the consideration that biochar may minimize N losses in the substrates, boost nutrient use efficiency, and particularly on nitrates, reducing their content in rocket leaves. Similar findings were reported by Haider et al. [76], who observed a reduction in tissue nitrate levels with biochar application. Based on our results, applying moderate biochar concentrations, such as 20%, in a peat-based substrate does not negatively affect rocket seed germination and plant growth, and improves leaf quality by reducing nitrate concentrations

## 5. Conclusions

Soilless cultivation systems are widely used for growing baby leaves like *Eruca vesicaria* due to their efficient management of substrates, water, plant nutrition, and environmental conditions, as well as their ability to mitigate nitrate accumulation and disease. However, the reliance on peat as a growing medium raises sustainability concerns, as peat extraction releases significant $CO_2$ into the atmosphere. This study investigated the potential of incorporating biochar at varying concentrations into peat-based substrates for rocket salad cultivation.

Our findings revealed that biochar concentrations up to 40% $v/v$ did not compromise seed germination compared to a commercial peat-based control. However, higher biochar levels (70% $v/v$) significantly reduced both germination and plant development. Moderate biochar additions (up to 20% $v/v$) maintained comparable plant growth and mineral content to the control, while higher concentrations (40% $v/v$) adversely affected growth and increased heavy metal levels, such as Zn, in the substrate. Notably, high biochar levels reduced nitrate accumulation in rocket leaves, potentially due to enhanced N-metabolizing enzyme activity and improved nitrogen use efficiency.

To the best of our knowledge, this study is the first to evaluate the effects of high biochar concentrations (>15% $v/v$) in peat-based substrates for rocket salad, providing valuable insights for more sustainable cultivation practices while addressing peat-related environmental concerns.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture15030302/s1, Figure S1: Correlation plot based on Pearson correlation coefficient (r) on rocket plant traits; Table S1. Physicochemical characteristics of biochar-enriched substrates used in this research with the thresholds' limits established by regional, National and European law. The means and standard deviations (in brackets) of BC0, BC5, BC10, BC20, and BC40 are published in Bini et al. [38].

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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