



Article

Assessing the Effectiveness of Vermi-Liquids as a Sustainable Alternative to Inorganic Nutrient Solutions in Hydroponic Agriculture: A Study on *Diplotaxis muralis*

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Abstract: Organic products are gaining popularity due to their positive impact on human health and the environment. While hydroponics is commonly used in vegetable production, it relies on mineral fertilizers derived from limited and non-renewable resources. As a result, farmers are actively seeking sustainable farming solutions. This study comprehensively evaluated the effectiveness of vermiliquids (organic nutrient solutions) as a replacement for conventional inorganic nutrient solutions in promoting growth and nutrient acquisition in *Diplotaxis muralis* plants in a controlled environment. The results showed that plant biomass and SPAD values of *D. muralis* grown in Hoagland solution and enhanced vermitea (vermitea having relatively low pH and high EC) were higher compared to standard vermitea (high pH and low EC). The findings also revealed improved nutrient assimilation of phosphorus, potassium, calcium, iron, manganese, copper, and zinc in the enhanced vermitea plants. The heavy metal contents in D. muralis leaves were evaluated, too, and they were found to fall significantly below the safe threshold, rendering them safe for human consumption. However, the standard vermitea, with its high pH and low EC, performed poorly as a hydroponic solution. This research suggests that enhanced vermitea can completely replace chemical nutrient solutions in hydroponic agriculture. This substitution could lead to reduced production costs and improved product quality.

Keywords: vermicompost tea; nutrients; rocket; organic fertilizer; sustainability; *Diplotaxis muralis*

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Citation: Rehman, S.u.; Aprile, A.; De Castro, F.; Negro, C.; Migoni, D.; Benedetti, M.; Sabella, E.; Fanizzi, F.P. Assessing the Effectiveness of Vermi-Liquids as a Sustainable Alternative to Inorganic Nutrient Solutions in Hydroponic Agriculture: A Study on *Diplotaxis muralis*. *Agronomy* 2024, 14, 1310. https://doi.org/10.3390/agronomy14061310

Academic Editor: Youssef Rouphael

Received: 13 May 2024 Revised: 7 June 2024 Accepted: 14 June 2024 Published: 18 June 2024



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1. Introduction

The expansion of urban areas has resulted in the conversion of agricultural lands into urban developments, leading to increased land-use limitations in urban regions. This poses a significant challenge in allocating sufficient land for agricultural purposes. To ensure food security, new crop production methods such as hydroponic vertical farming have emerged as a potential substitute for urban farming. Hydroponics involves growing crops in soilless media, using water and nutrient solutions [1]. This approach offers improved water and nutrient usage efficiency, as well as pest and disease management, compared to traditional farming methods [2]. It is remarkably beneficial in areas with limited arable land and degraded, contaminated, or arid soil. Therefore, hydroponic farming is a suitable option for urban areas. However, this method relies on mineral fertilizers derived from finite and non-renewable resources [3]. Concerns can be raised regarding the manufacturing process for macronutrients such as nitrogen, phosphorus, and potassium. For example, the production of ammonia, which accounts for 97% of nitrogenous fertilizers, requires a significant amount of energy (36.9 GJ/t of NH₃) and utilizes natural gas and hydrocarbons as fuel and feedstock [4]. The production of potassic and phosphatic fertilizers also involves

energy-intensive mining processes for potash and rock phosphate [5]. In hydroponics, plants assimilate nitrogen in the form of NO_3^- and NH_4^+ , and their continuous application can lead to the accumulation of NO_3^- in vegetables [6]. High nitrate contents in edible plants can be detrimental to both plant growth and human health [7].

The uneven distribution of synthetic fertilizers, combined with their reliance on non-renewable resources, makes them susceptible to economic instability during global crises [8]. Therefore, it is imperative to develop alternatives to synthetic fertilizers to address challenges related to agriculture and sustainability. Currently, the popularity of organic hydroponics is growing, and the organic food market is expanding [9]. Liquid organic solutions are the extracts of organic fertilizers that are diluted with water and transferred to a hydroponic system [10]. These solutions do not contain essential elements in their ionic form; hence the nutrients are not immediately accessible for plants. Additionally, the nutrients must undergo decomposition and mineralization by microorganisms [11]. Earthworms play a significant role in organic matter decomposition through the actions of microorganisms in their digestive tract, resulting in the production of vermicompost [12]. During this process, mucus is secreted, which enhances microbial and enzymatic activity, leading to increased mineralization of organic matter. Vermicompost (VC) is an organic fertilizer produced through the combined activity of earthworms and microbes. Due to microbial activity, the N, P, and K in VC become more bioavailable [13]. The liquid extract of VC is known as vermitea, which is a quite novel method of applying VC [14]. Standard vermitea requires a lower concentration of solid VC for larger production areas, while extracting essential nutrients from VC. Despite its positive effects on plant growth and yield, the use of vermitea has primarily been described as a foliar application on plants. However, its potential as an alternative to synthetic nutrient solutions in hydroponic farming has not been extensively studied.

In this study, we conducted an experiment using *Diplotaxis muralis* as a test plant to evaluate its growth and quality in hydroponic farming. The plant was grown using both organic and chemical nutrient solutions. *Diplotaxis muralis* is a fast-growing, perennial plant known for its pleasant aroma and spicy flavor. It is commonly used in Mediterranean cuisine as a salad ingredient and garnish [15] due to its high nutritional value, containing P, K, Ca, S, Fe, vitamins, and fiber [16]. *D. muralis* helps in the treatment of anemia, lung diseases, and lack of appetite. It can help treat triglycerides due to the presence of omega 3, a fatty acid that provides better blood circulation by unclogging the arteries [16]. However, some concerns, such as those surrounding nutrient balance, have to be addressed when growing rocket plants because they are efficient accumulators of nitrates, and the higher accumulation of nitrates is toxic to humans [7]. The economic importance of *Diplotaxis muralis* and its production challenges have significant implications for the agricultural industry, making the development of sustainable and efficient nutrient solutions for hydroponic agriculture crucial.

The main objective of this research was to develop and assess the effectiveness of robust organic nutrient solutions as alternatives to traditional chemical nutrient solutions to promote sustainable practices in hydroponic agriculture. Additionally, the study investigated the effect of vermitea on the nutrient uptake, growth, and quality of *Diplotaxis muralis* in hydroponic farming.

2. Materials and Methods

2.1. Preparation of Vermicompost Tea

Vermicompost tea was prepared using vermicompost made from green waste collected and processed at Compost Natura s.r.l. in Lecce, Italy. Green waste comprising biomass from pine, Mediterranean cypress, palm trees, and seagrass (*Posidonia oceanica*) contributed 20% of the total green waste. The waste was first moistened with tap water and allowed to pre-compost for 20 days. Then, earthworms of the species "*Eisenia fetida*" were added to the vermibed at a stocking density of 3 kg/m² to begin the composting process. The moisture content of vermibeds was regulated between 60 and 80% by sprinkling with water. The

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vermicomposting unit was maintained for 60 days, after which the vermicompost was collected once the composted material had been harvested using a 6 mm sized mesh. The air-dried vermicompost (40% moisture) was then used to make vermicompost tea following the method as described by [17], with minor modifications.

Preparation of Standard Vermitea (SVT) and Enhanced Vermitea (EVT):To prepare both the standard vermitea (SVT) and enhanced vermitea (EVT), we started with a 1:5 ratio of vermicompost to double distilled water (DDW), resulting in an initial extract with an electrical conductivity (EC) of approximately 2.0 mS/cm.

Standard Vermitea (SVT): The vermicompost used to obtain the first vermitea was further used for nutrient extraction, achieving a final dilution ratio of 1:10 (vermicompost:water). No additional pH adjustment was made, and the pH of the SVT was typically high, ranging between 7.5 and 8.0.

Enhanced Vermitea (EVT): The enhanced vermitea (EVT) was obtained with a single dilution of 1:5 (vermicompost:water). The pH of the EVT was then adjusted to a range of 5.8–6.3 by adding a few drops of diluted sulfuric acid ($10\%~H_2SO_4$). This process ensured that the EVT had a higher nutrient concentration and an optimized pH for plant uptake, similar to the Hoagland solution.

Both the EVT and SVT solutions exhibited distinct colors, indicative of the presence of organic compounds. The EVT had a dark brown color, while the SVT was yellowish-brown. The dark color of the EVT suggests a higher concentration of humic substances, as described by [18].

2.2. Experimental Design

The seeds of *Diplotaxis muralis* were germinated in plastic petri dishes containing moist filter paper at a temperature of 25 °C. After a germination period of 5–6 days, five sprouted seeds were transplanted into plastic pots (dimensions: $7 \times 7 \times 25$ cm), filled with perlite. The pots were then watered with deionized water and placed into a hydroponic system.

Hydroponic System: Fresh treatment solutions (Hoagland solution, enhanced vermitea [EVT], and standard vermitea [SVT]) were circulated through the pots. The nutrient solutions were delivered to the plant roots using a circulating system. The flow rate was maintained at approximately 2 L per hour per pot to ensure consistent hydration and nutrient supply. To maintain adequate oxygen levels in the nutrient solutions, an aquarium pump was used to continuously aerate the solutions. The pH values of the nutrient solutions were monitored and adjusted regularly. The initial pH of Hoagland solution and EVT was adjusted to the target range (5.8–6.3) using diluted sulfuric acid (10% $\rm H_2SO_4$). The pH was checked every two days using a pH meter (Waterproof Cyber-Scan PC 650 EUTECH Instruments). The nutrient solutions were renewed every two weeks to prevent nutrient depletion and accumulation of waste products. During the renewal process, the old solution was completely drained from the tanks, and a fresh solution was added. The plants were grown in a greenhouse at University of Salento, Lecce, Italy ($\rm 40^{\circ}20'12''~N; 18^{\circ}07'24''~E)$ under controlled conditions (14 h daylight, 25/18 °C day/night temperature, and 65% humidity).

The Hoagland solution was made by utilizing reverse osmosis water ($<30~\mu\text{S/cm}$). It consisted of 1.1 mM KNO₃, 0.2 mM NH₄NO₃, 3.0 mM Ca(NO₃)₂·2H₂O, 2.0 mM MgSO₄, 70 μ M H₃BO₃, 1.2 mM K₂HPO₄, 1.2 μ M Na₂MoO₄, 0.04 g/L FeEDDHA, 1.0 μ M ZnSO₄, 1.0 μ M CuSO₄, and 10 μ M MnSO₄. The EC of the Hoagland solution was adjusted to about 1.94 mS/cm. The initial pH of the Hoagland solution was adjusted between 5.8 and 6.3 (by addition of 10% H₂SO₄) and was continuously monitored.

2.3. Biomass Analysis

At the end of the growth period, which spanned around 45 days, the plants were harvested from the pots and the fresh biomass of plants was quantified using a digital weighing balance. The leaves of *Diplotaxis muralis* were then dried out in an 80 °C oven until a constant weight was achieved, at which point the dry weight was determined.

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2.4. Biochemical Analysis

The elemental composition of *Diplotaxis muralis* leaves was determined by the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) technique. A standardized procedure was used to analyze each sample. In summary, 0.5 g of each sample was mixed with $\rm H_2O_2$ (4 mL) and suprapure HNO₃ (6 mL), and then heated to 180 °C for 10 min in a microwave digestion system (Milestone Start D). The resulting solution was cooled and diluted with suprapure water, reaching an absolute volume of 20 mL. The mixture was filtered and analyzed for elemental composition using an ICP spectrometer. The spectrometer was calibrated using five standard solutions of specific concentrations (0.001, 0.01, 0.1, 0.5, and 1.0 mg/L) of the elements. The calibration lines showed strong correlation coefficients (r) above 0.99 for all measured elements. The results were calculated as the mean of three separate measurements, and the elemental concentrations are reported in ppm. Nitrate (N-NO₃⁻) levels were determined using spectrophotometry with a "HI 83225 multi-nutrient analyzer (Hanna Instruments, Woonsocket, RI, USA)". The pH and electrical conductivity (EC) were measured using electronic meters (Waterproof CyberScan PC 650, Eutech Instruments Pte Ltd., Singapore).

The chlorophyll levels in plant leaves were quantitatively determined using a SPAD Chlorophyll Meter (SPAD-502 Plus, Konica Minolta Sensing, Tokyo, Japan). For each plant, we selected leaves from the same layer to ensure consistency within each variant. Specifically, we chose fully expanded leaves from the middle portion of the plant, as these leaves are less likely to be affected by age-related changes compared to older leaves at the bottom or younger leaves at the top. Each selected leaf was measured at three different points along its midrib to account for variations in chlorophyll distribution. The three measurement points were chosen to be equidistant from the base to the tip of the leaf. To ensure repeatability, each measurement was performed in triplicate for each leaf, and the average value was recorded. Measurements were taken under consistent light conditions to eliminate any discrepancies caused by changes in light intensity. The data collected from the SPAD meter were recorded and analyzed to evaluate the nutritional status of the plants, specifically focusing on the availability of nitrogen, as indicated by the chlorophyll levels. The SPAD value for chlorophyll was measured at the beginning of the flowering stage.

2.5. Data Analysis

The data underwent analysis of variance (ANOVA) using the R software (R version 4.2.3). The differences in means were determined using the least significant difference (LSD) test with a significance level of p < 0.05.

3. Results and Discussion

3.1. Chemical Characteristics of Vermitea Solutions

The nutrient composition of vermi-liquids, specifically enhanced vermitea (EVT) and standard vermitea (SVT), is summarized in Table 1. Significant variations were observed among the different experimental media. The macronutrients (N, P, K, Ca, Mg) and micronutrients (Fe, Cu, Mn, Zn, B) are crucial for plant metabolism and growth [19]. Our analysis showed that EVT has higher concentrations of N, P, K, Mg, Fe, Cu, Zn, and Mn, whereas SVT has a lower nutrient level, as shown in Table 1. SVT also has a lower electrical conductivity and higher pH compared to EVT. Unlike nitrogen, phosphorus, and sulfur, which can be immobilized as part of organic molecules during composting, inorganic ions such as K^+ , Ca^{2+} , and Mg^{2+} typically remain in their ionic forms and are not significantly immobilized. These ions are retained in the compost in a readily available form for plant uptake. The benefits of using vermitea are not only due to its high nutrient levels but also because it contains metabolites and growth hormones that are typically absent in chemical nutrient solutions like Hoagland solution [20]. For example, the presence of water-soluble nutrients and other organic compounds in vermicompost extract, such as plant growth regulators and humic acids, promotes the germination of *Pinus pinaster* [21]. The pH and EC of solutions strongly influence nutrient uptake and plant growth. Elevated pH levels

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have a noteworthy impact on the acquisition of essential nutrients, particularly P, K, Ca, Mg, Cu, Mn, Zn, and Fe [22]. Furthermore, increasing the EC of the solution significantly improves the absorption of N, P, K, and Ca [23]. The electrical conductivity (EC) of the treatment solutions used in this study ranged from 1.29 to 1.99 mS/cm. Although the EC differences (1.99 vs. 1.29 mS/cm) may not be substantial enough alone to cause the observed differences in biomass, the combination of higher nutrient concentrations, the optimized pH, and the presence of beneficial organic compounds in the enhanced vermitea (EVT) likely contributed to the enhanced plant growth observed.

Table 1. Analytical characterization of vermi-liquids: enhanced vermitea (EVT) and standard vermitea (SVT) solutions used in the hydroponics system for this study.

| Nutrient | рН | EC (mS/cm) | Nutrients (mg/L) | | | | | | | | | |
|-----------|------|---------------|-------------------|------|-----|------|------|------|------|------|------|------|
| Solutions | | | NO ₃ - | P | K | Ca | Mg | Fe | Mn | Cu | Zn | В |
| HS * | 5.92 | 1.985 | 398.7 | 40.2 | 163 | 137 | 57.7 | 1.73 | 0.42 | 0.09 | 0.11 | 0.62 |
| EVT * | 6.28 | 1.995 | 265.8 | 20.9 | 178 | 119 | 39 | 0.29 | 0.18 | 0.03 | 0.10 | 0.42 |
| SVT * | 7.74 | 1.292 | 66.5 | 6.4 | 100 | 33.5 | 17.2 | 0.12 | 0.01 | 0.02 | 0.06 | 0.27 |

^{*} HS: Hoagland solution; EVT: enhanced vermitea; SVT: standard vermitea.

3.2. Shoot Dry Weight

The shoot dry weight of hydroponically grown *Diplotaxis muralis* was significantly affected by nutrient solutions (Figure 1). Plants grown in HS and EVT solutions exhibited superior dry weight metrics relative to those nurtured within the SVT solution, suggesting that discrepancies in nutrient concentrations across the treatments significantly contributed to the observed variances in dry weight outcomes.

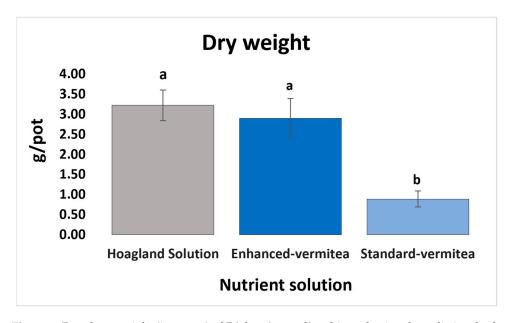


Figure 1. Dry shoot weight (in grams) of *Diplotaxis muralis* cultivated using three distinct hydroponic nutrient treatments: Hoagland solution (HS), enhanced vermitea (EVT), and standard vermitea (SVT). The x-axis signifies the nutrient solutions, while the y-axis illustrates the dry shoot weight. A one-way ANOVA test revealed significant differences among the different hydroponic solutions which is indicated by Letters (a, b).

N, P, and K are essential elements with both physiological and structural functions in plants. According to Stathopoulou et al. [24], higher concentrations of N, P, K, and Fe are related to enhanced biomass production in rocket plants. Our study also showed that the HS and EVT solutions had higher levels of nitrate, P, K, Ca, Mg, and other micronutrients, leading to increased biomass production compared to the SVT treatment, which had a

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lesser nutrient concentration. The pH and EC of the hydroponic solution also played a significant role in plant development. The higher pH and lower EC in the SVT solution contributed to lower biomass production (Table 1). The findings coincide with the outcomes of Wortman [25], who found that plants such as *Ocimum basilicum* L., *Brassica oleracea* L., *Capsicum annuum* L., and *Solanum lycopersicum* L. showed a reduction of up to 76% in yield when grown in nutrient solutions with low EC and high pH levels. The EC of the SVT solution was 1.292 mS/cm. In a study by Yang et al. [26], it was observed that lower EC values resulted in decreased growth and yield characteristics.

3.3. Diplotaxis muralis Nutritional Composition

3.3.1. SPAD Value of Diplotaxis muralis

The SPAD value is an indicator of nitrate concentration in plant leaves, and previous studies have reported a correlation between SPAD value and nitrate levels in spinach, palm oil seedlings, and forage grass [27–29]. Nitrogen is a component of proteins, which are functional compounds of chloroplasts [30]. Low nitrogen levels in the growth medium can negatively affect plant growth, as observed in the SVT solution, while high nitrate concentrations in edible plants can be toxic to both plant growth and human health [7].

In our experiment, the HS solution had a higher concentration of nitrate (398.7 mg/L) compared to EVT (265.8 mg/L) and the SVT solution, which had a very low concentration of nitrate (66.5 mg/L) (see Table 1). Despite the differences in nutrient levels between HS and EVT, statistical analysis showed no significant difference in the SPAD values of rocket plants (Figure 2). This could suggest that while nitrate concentration is a prime factor for plant growth, it is not the sole determinant of chlorophyll content in plants. Other nutrients or compounds present in EVT could contribute to chlorophyll production, effectively compensating for its lower nitrate contents compared to the Hoagland solution. The observed results can also be explained by the presence of humic acid in vermicompost tea, which activates the plasma membrane H⁺-ATPase enzyme, leading to acidification of the rhizosphere and increased uptake of NH₄⁺ and NO₃⁻, resulting in higher synthesis of chlorophyll molecules [31,32]. The results of this study are also supported by [33], explaining the positive impact of vermitea treatments on the growth parameters and photosynthetic pigments of maize and sunflower plants.

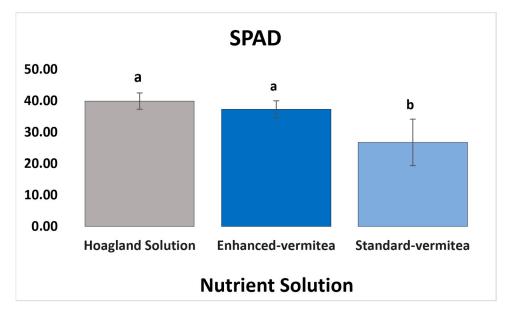


Figure 2. SPAD values of *Diplotaxis muralis* grown under three distinct hydroponic nutrient treatments: Hoagland solution (HS), enhanced vermitea (EVT), and standard vermitea (SVT). The x-axis shows the nutrient solutions, while the y-axis signifies the SPAD value. Letters (a, b) indicate significant differences between the treatments, as verified by the LSD test.

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Standard vermitea (SVT) has a lower SPAD value, indicating that its chlorophyll content is significantly lower than that of HS and EVT. This may be due to the higher EC in HS and EVT compared to SVT. These outcomes are in line with the findings of Yang, Samarakoon, Altland, and Ling [26], who observed that plants grown in solutions with an EC of 1.8 mS/cm had a higher SPAD value than those grown in solutions with an EC of 1.2 mS/cm.

According to these findings, EVT is an organic alternative that appears to have comparable performance in terms of chlorophyll content and is more sustainable and environmentally friendly. EVT can be the preferred option as it also contains other beneficial microorganisms or nutrients that are not present in HS due to its synthetic composition.

3.3.2. Phosphorus

Phosphorus plays a vital role in plant metabolism, specifically in the transfer of energy through ATP. Our research compared the levels of P in plants grown in chemical nutrient solutions and vermi-liquids. Figure 3 illustrates a notable difference in P absorption by rocket plants grown in EVT and SVT nutrient solutions. The plants grown in HS and EVT exhibited higher concentrations of phosphorus compared to those in SVT. The lower concentration of P in leaves can be attributed to the lower P concentration in SVT. Additionally, the higher pH of SVT solution probably inhibits P solubilization, leading to lower absorption by rocket plants.

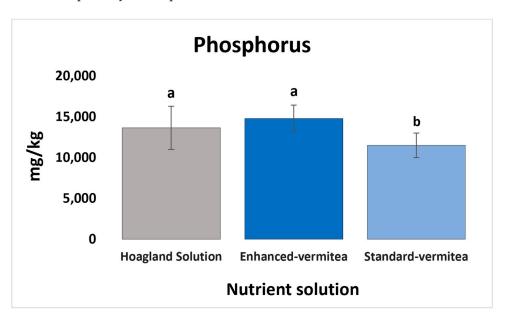


Figure 3. Comparison of phosphorus assimilation (mg/kg) by *Diplotaxis muralis* plants grown in various nutrient solutions: Hoagland solution (HS), enhanced vermitea (EVT), and standard vermitea (SVT). Letters above the bars indicate statistically similar groups identified through post hoc analysis.

The higher P content in enhanced vermitea solutions can be ascribed to the existence of beneficial microorganisms, such as phosphate-solubilizing bacteria (PSB), in vermicompost. These PSB have the capability to release phosphorus for plant absorption by producing organic acids and alkaline phosphatases, which promote P mineralization [34]. Our findings are consistent with previous studies conducted by Zandvakili, Barker, Hashemi, Etemadi, and Autio [30], and Ahmed et al. [35], which also observed increased phosphorus uptake in lettuce fertilized with organic methods.

3.3.3. Potassium

Potassium is an essential macronutrient that plays a crucial role in the growth and development of plants. It is responsible for the transport and absorption of nutrients, as well as increasing resistance to environmental and biological stressors. This leads to

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increased yields of high-quality crops and improved protection against plant diseases [36]. In this study, plants grown in HS and EVT solutions showed higher K levels than those cultivated in SVT nutrient solution (Figure 4).

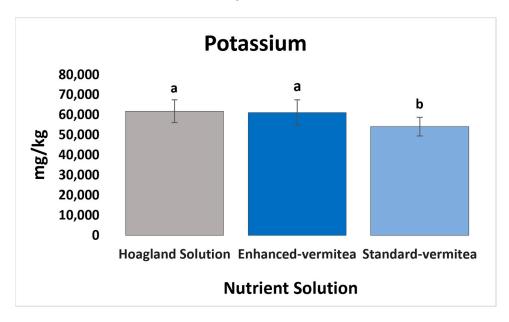


Figure 4. Potassium absorption in *Diplotaxis muralis* shoots cultivated in different hydroponic solutions: Hoagland solution (HS), enhanced vermitea (EVT), and standard vermitea (SVT). The bars indicate the mean potassium contents in rocket plants, with letters above denoting the level of significance determined through post hoc analysis.

Table 1 shows that the EVT solutions had higher K levels compared to the SVT nutrient solution. The SVT solution does not contain sufficient levels of K to support optimal growth. The increased concentration of potassium by rocket plants in the enhanced vermitea treatment can be attributed to changes in cellular charge. Humic acid molecules derived from vermicompost primarily have anionic characteristics and are absorbed into the cell, resulting in an increase in negative charges in the cytoplasm. This promotes the absorption of cations [37]. Rocket plants benefit from the higher K⁺ concentration in the enhanced vermitea solution, leading to its improved assimilation (Figure 4).

3.3.4. Calcium

It is noteworthy that the use of EVT and HS has had a positive impact on the accumulation of calcium in wild rocket leaves (see Figure 5). However, there was a significant difference observed between the effects of HS and EVT solutions. The decrease in Ca^{2+} assimilation by wild rocket plants grown in standard vermitea may be due to the low Ca^{2+} content in the nutrient solution (see Table 1). Furthermore, plants have a limited ability to absorb Ca^{2+} due to the concentration of available phosphate. The concentration of phosphate positively influences the plant's ability to take up Ca^{2+} ions [38]. Hoagland and enhanced vermitea solutions have higher concentrations of phosphorus, resulting in increased calcium uptake. Additionally, the alkaline pH of the nutrient solution in SVT may hinder the assimilation of Ca^{2+} .

Vermitea contains higher concentrations of microbial by-products and mineral nutrients in a form that is freely available for plant assimilation, which enhances their uptake in foliage [39]. During the brewing process of vermitea from solid vermicompost, plant growth regulators, soluble nutrients, microbes, and humic and fulvic acids are extracted [40]. These fulvic and humic acids make the mineral nutrients more accessible to plants and promote their uptake [41]. Humates in vermicompost tea have a strong affinity for cations and form complexes with them owing to the presence of O-, N-, and S-containing functional groups in the structure of humic substances, thereby increasing their bioavailability for plant

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absorption [42,43]. Humic substances also modify the root structure and activate proton pumps in maize and tomato, as explained by Canellas et al. [44] and Dobbss et al. [45].

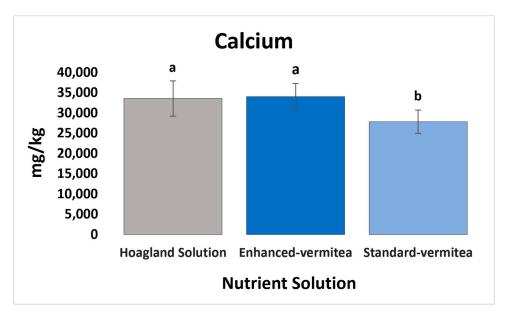


Figure 5. Calcium contents in *Diplotaxis muralis* plants leaves grown under three hydroponic nutrient treatments: Hoagland solution (HS), enhanced vermitea (EVT), and standard vermitea (SVT). The x-axis represents the nutrient solutions, while the y-axis shows the concentration of Ca²⁺ (mg/kg) in rocket plants. Letters (a, b) indicate significant differences between treatments, as determined by the LSD test.

This study investigated the absorption of nutrients and growth of *D. muralis* in different hydroponic solutions. The results demonstrate the complex relationship between plant nutrition and the important role of each macronutrient in promoting optimal plant health and productivity. The findings suggest that modifying hydroponic solutions, such as using vermitea-based solutions, can enhance the availability and balance of essential nutrients. This not only supports sustainable farming practices by increasing nutrient efficiency and reducing the use of chemical fertilizers, but also paves the way for further research on the molecular mechanisms involved in plant–nutrient interactions. Notably, the analysis revealed that plants grown in both the Hoagland solution (HS) and the enhanced vermitea (EVT) solution absorbed macronutrients at similar levels, indicating the potential of EVT as a beneficial option for organic farming. This highlights the potential of EVT as a sustainable alternative to traditional hydroponic solutions, offering a viable approach for promoting organic agricultural practices.

3.3.5. Micronutrients Assimilation

The use of enhanced vermitea (EVT) resulted in higher levels of micronutrient elements in *D. muralis* compared to other treatments, as shown in Figure 6. Gomes et al. [46] explained that the increased uptake of N, P, K, Mg, Ca, S, Mn, and Zn in shoots of *Garcinia mangostana* L. was due to the presence of humic acid derived from vermicompost. In our experiment, we also observed higher levels of Fe, Cu, Mn, and Zn in plants grown in EVT compared to other growth media. The uptake of Fe in rocket leaves was significantly higher in the EVT nutrient solution (Figure 6), which can be attributed to the capability of humic substances to form complexes with Fe. This complexation can also enhance the uptake of phosphorus, as phosphate can bind to humic substances through Fe bridges [47,48]. This process promotes the uptake of both P and Fe by plants [49], as observed in our study with the EVT nutrient solution. Furthermore, the increase in Fe and other micronutrient uptake in EVT may be due to the redox reactivity of humic substances [50]. The reduction of Fe³⁺ to Fe²⁺ at the root surface allows for the uptake of Fe²⁺ by young lateral roots [51]. This reduction of Fe³⁺

and other micronutrients is more significant at lower pH levels [43]. The lower contents of micronutrients in rocket plants grown in SVT solution may also be attributed to the higher pH of the nutrient solution.

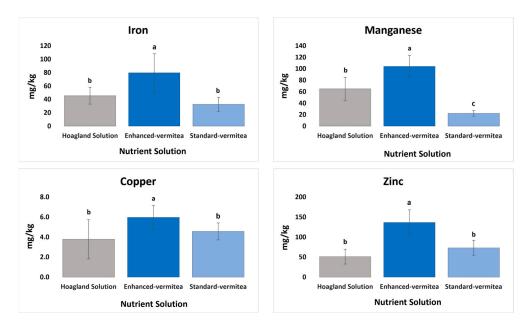


Figure 6. Micronutrient concentrations in *D. muralis* leaves. The graphs depict the absorption of nutrients in *D. muralis* plants cultivated in organic nutrient solutions (standard vermitea and enhanced vermitea) and a chemical nutrient solution (Hoagland solution). An ANOVA test revealed significant differences among the nutrient solutions. Post hoc analysis, utilizing Fisher's least significant difference (LSD) test, classified the nutrient solutions based on their statistical comparison in nutrient assimilation. Solutions with the same letters are not significantly different, while those with different letters demonstrate a significant variation in micronutrient uptake.

Figure 6 illustrates the concentrations of Mn, Cu, and Zn by rocket plants cultivated in different solutions: HS, EVT, and SVT. A significant difference was perceived in the uptake of these nutrients by the plant tissues. The plants grown in EVT exhibited a higher absorption of Mn compared to those grown in the other solutions. Standard vermitea (SVT), which had a lower concentration of Mn (0.01 mg/L), resulted in decreased uptake by the plants' leaves and led to a deficiency of Mn. A similar trend was noted in the absorption of Cu by rocket plants, likely due to the synergistic interaction between Cu and Mn (Figure 6).

Zinc is a crucial element for plant metabolism, enzymatic activity, and ion transport. Previous studies have demonstrated an antagonistic relationship between Zn and P, as a deficiency in phosphorus can stimulate the expression of genes related to Zn and Fe homeostasis [52]. Our study found that the HS solution had a higher concentration of P compared to the other treatments, but it resulted in lower absorption of Zn. The SVT nutrient solution had a significantly low amount of Zn, which hindered its absorption by the rocket plants (Figure 6).

The EVT solution contains a moderate concentration of P and a higher amount of Zn, resulting in increased Zn uptake and bioavailability to rocket plant leaves. Additionally, vermicompost promotes the growth of beneficial microbes, which produce phytohormones and enzymes that enhance plant growth [13]. Arancon et al. [53] discovered that vermitea, which contains plant hormones and humates, significantly increased the yield of lettuce and tomatoes. Furthermore, plant growth-promoting bacteria play a key function in the uptake of essential nutrients by plants. Zinc-solubilizing bacteria from vermicompost provides plants with sufficient zinc for optimal uptake [54]. In contrast, the chemical nutrient solution used in our experiment (Hoagland solution) lacks these beneficial microbes, plant

hormones, and humic substances. By comparison, vermicompost tea is rich in these components [20,53].

3.4. Heavy Metals Content in D. muralis Leaves

As previously discussed, vermitea has demonstrated efficacy as a nutrient source for hydroponically cultivated rocket plants, potentially serving as a viable alternative to mineral fertilizers. Nonetheless, vermitea derived from green waste vermicompost may contain varying levels of heavy metals detrimental to human consumers. Extensive research by multiple authors, including Soudek et al. [55] and Uchimura et al. [56], has documented the uptake and toxicity of heavy metals in both plants and humans.

Table 2 presents the concentrations of heavy metals in *D. muralis* leaves cultivated using organic nutrient solutions (standard vermitea and enhanced vermitea), alongside the maximum permissible limits for human consumption established by The Food and Agriculture Organization (FAO)/World Health Organization (WHO) [57], the European Commission [58], and China Food and Drug Administration [59]. The concentrations of heavy metals (Cd, Pb, Ni, Cr, Co, As) in the *D. muralis* leaves were found to be below the maximum allowable limits, indicating their safety for human consumption (Table 2). However, cadmium and chromium contents in test plants were close to the maximum allowed limit. To use these nutrient solutions safely, heavy metal concentrations in the solutions need to be further reduced. Several approaches, such as the selection of organic waste and the addition of a low-cost adsorbent like biochar to the substrate during vermicomposting, can be helpful for the non-toxic preparation of vermitea. The high surface area and cation exchange capacity increase the sorption of heavy metals to biochar surfaces, thus reducing the availability of heavy metals for plant uptake [12]. Furthermore, the type of cultivars used has an impact on how heavy metals are absorbed by rocket plants. Nasircilar et al. [60] explained that several rocket varieties respond differently to heavy metal accumulation. Thus, it is essential to select the varieties with less metal accumulation capability. Our study's findings suggest the potential to cultivate healthy and nutritious vegetables, even in contaminated areas, using cost-effective vermitea as a hydroponic nutrient solution.

Table 2. Heavy metal content in the leaves of *Diplotaxis muralis* grown in organic nutrient solutions and the maximum permissible limit in rocket leaves for human consumption. Mean values and standard deviations are given.

| Heavy Metals | Heavy Metal Contents in D. 1 | Maximum Permissible Limit | | |
|---------------|------------------------------|---------------------------|----------------------|--|
| Heavy Wietais | EVT | SVT | (mg kg $^{-1}$ dw *) | |
| Cadmium | 0.16 ± 0.01 | 0.15 ± 0.03 | 0.20 | |
| Lead | 0.14 ± 0.07 | 0.24 ± 0.05 | 0.30 | |
| Nickel | 0.25 ± 0.04 | 0.20 ± 0.05 | 1.50 | |
| Chromium | 0.43 ± 0.08 | 0.43 ± 0.07 | 0.50 | |
| Cobalt | 0.00 | 0.00 | - | |
| Arsenic | 0.13 ± 0.03 | 0.09 ± 0.06 | 1.00 | |

^{*} dw: dry weight.

Moreover, vermicompost serves as a cost-effective alternative to expensive chemical fertilizers. In Italy, solid vermicompost typically ranges from 0.70 to 0.90 EUR per kg. This price can be further reduced if vermicompost is produced on agricultural farms using farm residues as a substrate for vermicomposting. This shift towards a circular economy, where farm waste is transformed into fertilizer for use in crop production, can lead to even lower prices for vermicompost. Additionally, the production cost of enhanced vermitea (EVT) is decreased by using a small proportion of solid vermicompost (1:5, vermicompost to double distilled water). This makes EVT a cost-effective organic nutrient solution for hydroponics. Enhanced vermitea (EVT) shows potential as an alternative to chemical nutrient solutions in hydroponic agriculture, specifically for *Diplotaxis muralis*. However, further research is needed to confirm its effectiveness and safety in other crops.

4. Conclusions

In conclusion, our research provides strong evidence for the effectiveness of using organic nutrient solutions in hydroponic systems (Figure 7). Our comparison of Hoagland solution and enhanced vermitea (EVT) clearly shows that both solutions can adequately provide essential macronutrients, including nitrogen, phosphorus, potassium, and calcium, for *Diplotaxis muralis*. Additionally, EVT has the added benefit of promoting significantly higher absorption of micronutrients, specifically iron, manganese, copper, and zinc, compared to other solutions.

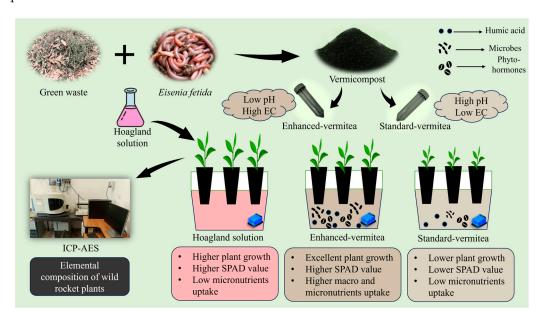


Figure 7. An overview of vermi-liquid production from green waste and its effects on plant growth, SPAD value, and nutrient assimilation in hydroponically cultivated *Diplotaxis muralis*. The use of enhanced vermitea treatment resulted in enhanced plant growth parameters and increased absorption of vital nutrients.

Organic nutrient solutions such as EVT offer sustainable alternatives to traditional hydroponic solutions, enhancing biomass production and SPAD values in *D. muralis* plants. These provide similar levels of chlorophyll to the Hoagland solution but also improve the overall health of *D. muralis* due to lower nitrate concentrations in the vermitea solutions. These enhancements in plant growth were observed despite the lower nitrate levels in the EVT solution compared to the Hoagland solution, highlighting the potential of EVT to provide a well-balanced nutrient profile while maintaining crop health and nutritional value.

The results of this research are particularly noteworthy due to the growing demand for environmentally friendly and cost-efficient farming methods. The use of enhanced vermitea is in line with the principles of a circular economy by utilizing organic waste, indicating a sustainable and financially viable approach for managing nutrients in hydroponic systems. Additionally, the reduced production expenses and enhanced product quality achieved with EVT make a strong case for its use as a complete replacement for chemical nutrient solutions in hydroponic environments.

Enhanced vermitea not only promotes strong plant growth and nutrient absorption, but also has potential for biofortifying *Diplotaxis muralis*. Biofortification, the practice of increasing the nutritional content of food crops, is a growing field that aims to address micronutrient deficiencies in human diets. The elevated levels of vital micronutrients such as iron, manganese, copper, and zinc observed in *D. muralis* when grown with EVT demonstrate the potential of this solution for biofortification. Moreover, the heavy metal contents in *D. muralis* leaves are below the permissible limits, and these leaves are thus safe for human ingestion.

By providing a nutrient solution containing bioavailable micronutrients and beneficial compounds, EVT has the potential to increase the micronutrient density of rocket leaves. This is particularly important as deficiencies in micronutrients, such as iron and zinc, are widespread among human populations worldwide, resulting in significant health issues. EVT's inherent properties, possibly due to the presence of humic substances and beneficial microbes, can enhance the concentration and bioavailability of these nutrients in plant tissues, making them more easily absorbed upon their application. This is a promising approach for producing nutrient-rich food crops that can contribute to improving nutritional outcomes for populations that rely on plant-based diets for their micronutrient intake.

The relevance of these findings lies in addressing the critical need for sustainable and efficient nutrient solutions in hydroponic systems. Given increasing urbanization and the corresponding reduction in arable land, hydroponic farming presents a viable solution for urban agriculture. The use of EVT not only supports sustainable agricultural practices but also offers a practical approach to improve the nutritional quality and safety of hydroponically grown crops. These findings contribute to the growing body of knowledge on the use of organic amendments in hydroponics and their impact on crop performance. Further research could investigate the specific ways in which EVT components, like humic substances and beneficial microorganisms, contribute to better plant growth and nutrient absorption, solidifying the move towards a more sustainable hydroponics industry.

Author Contributions: Conceptualization, S.u.R., A.A., M.B. and C.N.; methodology, S.u.R., A.A., M.B. and C.N.; formal analysis, S.u.R. and D.M.; data curation, S.u.R., F.D.C. and D.M.; writing—original draft preparation, S.u.R., F.D.C. and D.M.; supervision, A.A. and M.B.; writing—review and editing, E.S., A.A., M.B. and F.P.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ph.D. program entitled: "Development of new aerobic composting processes to reduce the environmental impact generated by waste disposal". (CUP:F85F21005750001) of "Dottorati su tematiche Green del PON R&I 2014–2020".

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors would like to express sincere gratitude to Paolo Marini of Compost Natura s.r.l., located at Via Mallacca Zummari 32, I-73010 Arnesano, Italy, for providing the platform for preparing the vermicompost tea used in this study.

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

References

- 1. Arancon, N.Q.; Schaffer, N.; Converse, C.E. Effects of coconut husk and sphagnum moss-based media on growth and yield of romaine and buttercrunch lettuce (*Lactuca sativa*) in a non-circulating hydroponics system. *J. Plant Nutr.* **2015**, *38*, 1218–1230. [CrossRef]
- 2. Bergstrand, K.-J.; Asp, H.; Hultberg, M. Utilizing anaerobic digestates as nutrient solutions in hydroponic production systems. Sustainability 2020, 12, 10076. [CrossRef]
- 3. Mehta, C.M.; Khunjar, W.O.; Nguyen, V.; Tait, S.; Batstone, D.J. Technologies to recover nutrients from waste streams: A critical review. *Crit. Rev. Environ. Sci. Technol.* **2015**, 45, 385–427. [CrossRef]
- 4. Basosi, R.; Spinelli, D.; Fierro, A.; Jez, S. Mineral nitrogen fertilizers: Environmental impact of production and use. *Fertil. Compon. Uses Agric. Environ. Impacts* **2014**, *8*, 3–43.
- 5. Cordell, D.; Drangert, J.-O.; White, S. The story of phosphorus: Global food security and food for thought. *Glob. Environ. Chang.* **2009**, *19*, 292–305. [CrossRef]
- 6. Gent, M.P. Solution electrical conductivity and ratio of nitrate to other nutrients affect accumulation of nitrate in hydroponic lettuce. *Hort. Sci.* **2003**, *38*, 222–227. [CrossRef]
- 7. Anjana, S.U.; Iqbal, M. Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. *Agron. Sustain. Dev.* **2007**, 27, 45–57. [CrossRef]
- 8. Szekely, I.; Jijakli, M.H. Bioponics as a Promising Approach to Sustainable Agriculture: A Review of the Main Methods for Producing Organic Nutrient Solution for Hydroponics. *Water* **2022**, *14*, 3975. [CrossRef]
- 9. Ezziddine, M.; Liltved, H.; Seljåsen, R. Hydroponic lettuce cultivation using organic nutrient solution from aerobic digested aquacultural sludge. *Agronomy* **2021**, *11*, 1484. [CrossRef]

10. Williams, K.; Nelson, J. Challenges of using organic fertilizers in hydroponic production systems. In Proceedings of the XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014), Queensland, Australia, 17–22 August 2014; Volume 1112, pp. 365–370.

- 11. Calvet, R.; Chenu, C.; Houot, S. Les Matières Organiques des Sols: Rôles Agronomiques et Environnementaux; Editions France Agricole: Paris, France, 2011.
- 12. Rehman, S.U.; De Castro, F.; Marini, P.; Aprile, A.; Benedetti, M.; Fanizzi, F.P. Vermibiochar: A Novel Approach for Reducing the Environmental Impact of Heavy Metals Contamination in Agricultural Land. *Sustainability* **2023**, *15*, 9380. [CrossRef]
- 13. Rehman, S.U.; De Castro, F.; Aprile, A.; Benedetti, M.; Fanizzi, F.P. Vermicompost: Enhancing plant growth and combating abiotic and biotic stress. *Agronomy* **2023**, *13*, 1134. [CrossRef]
- 14. Edwards, C.; Askar, A.; Vasko-Bennett, M.; Arancon, N. The suppression of plant pathogens by vermicompost aqueous solutions ('Teas'). In *Vermiculture Technology: Earthworm, Organic Wastes, and Environmental Management*; Taylor and Francis: Abingdon, UK, 2011; pp. 183–208.
- 15. Garibaldi, A.; Gilardi, G.; Pasquali, M.; Kejji, S.; Gullino, M.L. Seed transmission of Fusarium oxysporum of Eruca vesicaria and Diplotaxis muralis/Samenübertragung von Fusarium oxysporum bei Eruca vesicaria und Diplotaxis muralis. Z. Für Pflanzenkrankh. Pflanzenschutz/J. Plant Dis. Prot. 2004, 111, 345–350.
- 16. Ferreira, M.P.C. Study of the Bioactivity of Medicinal Plants' Extracts: Diplotaxis muralis and Cochlospermum angolensis; Instituto Politecnico do Porto: Porto, Portugal, 2018.
- 17. Weltzien, H.C. Biocontrol of foliar fungal diseases with compost extracts. In *Microbial Ecology of Leaves*; Springer: New York, NY, USA, 1991; pp. 430–450.
- 18. Maximova, A.; Gummer, Y.; Khan, I.; Kurovsky, A.; Kornievskaya, E.; Babenko, A. Effect of vermitea on morphological parameters of wheat seedlings. In Proceedings of the E3S Web of Conferences, 2023, Dushanbe, Republic of Tajikistan, 25–27 October 2023; p. 01038.
- 19. Pandey, N. Role of plant nutrients in plant growth and physiology. In *Plant Nutrients and Abiotic Stress Tolerance*; Springer: Singapore, 2018; pp. 51–93.
- 20. Arancon, N.Q.; Pant, A.; Radovich, T.; Hue, N.V.; Potter, J.K.; Converse, C.E. Seed germination and seedling growth of tomato and lettuce as affected by vermicompost water extracts (teas). *Hort. Sci.* **2012**, *47*, 1722–1728. [CrossRef]
- 21. Lazcano, C.; Sampedro, L.; Zas, R.; Domínguez, J. Vermicompost enhances germination of the maritime pine (*Pinus pinaster* Ait.). *New For.* **2010**, *39*, 387–400. [CrossRef]
- 22. Bugbee, B. Nutrient management in recirculating hydroponic culture. In Proceedings of the South Pacific Soilless Culture Conference-SPSCC 648, Palmerston North, New Zealand, 10–13 February 2003; pp. 99–112.
- 23. Samarakoon, U.C.; Weerasinghe, P.; Weerakkody, W. Effect of electrical conductivity [EC] of the nutrient solution on nutrient uptake, growth and yield of leaf lettuce (*Lactuca sativa* L.) in stationary culture. *Trop. Agric. Res.* **2006**, *18*, 13.
- 24. Stathopoulou, P.; Tsoumalakou, E.; Levizou, E.; Vanikiotis, T.; Zaoutsos, S.; Berillis, P. Iron and potassium fertilization improve rocket growth without affecting tilapia growth and histomorphology characteristics in aquaponics. *Appl. Sci.* **2021**, *11*, 5681. [CrossRef]
- 25. Wortman, S.E. Crop physiological response to nutrient solution electrical conductivity and pH in an ebb-and-flow hydroponic system. *Sci. Hortic.* **2015**, *194*, 34–42. [CrossRef]
- Yang, T.; Samarakoon, U.; Altland, J.; Ling, P. Photosynthesis, biomass production, nutritional quality, and flavor-related phytochemical properties of hydroponic-grown arugula (*Eruca sativa* Mill.)'standard'under different electrical conductivities of nutrient solution. *Agronomy* 2021, 11, 1340. [CrossRef]
- 27. Liu, Y.-J.; Tong, Y.-P.; Zhu, Y.-G.; Ding, H.; Smith, F.A. Leaf chlorophyll readings as an indicator for spinach yield and nutritional quality with different nitrogen fertilizer applications. *J. Plant Nutr.* **2006**, *29*, 1207–1217. [CrossRef]
- 28. Lavres Junior, J.; Santos Junior, J.d.D.G.d.; Monteiro, F.A. Nitrate reductase activity and spad readings in leaf tissues of guinea grass submitted to nitrogen and potassium rates. *Rev. Bras. Ciência Solo* **2010**, *34*, 801–809. [CrossRef]
- 29. Law, C.; Husni, A.Z.M.; Akmar, A.S.N. Leaf nitrogen content in oil palm seedlings and their relationship to SPAD chlorophyll meter readings. *J. Oil Palm Environ. Health (JOPEH)* **2014**, *5*. [CrossRef]
- 30. Zandvakili, O.R.; Barker, A.V.; Hashemi, M.; Etemadi, F.; Autio, W.R. Comparisons of commercial organic and chemical fertilizer solutions on growth and composition of lettuce. *J. Plant Nutr.* **2019**, 42, 990–1000. [CrossRef]
- 31. Zandonadi, D.B.; Canellas, L.P.; Façanha, A.R. Indolacetic and humic acids induce lateral root development through a concerted plasmalemma and tonoplast H+ pumps activation. *Planta* **2007**, 225, 1583–1595. [CrossRef] [PubMed]
- 32. Canellas, L.P.; Olivares, F.L. Physiological responses to humic substances as plant growth promoter. *Chem. Biol. Technol. Agric.* **2014**, *1*, 3. [CrossRef]
- 33. Darwesh, O.M.; Elshahawy, I.E. Management of sunflower charcoal-rot and maize late-wilt diseases using the aqueous extract of vermicompost (vermitea) and environmental-safe biochar derivative (wood vinegar). *Sci. Rep.* **2023**, *13*, 17387. [CrossRef] [PubMed]
- 34. Sharma, S.B.; Sayyed, R.Z.; Trivedi, M.H.; Gobi, T.A. Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* **2013**, *2*, 587. [CrossRef] [PubMed]
- 35. Ahmed, Z.F.; Alnuaimi, A.K.; Askri, A.; Tzortzakis, N. Evaluation of Lettuce (*Lactuca sativa* L.) production under hydroponic system: Nutrient solution derived from fish waste vs. Inorganic nutrient solution. *Horticulturae* **2021**, 7, 292. [CrossRef]

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36. Epstein, E.; Bloom, A. Inorganic components of plants. In *Mineral Nutrition of Plants: Principles and Perspectives*, 2nd ed.; Sinauer Associates, Inc.: Sunderland, MA, USA, 2005; pp. 44–45.

- 37. COSTA, C.d.N. Efeito das Substancias Humicas No Desenvolvimento Radicular da Cebola (Allium cepa L.) e na Cinetica de Absorcao de Fosforo e Potassio; Dissertação (Mestrado em Agronomia)—Universidade Federal de Pelotas, Pelotas, 2001.
- 38. Jakobsen, S.T. Interaction between plant nutrients: IV. Interaction between calcium and phosphate. *Acta Agric. Scand. B-Plant Soil Sci.* 1993, 43, 6–10. [CrossRef]
- 39. Xu, H.-L.; Wang, X.; Wang, J. Effect of a microbial inoculant on stomatal response of maize leaves. *J. Crop Prod.* **2001**, *3*, 235–243. [CrossRef]
- 40. Edwards, C.A.; Arancon, N.Q.; Greytak, S. Effects of vermicompost teas on plant growth and disease. Biocycle 2006, 47, 28.
- 41. Singh, R.; Sharma, R.; Kumar, S.; Gupta, R.; Patil, R. Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (Fragaria x ananassa Duch.). *Bioresour. Technol.* **2008**, *99*, 8507–8511. [CrossRef] [PubMed]
- 42. Garcia-Mina, J.; Antolin, M.; Sanchez-Diaz, M. Metal-humic complexes and plant micronutrient uptake: A study based on different plant species cultivated in diverse soil types. *Plant Soil* **2004**, *258*, 57–68. [CrossRef]
- 43. Zanin, L.; Tomasi, N.; Cesco, S.; Varanini, Z.; Pinton, R. Humic substances contribute to plant iron nutrition acting as chelators and biostimulants. *Front. Plant Sci.* **2019**, *10*, 675. [CrossRef]
- 44. Canellas, L.P.; Olivares, F.L.; Okorokova-Façanha, A.L.; Façanha, A.R. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H+-ATPase activity in maize roots. *Plant Physiol.* **2002**, *130*, 1951–1957. [CrossRef] [PubMed]
- 45. Dobbss, L.B.; Pasqualoto Canellas, L.; Lopes Olivares, F.b.; Oliveira Aguiar, N.l.; Peres, L.z.E.q.P.; Azevedo, M.; Spaccini, R.; Piccolo, A.; Facanha, A.R. Bioactivity of chemically transformed humic matter from vermicompost on plant root growth. *J. Agric. Food Chem.* **2010**, *58*, 3681–3688. [CrossRef] [PubMed]
- Gomes, G.A.; Pereira, R.A.; Sodré, G.A.; Gross, E. Humic acids from vermicompost positively influence the nutrient uptake in mangosteen seedlings. *Pesqui. Agropecuária Trop.* 2019, 49, e55529. [CrossRef]
- 47. Gerke, J. Humic (organic matter)-Al (Fe)-phosphate complexes: An underestimated phosphate form in soils and source of plant-available phosphate. *Soil Sci.* **2010**, *175*, 417–425. [CrossRef]
- 48. Urrutia, O.; Guardado, I.; Erro, J.; Mandado, M.; García-Mina, J.M. Theoretical chemical characterization of phosphate-metal-humic complexes and relationships with their effects on both phosphorus soil fixation and phosphorus availability for plants. *J. Sci. Food Agric.* **2013**, *93*, 293–303. [CrossRef]
- 49. Urrutia, O.; Erro, J.; Guardado, I.; San Francisco, S.; Mandado, M.; Baigorri, R.; Claude Yvin, J.; Ma Garcia-Mina, J. Physicochemical characterization of humic-metal-phosphate complexes and their potential application to the manufacture of new types of phosphate-based fertilizers. *J. Plant Nutr. Soil Sci.* **2014**, 177, 128–136. [CrossRef]
- 50. Struyk, Z.; Sposito, G. Redox properties of standard humic acids. Geoderma 2001, 102, 329–346. [CrossRef]
- 51. Brown, J.C. Mechanism of iron uptake by plants. Plant Cell Environ. 1978, 1, 249–257. [CrossRef]
- 52. Misson, J.; Raghothama, K.G.; Jain, A.; Jouhet, J.; Block, M.A.; Bligny, R.; Ortet, P.; Creff, A.; Somerville, S.; Rolland, N. A genome-wide transcriptional analysis using Arabidopsis thaliana Affymetrix gene chips determined plant responses to phosphate deprivation. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11934–11939. [CrossRef] [PubMed]
- 53. Arancon, N.Q.; Owens, J.D.; Converse, C. The effects of vermicompost tea on the growth and yield of lettuce and tomato in a non-circulating hydroponics system. *J. Plant Nutr.* **2019**, *42*, 2447–2458. [CrossRef]
- 54. Karnwal, A. *Pseudomonas* spp., a zinc-solubilizing vermicompost bacteria with plant growth-promoting activity moderates zinc biofortification in tomatoes. *Int. J. Veg. Sci.* **2021**, 27, 398–412. [CrossRef]
- 55. Soudek, P.; Petrová, Š.; Vaněk, T. Heavy metal uptake and stress responses of hydroponically cultivated garlic (*Allium sativum* L.). *Environ. Exp. Bot.* **2011**, 74, 289–295. [CrossRef]
- 56. Uchimiya, M.; Bannon, D.; Nakanishi, H.; McBride, M.B.; Williams, M.A.; Yoshihara, T. Chemical speciation, plant uptake, and toxicity of heavy metals in agricultural soils. *J. Agric. Food Chem.* **2020**, *68*, 12856–12869. [CrossRef] [PubMed]
- 57. Codex Alimentarius Commission. *General Standard for Contaminants and Toxins in Food and Feed (CXS 193–1995)*; Amendment; Food and Agriculture Organization; Rome, Italy, 2019.
- 58. Commission, E. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *Off. J. Eur. Union* **2023**, *119*, 103–157.
- 59. USDA-FAS. China Releases the Standard for Maximum Levels of Contaminants in Foods; USDA-FAS: Washington, DC, USA, 2018.
- 60. Nasircilar, A.G.; Ulukapi, K.; Topcuoglu, B.; Kurubas, S.; Erkan, M. Salt and heavy metal stress responses and metal uptake potentials of some leafy vegetables. *Agrosyst. Geosci. Environ.* **2024**, *7*, e20487. [CrossRef]

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