








## Article

# Suitability of Treated Domestic and Urban Wastewaters for the Hydroponic Cultivation of Rocket (*Eruca vesicaria* [L.] Cav.)

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## Abstract

Reusing treated wastewater (TWW) in agriculture may reduce water use pressure. While TWW is often used for soil irrigation, its application in hydroponics remains limited. In these systems, TWW can serve as a source of nutrients for plants while also being further reclaimed. We evaluated two TWWs of different origin and composition for hydroponic rocket cultivation. Each TWW was tested in its native form (TWW1 and TWW2) and after dilution and supplementation with mineral salts (TWW1\_DH and TWW2\_DH), using a Hoagland nutrient solution as a control. Yield and qualitative aspects of the product, including health risk factors (nitrates and heavy metals), were assessed. Rocket grown in TWW1 reached the harvesting stage, but with a significant yield reduction compared to the control (−40%). In TWW2, plants reached only the cotyledon stage and were not harvested. Two harvests were obtained in TWW1\_DH and TWW2\_DH, with yields comparable to the control or even significantly higher (+25%) in the first harvest in TWW1\_DH. No health concerns were detected, with values of Health Risk Index < 1 for all the heavy metals and nitrate levels (~3000 mg kg<sup>−1</sup> FW) well below EU limits. The study highlights the potential of TWW for the hydroponic cultivation of rocket, but also highlights the need to tailor its use based on composition.

**Keywords:** water scarcity; domestic and urban wastewater; soilless cultivation; nutrient solution; vegetables; heavy metals; health risk



Academic Editor: Genhua Niu

Received: 11 August 2025

Revised: 4 September 2025

Accepted: 12 September 2025

Published: 14 September 2025

**Citation:** Bini, L.; Baldi, A.; Passaseo, D.; Biricolti, S.; Giordani, E.; Lippi, P.; Zezza Mottola Di Amato, F.; Fibbi, D.; Del Bubba, M.; Lenzi, A. Suitability of Treated Domestic and Urban Wastewaters for the Hydroponic Cultivation of Rocket (*Eruca vesicaria* [L.] Cav.). *Horticulturae* **2025**, *11*, 1115. <https://doi.org/10.3390/horticulturae11091115>

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## 1. Introduction

Water is the basis of life, and its adequate availability, both in quantity and quality, is crucial for ecosystems and human society [1]. Today, increasing water demand is coupled with shrinking water availability, driven by a combination of factors such as climate change, population growth, urbanization, human activity, and inefficient water management [2,3]. This scenario makes water scarcity one of the most critical global issues, with over 2 billion people already living in areas affected by severe water stress [4]. As freshwater resources become increasingly limited, there is a growing urgency for sustainable water use, particularly in agriculture, which accounts for about 70% of the freshwater withdrawals in the world [5].

At the same time, an estimated  $359.4 \times 10^9 \text{ m}^3$  of wastewater is generated annually from domestic and manufacturing sources worldwide, with nearly 48% discharged into the environment untreated, causing pollution problems [6]. Reusing wastewater in agriculture may reduce water use pressure and mitigate water pollution [7]. Moreover, wastewater can be a source of nutrients for crop plants [8–10]. If fully recovered, the nutrients embedded in wastewater, which include 16.6, 3.0, and 6.3 million metric tons of nitrogen, phosphorus, and potassium, respectively, would offset 13.4% of global agricultural demand [9]. On the other hand, wastewater often contains undesirable chemical constituents and microorganisms, including human pathogens, which pose health and environmental risks [7]. The use of advanced processes for wastewater treatment, such as filtration, biological treatment, and disinfection, can make wastewater safe for irrigation [11]. However, hazards can still arise depending on the microbial and chemical composition of the treated wastewater [12], which, in turn, depends on its origin (domestic, industrial, or agricultural) and the level of treatment it has undergone [13]. Salinity, toxic ions, and heavy metals are among the major concerns from a chemical point of view [14,15]. Excessive salt content, especially elements like Na and Cl, is well-known to exert negative effects on soil (e.g., increased soil salinity and pH, and structure degradation) and crops (e.g., physiological drought, toxicity, and reduced yield), posing significant limitations to the use of treated wastewater for irrigation [16]. Serious risks to both ecosystems and human health may arise from heavy metals such as lead, cadmium, mercury, arsenic, and chromium, which can contaminate wastewater from both natural (e.g., soil erosion and volcanic activity) and human sources [17]. By passing from the soil to the edible plants, heavy metals enter the food chain and, even at low concentrations, can cause long-term health problems [18]. Nitrate content is another factor to consider in the management of irrigation with wastewater. Nitrate is a source of nitrogen for plants, but when present at high concentrations, it can contaminate groundwater and crops, with safety implications due to accumulation in drinking water and produce [19–21].

Several countries use wastewaters or treated wastewaters for soil irrigation purposes [13,22], while the application of wastewater in hydroponic cultivation systems remains largely limited to laboratory or pilot-scale studies [23]. Hydroponics is a cultivation technique for vegetables, herbs, and flowers in which plants are grown without soil, and their water and mineral requirements are met by administering a nutrient solution (water with dissolved minerals). Compared to soil cultivation, hydroponics reduces the risk of soil-borne diseases and allows for precise control over nutrients, leading to faster plant growth, higher productivity, and better quality, while decreasing the use of fertilizers and improving land and water use efficiency [24]. According to AlShrouf [25], hydroponic cultivation uses only 10% of water resources in comparison with conventional agriculture. Thanks to plants' ability to uptake nutrients, toxic metals, and emerging contaminants, hydroponic systems have also been demonstrated to be an efficient treatment for wastewater, with the added benefit of producing economically relevant crops at the same time [23,26]. Nevertheless, insufficient nutrient content may limit the use of wastewater (or treated wastewater) as the sole source of nutrients for plants grown hydroponically, making it necessary to supplement it with additional nutrients to meet the full requirements of the crops [27]. On the other hand, excessive nutrients or the presence of non-nutrient components may hinder the application of wastewater or, at least, require its dilution. Anyway, when hydroponics uses wastewater, the water savings from replacing, even if only partially, freshwater with wastewater, combined with the savings generated by hydroponics itself, further increase the sustainability of this cultivation system.

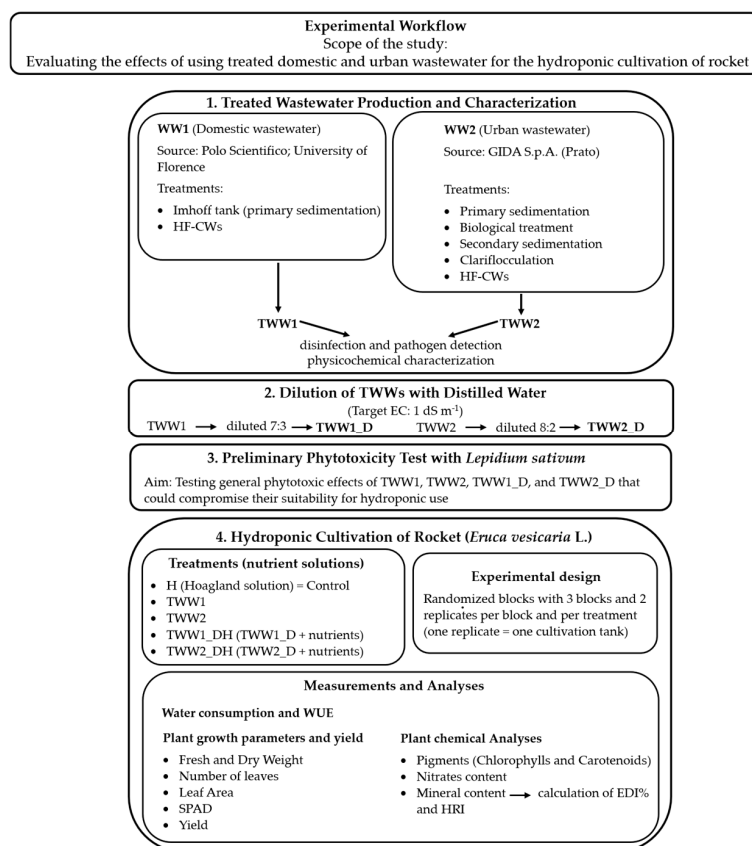
The risks to public health and environment associated with wastewater reuse have led organizations like the World Health Organization (WHO), the Food and Agriculture

Organization of the United Nations (FAO), and the Environmental Protection Agency (EPA) to draft guidelines for the safe use of it, which have served as the basis for the formulation of the regulations in many countries in the world [7]. Current agricultural water reuse regulations and guidelines vary significantly across different countries [28]. In Italy, the Ministerial Decree (MD) 185/2003 [29], following the principle of maximum precaution, establishes that urban wastewater must undergo the highest level of treatment and sets nonspecific requirements regardless of the intended final use (the “fit for all” approach). More recently, the EU Reg. 741/2020 [30] has focused on the reuse of wastewater in agriculture, with specific quality requirements depending on crop type and irrigation system (the “fit for purpose” approach). However, no reference to hydroponic systems is included in the regulation.

Based on the hypothesis that treated wastewater can serve as a source of both water and nutrients in hydroponic systems, this study aimed to assess the feasibility of using two different types of treated wastewater (domestic and urban) for the hydroponic cultivation of rocket (*Eruca vesicaria* (L.) Cav.). Rocket is a popular leafy salad crop whose leaves are particularly appreciated for their pungent taste and strong flavor, as well as for the presence of a range of health-promoting phytochemicals [31]. On the other hand, it is also a high-nitrate accumulating vegetable, which raises concerns for human health [32]. Both yield and qualitative aspects of the product, including nitrate and heavy metal content, were evaluated in our study.

## 2. Materials and Methods

To aid comprehension of the text, the materials and methods adopted in the present study are summarized in a flowchart illustrating the overall experimental design and the sequence of the main operational steps (Figure 1).



**Figure 1.** Overview of the experimental workflow.

### 2.1. Treated Wastewaters Production, Characterization, and Dilution

In this study, two types of treated wastewater (TWW1 and TWW2) were used to grow rocket salad (*Eruca vesicaria* [L.] Cav.) in a floating hydroponic system. The TWWs were produced from two different wastewaters (WW1 and WW2), which differed in their origin: (i) WW1, a domestic wastewater sourced from the *Polo Scientifico e Tecnologico* of the University of Florence (Sesto Fiorentino, Florence, Italy), and (ii) WW2, an urban wastewater containing both domestic and textile industrial inputs, derived from a treatment chain consisting in primary sedimentation, biological treatment, secondary sedimentation, and clariflocculation, which was provided by GIDA S.p.A. (*Gestione Impianti Depurazione Acqua*, Prato, Italy). Further details regarding WW2 are described in Ayadi et al. [31].

To improve the wastewater quality, attempting to meet the irrigation standards outlined in EU Reg. 741/2020 [30] and Italian MD 185/2003 [29], both wastewaters were treated by submerged horizontal flow constructed wetlands (HF-CWs) and then disinfected with sodium hypochlorite. In detail, WW1 was subjected to primary sedimentation in an Imhoff tank prior to HF-CW treatment, while WW2 was directly treated by HF-CW; accordingly, the HF-CWs acted as secondary treatment and quaternary treatment for WW1 and WW2, respectively. Each HF-CW consisted of a plexiglass tank (1.60 m × 0.55 m × 0.70 m) filled with a gravel substrate (7–10 mm in diameter), with coarser gravel (30–50 mm) near the inlet and outlet, and planted with *Phragmites australis* (Cav.) Trin. ex Steud. to enhance water purification. Both HF-CWs were subjected to forced oxygenation through solar-powered aeration systems. The disinfection treatment with sodium hypochlorite was carried out by adding two different concentrations of sodium hypochlorite to TWW1 (1.5 mg/L) and TWW2 (2 mg/L), depending on the concentration of organic carbon and ammonia (to reach the disinfection break point) in the TWWs [33]. Following these disinfection treatments, the absence of pathogenic species, in particular *Escherichia coli*, was verified in both the TWWs. This microorganism was selected according to EU Reg. 741/2020 [30].

The TWW1 and TWW2 were characterized for their physicochemical properties (Table 1). In more detail, pH, electrical conductivity (EC), dissolved oxygen (DO), bicarbonates ( $\text{HCO}_3^-$ ), chemical oxygen demand (COD), total suspended solids (TSS), total nitrogen (TN), ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrite nitrogen ( $\text{NO}_2^-\text{-N}$ ), and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) were determined according to Ayadi et al. [31]. Analyses of sulfates ( $\text{SO}_4^{2-}$ ), total phosphorus (TP), orthophosphates ( $\text{PO}_4^{3-}$ ), and chlorides ( $\text{Cl}^-$ ) were determined following standard EU procedures [34–36], and a number of elements (K, Ca, Mg, Fe, Zn, Cu, Mn, B, Mo, Na, Al, Pb, Cd, Ni, As, Se, Sn, Ba, Cr, and Sb) were detected by ICP–OES, after microwave digestion of 12.5 mL of TWWs with 3.5 mL of  $\text{HNO}_3$  65% and 0.5 mL  $\text{H}_2\text{O}_2$  30% according to Fibbi et al. [37].

**Table 1.** Physicochemical properties of the two TWWs used in this study. When possible, the values of the two TWWs were compared with the limits established by Italian Ministerial Decree 185/2003 [29] on the reuse of wastewaters in agriculture. All values are expressed as  $\text{mg L}^{-1}$ , with the exception of electrical conductivity (EC,  $\text{dS m}^{-1}$ ) and pH.

Parameters	TWW1	TWW2	MD 185/2003
pH	7.3	8.5	6–9.5
EC	3.05	4.8	3
DO	6.1	5.8	n.a.
$\text{HCO}_3^-$	51	449	n.a.
COD	12	21	100
TSS	3.5	4	10
TN	71	0.5	15

Table 1. Cont.

Parameters	TWW1	TWW2	MD 185/2003
NH <sub>4</sub> <sup>+</sup> -N	0.001	0.002	2
NO <sub>3</sub> <sup>-</sup> -N	60.5	0.2	n.a.
NO <sub>2</sub> <sup>-</sup> -N	0.28	0.1	n.a.
TP	44	1.05	2
PO <sub>4</sub> <sup>3-</sup>	20.25	0.98	n.a.
K	39	7.2	n.a.
SO <sub>4</sub> <sup>2-</sup>	62	336	500
Ca	157	104	n.a.
Mg	18	20	n.a.
Fe	0.0125	0.0125	2
Zn	0.0535	0.0515	0.1
Cu	<0.05	<0.05	1
Mn	<0.05	<0.05	0.2
B	0.0905	0.1155	1
Mo	<0.02	0.065	n.a.
Na	355	808	n.a.
Cl	731	1202	250
Al	0.035	0.0215	1
Pb	<0.05	<0.05	0.1
Cd	<MDL	<MDL	0.005
Ni	<0.05	<0.05	0.2
As	<0.02	<0.02	0.02
Se	0.0095	0.01	0.01
Sn	<0.02	<0.02	3
Ba	0.1195	0.1	10
Cr	<0.01	<0.01	0.1
Sb	<0.02	0.065	n.a.

n.a. = not applicable/not available; MDL = method detection limit.

TWW1 and TWW2 were diluted to achieve a final EC of 1 dS m<sup>-1</sup>, a level considered suitable for irrigation water to be used in soilless cultivation systems [32]. Specifically, TWW1 was diluted with distilled water at a 7:3 ratio (water–TWW1), while TWW2 was diluted at an 8:2 ratio (water–TWW2). The resulting waters were named TWW1\_D and TWW2\_D, respectively.

## 2.2. Phytotoxicity Test

TWW1, TWW2, TWW1\_D, and TWW2\_D were subjected to a phytotoxicity test on *Lepidium sativum* L., according to previous procedures [38] with minor modifications. *Lepidium sativum* seeds (Four Sementi, Bolzano, Italy) were soaked in distilled water for one hour. Subsequently, seeds were placed in Petri dishes 9 cm in diameter (10 seeds per dish) containing filter paper to avoid bubbling, and 1 mL of TWW1, TWW2, TWW1\_D, TWW2\_D, or distilled water (control) was added to each dish. Five dishes were prepared for each treatment. Then, the plates were sealed with parafilm to retain moisture and incubated in a germination chamber at 27 °C for 48 h. After incubation, the number of germinated seeds and radicle length of the seedlings were measured on each plate. Collected data were used to calculate the germination index (GI, %) with the following equation (Equation (1)):

$$GI = \frac{(G_{TWW} \times L_{TWW})}{(G_c \times L_c)} \times 100 \quad (1)$$

where  $G_{TWW}$  represents the number of seeds germinated in each TWW (TWW1, TWW1\_D, TWW2, or TWW2\_D);  $L_{TWW}$  is the average radical length (mm) reached by the seedlings

in each TWW;  $G_c$  represents the number of seeds germinated in the control, and  $L_c$  is the radical length (mm) measured in the control.

Moreover, GI was used to calculate the germination inhibition (I, %) with the following formula (Equation (2)):

$$I = 100 - GI \quad (2)$$

### 2.3. Plant Material and Growing Conditions

The experiment was conducted at the Department of Agriculture, Food, Environment, and Forestry (DAGRI), University of Florence, Italy. Rocket was cultivated in a floating hydroponic system using the following nutrient solutions: (i) a full-strength Hoagland nutrient solution according to Rajan et al. [34] with minor modifications (Table 2), as a control (H); (ii) TWW1\_D with the addition of nutrients at a concentration equivalent to the full-strength Hoagland solution (TWW1\_DH); (iii) TWW2\_D with the addition of nutrients at a concentration equivalent to the full-strength Hoagland solution and 95% sulfuric acid ( $45.33 \text{ mg L}^{-1}$ ) for bicarbonate neutralization (TWW2\_DH); (iv) untreated TWW1; and (v) untreated TWW2. The nutrient content of TWW1\_D and TWW2\_D was considered negligible given the low concentrations of macro- and micronutrients in the starting wastewaters TWW1 and TWW2 (Table 1). The initial pH and EC of TWW1\_DH and TWW2\_DH were 6.0 and 6.0, and 2.6 and 2.5  $\text{dS m}^{-1}$ , respectively, as compared to 5.8 and 1.7  $\text{dS m}^{-1}$  of the Hoagland solution.

**Table 2.** Full-strength Hoagland nutrient solution composition (macro and micronutrients) used for rocket growth.

Formula	Content ( $\text{mg L}^{-1}$ )
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	1180
$\text{KNO}_3$	505
$\text{KH}_2\text{PO}_4$	136
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	493
Fe-EDDHA	46.6
$\text{H}_3\text{BO}_3$	2.86
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.81
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.22
$\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$	0.08
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.03

Rocket seeds (Franchi Sementi Spa, Grassobbio, Bergamo, Italy) were sown in polystyrene cell trays ( $23 \text{ cm} \times 13.5 \text{ cm}$ , 28 cells) filled with 0.5–3 mm vermiculite (Perlite Italiana, Corsico, MI, Italy), with four seeds per cell. Germination occurred within two days in a climate-controlled chamber at  $20^\circ\text{C}$  in complete darkness. After germination, the trays were transferred into polyethylene tanks ( $26 \text{ cm} \times 18 \text{ cm} \times 8 \text{ cm}$ ) containing 1.8 L of nutrient solution. Six replicate tanks were used for each treatment, arranged in a randomized block design (three blocks with two tanks per block and per treatment) in a growth chamber at  $22^\circ\text{C} \pm 1^\circ\text{C}$  with a 16-h photoperiod provided by LED lights delivering  $55\text{--}100 \mu\text{mol m}^{-2} \text{ s}^{-1}$  PPFD. During cultivation, the nutrient solution was oxygenated manually, and the consumed volume was replenished once/twice a week, recording the added amount. Rocket was harvested at the baby leaf stage. When resprouting made it possible, two harvests were carried out (4 and 8 weeks after sowing, respectively). At the time of the first harvest, the residual nutrient solution was replaced with fresh nutrient solution. At both harvests, data on the added and residual volumes were used to calculate the total volume of the nutrient solution consumption. In addition, this volume was used to

calculate the water use efficiency (WUE) by dividing the nutrient solution consumption (L) by the total rocket production (kg) according to Palmitessa et al. [39].

#### 2.4. Plant Growth Parameters and Yield

At harvest, two representative plants per tank (12 plants per treatment) were used to measure the growth parameters of individual plants: fresh weight (FW), dry weight (DW) after oven drying at 80 °C for 48 h or until a constant weight, plant height, number of leaves, and leaf area (LA) measured by a LI-3000 planimeter (LI-COR, Lincoln, NE, USA). The total fresh weight of rocket harvested from each tray was also measured to calculate the yield ( $\text{kg m}^{-2}$ ). In addition, SPAD values were recorded on three fully expanded leaves of different plants (18 leaves per treatment) using the SPAD-502 dual-wavelength meter (Minolta Camera Co., Ltd., Osaka, Japan).

#### 2.5. Plant Chemical Analysis

##### 2.5.1. Pigment Concentration

Extraction of chlorophylls and carotenoids was carried out by placing fresh leaf discs 1 cm in diameter and of known weight (three discs from three leaves of different plants collected in each tank, six samples per treatment) in 5 mL of 99.9% methanol as a solvent. The samples were stored in darkness at 4 °C for 24 h. Pigment concentration was quantified immediately after extraction. Specifically, absorbance was measured at wavelengths of 665.2 nm and 652.4 nm for chlorophylls, and at 470 nm for total carotenoids using a Hitachi U-2000 spectrophotometer (Hitachi High Tech Corporation, Tokyo, Japan). Concentrations of both chlorophylls and carotenoids were calculated using the equations provided by Lichtenthaler [40].

##### 2.5.2. Mineral Content

The element concentration (Al, As, B, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, V, and Zn) was measured using an inductively coupled plasma optical emission spectrometry (ICP-OES) with an iCAP 7000 Plus system (Thermo Fisher Scientific, Waltham, MA, USA). Samples underwent microwave-assisted acidic digestion in accordance with standard procedures using a Mars Microwave Digestor (CEM, Matthews, NC, USA). For the analysis, 5 g of oven-dried, finely crushed sample were weighed and placed into a microwave digestion vessel with 2 mL of nitric acid. The vessel was sealed, and digestion was carried out using a controlled heating program (190 °C in 15 min, followed by a 15 min hold at 190 °C). After cooling, the vessels were opened, and the resulting digestion mixture was transferred to a 50 mL volumetric flask, diluted with deionized water, and analyzed by ICP-OES following DIN EN ISO 17294-2 standards [41]. The results were reported on a dry weight basis ( $\text{mg kg}^{-1}$  DW). Three samples per treatment were analyzed, each obtained by a bulk from two trays.

##### 2.5.3. Nitrate Content

Nitrate concentration was analyzed using the salicylic acid method described by Cataldo et al. [42]. First, 100 mg of dried rocket leaves were finely ground with a mortar and pestle. The powdered sample was then suspended in 30 mL of distilled water and stirred for 2 h at room temperature. Subsequently, 200  $\mu\text{L}$  of the extract was mixed with 800  $\mu\text{L}$  of 5% salicylic acid in 95% sulfuric acid, followed by the addition of 30 mL of 1.5 N NaOH. After cooling to room temperature, the absorbance of the solution was measured at 410 nm using a Hitachi U-2000 spectrophotometer (Hitachi High Tech Corporation, Tokyo, Japan). Six samples (one from each tank) were analyzed for each treatment. Nitrate concentrations were calculated based on a calibration curve prepared with  $\text{KNO}_3$  standards and expressed on a fresh weight (FW) basis by accounting for the dry-to-fresh-weight ratio.

## 2.6. Contribution to Dietary Mineral Intake and Health Risk Assessment

The contribution to dietary mineral intake and health risk associated with the consumption of the rocket grown in the control, TWW1\_DH, and TWW2\_DH nutrient solutions was calculated for the elements that were above the detection limits of ICP-OES. Rocket grown in TWW1 was excluded from the calculation, as it was deemed unmarketable.

To evaluate the nutritional contribution, the Estimated Dietary Intake (EDI, mg day<sup>-1</sup>) of essential minerals for humans was calculated using the following equation (Equation (3)):

$$EDI = C_{\text{mineral}} \times \left( \frac{CP}{1000} \right) \quad (3)$$

where  $C_{\text{mineral}}$  is the element concentration in the produce (mg kg<sup>-1</sup> FW) and CP is the daily consumed portion, assumed to be 50 g of rocket leaves per person [43,44].

The EDI was then expressed as a percentage (EDI%) of the Recommended Dietary Intake (RDI, mg day<sup>-1</sup>) for Ca, Cu, Fe, K, Mg, Mo, Na, P, Se, and Zn, or the Adequate Intake (AI, mg day<sup>-1</sup>) for Cr and Mn, as defined by the Italian Society of Human Nutrition (SINU), based on intake recommendations for an average adult male [45].

To assess the potential health risks associated with the intake of heavy metals through rocket consumption, the Health Risk Index (HRI) was calculated using the formula (Equation (4)):

$$HRI = EDI_{\text{BW}} / \text{RfD} \quad (4)$$

where  $EDI_{\text{BW}}$  is the EDI (as referred above) per kilogram of body weight (BW) (mg kg<sup>-1</sup> BW day<sup>-1</sup>), and RfD is the oral reference dose (mg kg<sup>-1</sup> BW day<sup>-1</sup>), defined as the estimated daily exposure that is not expected to cause adverse health effects over a lifetime, according to the US Environmental Protection Agency (US-EPA) [46,47]. For this assessment, an average adult body weight of 70 kg was assumed, as reported in previous studies [48].

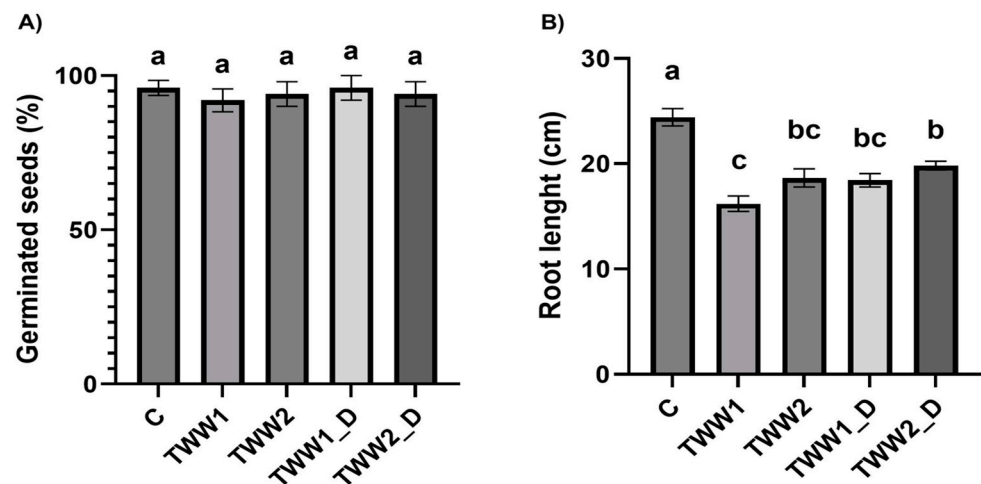
## 2.7. Statistical Analysis

Statistical analyses were performed through a One-Way ANOVA, followed by the Tukey–Kramer multiple comparison post hoc test, with a significant confidence level at 95% ( $p < 0.05$ ), using IBM SPSS Statistics v. 29.0.2 (IBM, Armonk, NY, USA). Data from the two harvests were analyzed separately. Histograms were designed using GraphPad Prism v. 10.3.1 software (GraphPad Software, Boston, MA, USA).

# 3. Results

## 3.1. Phytotoxicity Test

Figure 2 reports the effects of both native TWWs in their native form and their dilutions on seed germination and root length of *L. sativum*. In terms of germination percentage (Figure 2A), the statistical analysis revealed no significant differences between the different waters, both with respect to each other and compared to the control (distilled water). The average germination percentages were 96% for the control (C), 92% for TWW1, 94% for TWW2, 96% for TWW1\_D, and 94% for TWW2\_D. Conversely, root length displayed notable variations (Figure 2B). The control exhibited longer roots ( $24.41 \pm 0.83$  cm) than all the TWW treatments. Among the TWWs, TWW1 produced the shortest roots ( $16.2 \pm 0.74$  cm), with significant differences not only compared to the control but also to TWW2\_D ( $19.82 \pm 0.42$  cm). Seedlings germinated in TWW2 and TWW1\_D showed intermediate root lengths ( $18.65 \pm 0.87$  cm and  $18.43 \pm 0.64$  cm, respectively), with values not different compared with both TWW1 and TWW2\_D.



**Figure 2.** Effect of the TWWs (see Section 2.1) on seed germination (A) and root length (B) in *Lepidium sativum*. Different letters show statistically significant differences among treatments ( $p < 0.05$ ). C (control) = distilled water.

The GI and I of the TWWs, calculated in relation to germination in distilled water, are shown in Table 3. The lowest GI value was recorded for TWW1, while the highest was observed for TWW2\_D. Conversely, TWW1 exhibited the greatest I value, whereas TWW2\_D showed the lowest one. TWW2 and TWW1\_D displayed intermediate values.

**Table 3.** Germination index (GI) and germination inhibition (I) of the different TWWs (see Section 2.1) detected in *Lepidium sativum* in relation to the control (distilled water).

Treatment	GI (%)	I (%)
TWW1	61.7	38.3
TWW2	74.8	25.2
TWW1_D	75.5	24.5
TWW2_D	79.5	20.5

### 3.2. Plant Growth and Yield

The use of native TWWs negatively impacted the growth of rocket plants. Specifically, plants grown in TWW2 did not grow beyond the cotyledon stage, while those in TWW1 reached the baby leaf stage but exhibited low yield (as discussed later) and extensive leaf yellowing, rendering the product unmarketable. As a result, this treatment was excluded from the second cultivation cycle. In contrast, control plants and those grown in TWW1\_DH and TWW2\_DH showed a resprouting suitable for subjecting them to a second harvest. Table 4 summarizes the yield and biometric traits of rocket plants grown in hydroponic systems over the two harvests. The first harvest yield was significantly influenced by the use of the TWWs. Notably, TWW1\_DH achieved significantly higher production compared to the control, while TWW2\_DH did not differ from the control. In contrast, the cultivation in TWW1 resulted in a dramatic decline in yield (~40% and 52% lower than the control and TWW1\_DH, respectively). At the second harvest, no significant differences in yield were observed among the control, TWW1\_DH, and TWW2\_DH treatments.

Detailed analyses of individual plant traits, including FW, DW, plant height, number of leaves, and LA, revealed the same trend across both harvests. No significant differences were detected between the control, TWW1\_DH, and TWW2\_DH for these parameters. In contrast, plants grown in TWW1 (first harvest) exhibited significantly reduced values for all the parameters (Table 4).

**Table 4.** Yield and biometric parameters of rocket grown hydroponically using different TWWs (see Section 2.3). H (control) = Hoagland nutrient solution. Different letters in the same column show statistically significant differences among treatments ( $p < 0.05$ ). Data from the two harvests were analyzed separately.

	Yield (kg m <sup>-2</sup> )	Fresh Weight (g Plant <sup>-1</sup> )	Dry Weight (g Plant <sup>-1</sup> )	Plant Height (cm)	Number of Leaves	Leaf Area (cm <sup>2</sup> Plant <sup>-1</sup> )
Harvest 1						
H	2.21 b	2.91 a	0.21 a	15.22 a	5.83 a	71.67 a
TWW1	1.31 c	1.68 b	0.12 b	11.37 b	4.83 b	35.51 b
TWW1_DH	2.78 a	3.11 a	0.20 ab	15.68 a	5.50 ab	66.73 a
TWW2_DH	2.47 b	3.01 a	0.21 a	15.73 a	5.50 ab	65.10 a
Harvest 2						
H	2.50 a	3.99 a	0.33 a	14.83 a	6.92 a	80.61 a
TWW1_DH	2.92 a	4.50 a	0.37 a	15.58 a	7.08 a	81.81 a
TWW2_DH	2.58 a	3.89 a	0.35 a	15.21 a	6.92 a	76.74 a

### 3.3. SPAD and Pigments

Table 5 presents the results for SPAD values and pigment content of rocket plants grown using the TWWs across two harvests. In the first harvest, SPAD values of plants grown in TWW1, TWW1\_DH, and TWW2\_DH were comparable to the control but higher than those of plants grown with undiluted TWW1. The trend of SPAD values was reflected in terms of pigment concentration. In fact, TWW1 showed significantly lower values for both chlorophylls and carotenoids than the control. No differences between the treatments were noted for any parameter during the second harvest (Table 5).

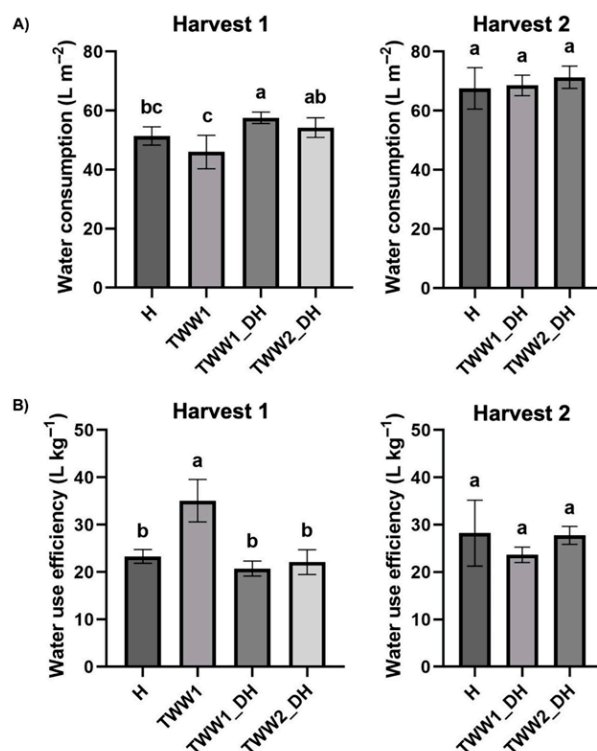
**Table 5.** SPAD and pigment content of rocket grown hydroponically using different TWWs (see Section 2.3). H (control) = Hoagland nutrient solution. Different letters in the same column show statistically significant differences among treatments ( $p < 0.05$ ). Data from the two harvests were analyzed separately.

Treatment	SPAD SPAD	Chl a (mg g <sup>-1</sup> FW)	Chl b (mg g <sup>-1</sup> FW)	Chl a + b (mg g <sup>-1</sup> FW)	Carotenoids (mg g <sup>-1</sup> FW)
Harvest 1					
H	35.88 ab	1.57 a	0.53 a	2.10 a	0.29 a
TWW1	33.04 b	1.14 b	0.39 b	1.53 b	0.020 b
TWW1_DH	38.77 a	1.43 ab	0.51 a	1.94 ab	0.25 ab
TWW2_DH	40.37 a	1.49 a	0.52 a	2.00 a	0.26 ab
Harvest 2					
H	43.02 a	1.08 a	0.52 a	1.62 a	0.13 a
TWW1_DH	44.13 a	0.96 a	0.47 a	1.44 a	0.12 a
TWW2_DH	44.31 a	1.01 a	0.50 a	1.53 a	0.11 a

### 3.4. Consumption of Nutrient Solution and WUE

Significant differences in the volume of consumed nutrient solution were observed among the treatments during the first harvest (Figure 3A). TWW1 exhibited the lowest consumption ( $45.94 \pm 5.66$  L m<sup>-2</sup>), significantly different from TWW1\_DH and TWW2\_DH but not from the control ( $51.37 \pm 3.10$  L m<sup>-2</sup>). Consumption higher than the control was recorded in TWW1\_DH ( $57.58 \pm 1.95$  L m<sup>-2</sup>). In terms of WUE (expressed as L of water consumed per kg of product), TWW1 proved to be less efficient than the other treatments, with a WUE of  $35.07 \pm 4.48$  L kg<sup>-1</sup> (Figure 3B). The control, TWW1\_DH, and TWW2\_DH

showed similar WUE values, averaging  $22.05 \text{ L kg}^{-1}$ . During the second harvest, these treatments also did not show any significant differences in either solution consumption or WUE (Figure 3A,B).



**Figure 3.** Consumption of the different nutrient solutions (see Section 2.3) (A) and water use efficiency (B) during the two cycles (harvest 1 and harvest 2) of rocket. Different letters show statistically significant differences among treatments ( $p < 0.05$ ). Data from the two harvests were analyzed separately.

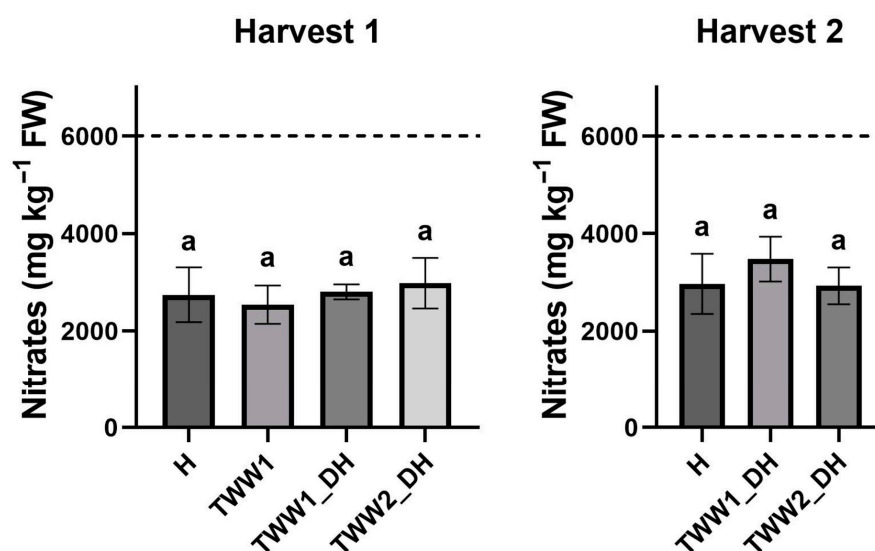
### 3.5. Mineral Content and Nitrates

The concentrations of elements found in rocket plants grown with the different nutrient solutions are reported in Table 6. Aluminum, As, Cd, Hg, Ni, Pb, Se, and V were not detected. In the first harvest, TWW1 led to a substantial reduction in K and P contents and a noticeable increase in Na concentration in comparison with the other treatments. As compared to the control, K and P decreased by 43.49% and 37.14%, respectively, and Na increased from  $0.42 \pm 0.05 \text{ mg kg}^{-1} \text{ DW}$  to as much as  $11.52 \pm 2.49 \text{ mg kg}^{-1} \text{ DW}$ . A significant increase in Na content was also found in rocket grown with the solutions obtained from the two diluted TWWs, which, for the other elements, did not significantly differ from the control. TWW1 also revealed significantly higher concentrations of Cr and Cu in comparison with TWW2\_DH. No differences between the treatments were found for Ca, Mg, S, B, Mn, and Zn at the first harvest. At the second harvest, macroelements (Ca, K, Mg, and P) did not show significant differences between the three nutrient solutions. Again, the use of the TWWs led to a significant increment of Na content compared to the control. In contrast, B, Cr, Cu, Mn, and Zn were significantly lower in plants grown in TWW1\_DH and TWW2\_DH than in control plants, while no differences were found for S, Fe, and Mo. (Table 6).

The use of the TWWs did not influence nitrate concentrations in rocket leaves (Figure 4), leading to contents not different from those detected in the control plants for both harvests. All the measured values were far below the maximum permissible limits for rocket commercialization, as established by EU Reg. 1258/2011 [49].

**Table 6.** Element concentrations of rocket grown hydroponically using different TWWs (see Section 2.3). H (control) = Hoagland nutrient solution. Different letters within the same row indicate statistically significant differences among treatments ( $p < 0.05$ ). Data from the two harvests were analyzed separately. Elements in roman are expressed in  $\text{g kg}^{-1}$  dw, elements in italics are expressed in  $\text{mg kg}^{-1}$  dw.

Element	Harvest 1				Harvest 2		
	H	TWW1	TWW1_DH	TWW2_DH	H	TWW1_DH	TWW2_DH
Ca	44.98 a	48.43 a	46.35 a	42.06 a	43.96 a	41.78 a	40.70 a
K	61.17 a	34.57 b	65.87 a	58.26 a	60.14 a	57.15 a	55.73 a
Mg	17.98 a	15.25 a	18.47 a	16.98 a	20.81 a	18.85 a	18.27 a
Na	0.42 c	11.52 a	4.78 b	5.74 b	0.60 b	8.85 a	10.57 a
P	7.70 a	4.84 b	8.01 a	7.67 a	7.35 a	6.42 a	6.98 a
S	14.72 a	12.15 a	15.61 a	14.98 a	24.07 a	20.95 a	20.50 a
<i>B</i>	52.58 a	53.48 a	51.37 a	46.51 a	108.55 a	82.97 b	81.19 b
<i>Cr</i>	0.66 ab	3.89 a	0.61 ab	0.35 b	2.22 a	1.27 b	0.71 b
<i>Cu</i>	4.79 ab	6.49 a	4.65 ab	4.29 b	8.88 a	5.81 b	5.03 b
<i>Fe</i>	138.75 a	140.09 a	129.16 a	123.22 a	168.80 a	180.06 a	192.97 a
<i>Mn</i>	194.49 a	143.47 a	135.29 a	156.83 a	196.28 a	116.27 b	129.33 b
<i>Mo</i>	2.89 a	2.97 a	3.46 a	3.73 a	2.62 a	3.46 a	2.90 a
<i>Zn</i>	33.52 a	43.93 a	35.27 a	31.82 a	42.20 a	31.26 b	31.01 b



**Figure 4.** Nitrate concentration of rocket grown hydroponically using two TWWs (see Section 2.3). H (control) = Hoagland nutrient solution. Letters show statistically significant differences among treatments ( $p < 0.05$ ). The dashed line represents the maximum threshold for nitrate content in rocket leaves allowed by EU Reg. 1258/2011 [49]. Data from the two harvests were analyzed separately.

### 3.6. Contribution to Mineral Dietary Intake and Health Risk Assessment

Table 7 shows the EDI% values resulting from a daily rocket consumption of 50 g. In the first harvest, values exceeding 20% were recorded for Mg across all treatments, as well as for Mn in the control. Moderate percentages, ranging from 10% to 20%, were observed for Ca, Mn, and Mo. The lowest contribution was found for Zn and Na, with an average value across treatments of 0.9% and 0.8% for Zn and Na, respectively. For the latter, however, it is noteworthy that the contribution resulting from the consumption of the control plants was negligible (0.1%). In contrast, although still low, higher EDI% values were observed for rocket grown in TWW\_DH. Specifically, the increase compared to the control was 12 times and 15 times higher for TWW1\_DH and TWW2\_DH, respectively. In the second harvest, the highest EDI% was found for Mg in control plants, with a value

above 35%. Moderate contributions were found for Ca, Mn, and Mo. As observed in the first cycle, the lowest EDI% values were recorded for Zn (1.2% on average) and Na (1.8% on average). Marked variability in EDI% values among treatments was observed for Cr and Mn, with the highest contribution to AI recorded for the control plants. In contrast, Na levels showed 13 times and 17 times increases in TWW1\_DH and TWW2\_DH, respectively, compared to the control.

**Table 7.** Estimated dietary intake expressed as a percentage (EDI%) of the recommended dietary intake (RDI) or adequate intake (AI) resulting from the daily consumption of 50 g of rocket grown hydroponically using two TWWs (see Section 2.3). H (control) = Hoagland nutrient solution.

Element	RDI/AI <sup>a</sup> (mg day <sup>−1</sup> )	Harvest 1			Harvest 2		
		H	TWW1_DH	TWW2_DH	H	TWW1_DH	TWW2_DH
Ca	<b>1000</b>	13.8	14.0	13.3	18.5	15.7	16.8
K	3900	4.8	5.1	4.7	6.5	5.5	5.9
Mg	<b>240</b>	22.9	23.3	22.3	36.7	29.5	31.4
Na	<b>1500</b>	0.1	1.0	1.2	0.2	2.2	2.9
P	<b>700</b>	3.4	3.5	3.5	4.4	3.5	4.1
Cr	<i>0.035</i>	5.8	5.3	3.3	26.9	13.7	8.4
Cu	<b>0.9</b>	1.6	1.6	1.5	4.2	2.4	2.3
Fe	<b>10</b>	4.3	3.9	3.9	7.1	6.8	8.0
Mn	2.7	22.0	15.2	18.3	30.6	16.2	19.8
Mo	<b>0.045</b>	13.7	16.1	18.1	17.2	20.1	18.4
Zn	<b>11</b>	0.9	0.9	0.8	1.5	1.0	1.1

<sup>a</sup> RDI (bold) and AI (italic) according to SINU (2014).

Regarding the health risk assessment, for all the elements, the calculated EDI<sub>BW</sub> values were much lower than the RfD established by the US-EPA [44,45] and thus the HRI values were far below 1.0 (Table 8).

**Table 8.** Estimated daily intake per kg of body weight (EDI<sub>BW</sub>) and health risk index (HRI) resulting from the daily consumption of 50 g of rocket grown hydroponically using two TWWs (see Section 2.3). H (control) = Hoagland nutrient solution.

Element		H	Harvest 1		H	Harvest 2	
			TWW1_DH	TWW2_DH		TWW1_DH	TWW2_DH
B	EDI <sub>BW</sub>	$2.30 \times 10^{-3}$	$2.22 \times 10^{-3}$	$2.09 \times 10^{-3}$	$6.56 \times 10^{-3}$	$4.46 \times 10^{-3}$	$4.78 \times 10^{-3}$
(RfD 0.2)	HRI	$1.15 \times 10^{-2}$	$1.11 \times 10^{-2}$	$1.05 \times 10^{-2}$	$3.28 \times 10^{-2}$	$2.23 \times 10^{-2}$	$2.29 \times 10^{-2}$
Cr	EDI <sub>BW</sub>	$2.89 \times 10^{-5}$	$2.63 \times 10^{-5}$	$1.62 \times 10^{-5}$	$1.34 \times 10^{-5}$	$6.86 \times 10^{-5}$	$4.18 \times 10^{-5}$
(RfD 0.0009)	HRI	$3.21 \times 10^{-2}$	$2.92 \times 10^{-2}$	$1.80 \times 10^{-2}$	$1.49 \times 10^{-1}$	$7.62 \times 10^{-2}$	$4.64 \times 10^{-2}$
Cu	EDI <sub>BW</sub>	$2.09 \times 10^{-4}$	$2.01 \times 10^{-4}$	$1.93 \times 10^{-4}$	$5.39 \times 10^{-4}$	$3.11 \times 10^{-4}$	$2.96 \times 10^{-4}$
(RfD 0.04)	HRI	$5.23 \times 10^{-3}$	$5.02 \times 10^{-3}$	$4.83 \times 10^{-3}$	$1.35 \times 10^{-2}$	$7.79 \times 10^{-3}$	$7.41 \times 10^{-3}$
Fe	EDI <sub>BW</sub>	$6.08 \times 10^{-3}$	$5.58 \times 10^{-3}$	$5.57 \times 10^{-3}$	$1.02 \times 10^{-2}$	$9.70 \times 10^{-3}$	$1.14 \times 10^{-2}$
(RfD 0.7)	HRI	$8.68 \times 10^{-3}$	$7.96 \times 10^{-3}$	$7.96 \times 10^{-3}$	$1.45 \times 10^{-2}$	$1.39 \times 10^{-2}$	$1.63 \times 10^{-2}$
Mn	EDI <sub>BW</sub>	$8.50 \times 10^{-3}$	$5.86 \times 10^{-3}$	$7.05 \times 10^{-3}$	$1.18 \times 10^{-2}$	$6.25 \times 10^{-3}$	$7.62 \times 10^{-3}$
(RfD 0.14)	HRI	$6.07 \times 10^{-2}$	$4.18 \times 10^{-2}$	$5.04 \times 10^{-2}$	$8.44 \times 10^{-2}$	$4.47 \times 10^{-2}$	$5.44 \times 10^{-2}$
Mo	EDI <sub>BW</sub>	$1.27 \times 10^{-4}$	$1.50 \times 10^{-4}$	$1.68 \times 10^{-4}$	$1.60 \times 10^{-4}$	$1.86 \times 10^{-4}$	$1.71 \times 10^{-4}$
(RfD 0.05)	HRI	$2.54 \times 10^{-2}$	$2.99 \times 10^{-2}$	$3.36 \times 10^{-2}$	$3.20 \times 10^{-2}$	$3.73 \times 10^{-2}$	$3.41 \times 10^{-2}$
Zn	EDI <sub>BW</sub>	$1.47 \times 10^{-3}$	$1.52 \times 10^{-3}$	$1.43 \times 10^{-3}$	$2.53 \times 10^{-3}$	$1.68 \times 10^{-3}$	$1.83 \times 10^{-3}$
(RfD 0.3)	HRI	$4.90 \times 10^{-3}$	$5.08 \times 10^{-3}$	$4.77 \times 10^{-3}$	$8.44 \times 10^{-3}$	$5.60 \times 10^{-3}$	$6.08 \times 10^{-3}$

#### 4. Discussion

The combination of hydroponic cultivation with the use of wastewater may result in a sort of mutualism: on one hand, hydroponics has been demonstrated to be an effective

method for reclaiming contaminated waters, with the effect of reducing the pollutant load on the environment [50,51]; on the other hand, wastewater may be a direct source of water and nutrients for plants, leading to a saving of inputs in agriculture [52]. The limits of the approach are closely related to the composition of the wastewater used, which can contain factors responsible for toxic effects on plants (e.g., Na and Cl) or contamination of the produce (e.g., heavy metals), but, at the same time, may not contain enough nutrients to sustain crops [26]. Therefore, the possible success of agricultural wastewater reuse using hydroponic systems is strongly dependent on the composition of the wastewater [53].

Although several studies have demonstrated the potential of wastewater for agricultural reuse, further research is still needed to explore its long-term benefits and limitations [54]. The study on the effect of treated wastewater on hydroponically grown vegetables can provide useful information not only for growers but also for policymakers, considering that current legislation does not address the case of hydroponics.

The present study investigates the possibility of using two types of treated wastewater for the hydroponic cultivation of rocket, employing them both in their native form (TWW1 and TWW2) and after dilution and supplementation with mineral salts to provide adequate nutrients to the plants (TWW1\_DH and TWW2\_DH). Both TWW1 and TWW2, but especially the latter, showed high EC, Na, and Cl content, with Cl exceeding the limit admitted by Italian MD 185/2003 [29] on the reuse of wastewaters in agriculture (Table 1). Instead, the two TWWs seemed to be safe in terms of heavy metals, whose values were below the admitted limits or even below the limits of detection. In terms of nutrient content, TWW1, consistent with the usual composition of municipal wastewater reported by other authors [26], was richer than TWW2 in N, P, K, and Ca with concentrations corresponding to 28.9%, 21.3%, 165%, and 78.5%, respectively, of those in the Hoagland nutrient solution (H), which was used as the control in the study. Both the TWWs contained enough S, with TWW2 even exceeding that in H, even though remaining within the limits established by the MD 185/2003. In addition, both TWWs showed interesting concentrations in Mg (37.5% and 41.75% of the concentration present in H for TWW1 and TWW2, respectively). Among the microelements, only Zn was provided in a sufficient amount as compared to H. In TWW1, the concentrations of TN (of which about 85% in a soluble form, mainly nitrate) and TP (15% in a soluble form) were above the limits set by MD 185/2003 [29]. Nevertheless, it should be considered that the impact of these elements is much different when the water is used as irrigation water released into the soil, with risks of environmental pollution (which was likely in the mind of the policymaker), compared to when it is used in hydroponics as a nutrient solution (where high N and P concentrations are necessarily high to cope with plant requirements). After the dilution applied to bring the TWWs to an acceptable EC ( $1 \text{ dS m}^{-1}$ ) for a starting water to be used in preparing a nutrient solution [55], the nutrient concentrations of the waters (TWW1\_D and TWW2\_D) became negligible, although the legal limits for N and P were still exceeded. In contrast, the dilution was effective in reducing Cl concentrations below the legal limit.

Physicochemical analysis provides valuable information on waste toxicity, but it is not sufficient for comprehensively assessing its possible impacts on living organisms and is therefore often complemented by specific toxicity tests [56]. These tests are usually based on the assessment of seed germination and root elongation, and one of the most used species is *Lepidium sativum* due to its heightened sensitivity to contaminants such as heavy metals, adaptability to humid conditions, rapid growth, and widespread availability [56]. In the phytotoxicity test with *L. sativum* carried out in this study, none of the TWWs affected the seed germination rate, but all caused significant reductions in root length compared to distilled water (Figure 2). The germination index (GI) ranged from 61.73% (TWW1) to 79.49% (TWW2\_D) (Table 3). As reported by Mañas and De las

Heras [38] ( $GI \geq 80\%$  = s minimal or no presence of phytotoxic substances;  $GI \leq 50\%$  = high phytotoxicity;  $GI$  51% and 79% = moderate phytotoxicity), all the tested TWWs fell in the category of moderate phytotoxicity. TWW2\_D was particularly close to a condition of no toxicity. Mancini et al. [57] consider water to be phytotoxic when it causes germination inhibition (I) greater than 30%. Therefore, based on this parameter, only TWW1 shows slight phytotoxicity ( $I = 38.3\%$ ) among the TWWs examined. We hypothesize that the moderate phytotoxicity observed could be due to a detrimental effect exerted by Na and Cl [58], whose concentration remained quite high even after dilution (approximately 106 and 160  $\text{mg L}^{-1}$  Na and 219 and 249  $\text{mg L}^{-1}$  Cl in TWW1\_D and TWW2\_D, respectively). Nevertheless, some factor not detected by the analysis likely played a role in the case of TWW1, whose Na and Cl content were lower than that of TWW2.

In the hydroponic cultivation of rocket, the TWWs in their native form exerted a negative effect on plant growth, especially TWW2. In this last case, plants reached the cotyledon stage but then stopped growing, and, therefore, were not harvested. Plants grown in TWW1 reached the baby leaf stage and were harvested, but the yield was significantly reduced. Furthermore, leaves showed extensive yellowing, further confirmed by lower SPAD values and chlorophyll concentration in the leaf tissues (Table 5), and were considered unmarketable. We believe that plants in TWW2 suffered from both the effects of high EC and specific toxicity by Na and Cl, coupled with a lack of nutrients (especially N). Sodium, in particular, might have played a crucial role. Actually, it is well-known that high levels of Na in plants lead to reduced biomass accumulation, as well as osmotic, oxidative, and ionic stress, and growth inhibition caused by alterations in cellular biochemistry [59,60]. In TWW1, plants suffered for the same reasons, but the EC and Na and Cl content were lower; moreover, the lack of nutrients was less severe, allowing the plants to reach a more advanced growth stage. However, water and nutrient uptake during the cycle were reduced, as supported by the lower volume of absorbed solution (approximately 10% less than the control) and the content of certain minerals (mainly macroelements) in rocket leaves that was reduced as well, as discussed below. Yield decreased by as much as 40%, having a greater impact on WUE, which was therefore significantly lower (Figure 3). Other authors have reported poor growth and productivity in vegetable crops grown hydroponically with treated wastewater due to nutrient deficiency and imbalance [27,54]. Egbuikwem et al. [54] did not observe any negative impact of treated wastewater on the seed germination of lettuce and beet but reported subsequent effects on the plants. They observed stunted growth in plant root systems, which led to limited access to nutrients, and, consequently, a reduction in vegetative growth, impaired phenological development, and decreased chlorophyll production.

Krishnasamy et al. [59] demonstrated that, due to the presence of toxicity factors, the dilution of anaerobic effluents from food and vegetable waste digestion was necessary for their use in the hydroponic cultivation of silverbeet. Nevertheless, the dilution caused a reduction in nutrient availability, which was responsible for a lower plant growth compared to that obtained with a commercial hydroponic nutrient solution [61]. In our study, the dilution of the TWWs coupled with nutrient supplementation led to good results. Using TWW1\_DH and TWW2\_DH, rocket growth and pigment content were comparable to those obtained with H, or even better. Actually, at the first harvest, TWW1\_DH achieved a higher yield than the control, coupled with a greater solution uptake. We hypothesize that rocket plants could have taken advantage of the extra, although small, amount of some nutrients, especially N, present in TWW1\_DH as compared to H, and derived from the TWW1 component, or that TWW1\_DH may contain some unidentified substance with a biostimulant effect, presumably on the efficiency of the root system. In fact, several studies

provide evidence that urban wastewaters can contain chemical compounds acting as plant biostimulants [62–64].

The concentrations of macroelements in rocket leaves closely reflected the composition of the respective nutrient solutions, particularly in the case of P and K, which were significantly reduced in plants supplied with TWW1 (−37 and 43.5%, respectively, as compared to control plants). Conversely, plants grown with TWW1 exhibited elevated levels of Na, consistent with the high salinity of this solution. A similar trend was observed in both TWW1\_DH and TWW2\_DH treatments across the two harvests, with leaf Na concentrations found to be from 11 to 17 times higher than in the control. This significant increase was also reflected in the EDI% values, which, nevertheless, were still low enough (maximum EDI% = 2.89% in the case of TWW2\_DH) to exclude any health risks. Sodium, actually, is an essential nutrient, but can be associated with the onset of serious diseases in case of excessive consumption [65]. Considering that the primary sources of dietary Na are processed foods and table salt, rather than vegetables [66], high EDI% values in rocket would have represented a potential concern.

In the second harvest, a reduction in the concentration of some micronutrients (i.e., B, Cr, Cu, Mn, and Zn) was observed in plants grown with TWW1\_DH and TWW2\_DH. This phenomenon may be attributed to the high EC, which may hinder nutrient uptake due to an osmotic effect and/or antagonistic interactions among elements. Our results are in agreement with those reported by Huang et al. [67], who found that increasing EC levels led to decreased accumulation of micronutrients such as Mn and Zn in rice plants, while Fe remained unaffected. The EDI% values calculated for all the TWW treatments were generally comparable to those of the control, although more marked variability was observed among them in the second harvest. Considering the average values across the treatments, the daily consumption of 50 g of rocket would contribute most to the dietary intake of Mg, followed by Mn, Mo, and Ca.

The consumption of rocket grown with TWWs would not pose health risks either in terms of nitrate or heavy metals. In any treatment, nitrate concentration was well below the limits set by the EU for the commercialization of this crop. Interestingly, although TWW1 contained only about one-third of the nitrate present in the other solutions, plants grown in it accumulated similar amounts of nitrate. That could be explained considering the natural tendency of rocket to accumulate these compounds [68]. With regard to heavy metals, consistent with their low levels in the native TWWs, the concentrations found in rocket tissues resulted in HRI values below 1 for all the elements across all treatments, indicating no health concern from this perspective.

## 5. Conclusions

Our results demonstrate that treated wastewater can be effectively used in hydroponic cultivation, provided it is properly adjusted to meet plant needs. This requires careful consideration of treated wastewater composition, which varies depending on its source and the treatment processes it has undergone. High salinity levels, especially when caused by Na and Cl, along with insufficient nutrient content, can be critical factors limiting the use of treated wastewater in its native form. However, if appropriately diluted and enriched with nutrients, treated wastewater can support satisfactory plant growth. Depending on the dilution degree, varying levels of freshwater replacement can be achieved. In this study, a 20–30% reduction in freshwater use was successfully implemented, maintaining yield and ensuring product safety. However, it would be worth evaluating lower dilution levels, which would allow for greater freshwater saving and possibly a reduction in nutrient supplementation. On the other hand, the potential transfer of contaminants to products (e.g., heavy metals) should always be carefully assessed when hydroponic systems are

intended for food production. Therefore, higher dilutions may be required to exclude health risks.

We believe that our findings may offer valuable insights for both producers and policymakers, particularly given the current lack of regulatory frameworks governing the use of wastewater in hydroponic systems.

**Author Contributions:** Conceptualization, S.B., E.G., M.D.B. and A.L.; methodology, L.B., A.B. and A.L.; software, L.B., A.B., P.L., F.Z.M.D.A. and A.L.; validation, L.B., A.B., D.P., F.Z.M.D.A. and A.L.; formal analysis, L.B., A.B., P.L., F.Z.M.D.A. and A.L.; investigation, L.B., D.P., F.Z.M.D.A. and A.L.; resources, E.G., D.F., M.D.B. and A.L.; data curation, L.B., A.B. and A.L.; writing—original draft preparation, L.B., A.B. and A.L.; writing—review and editing, L.B., A.B., D.P., S.B., E.G., P.L., F.Z.M.D.A., D.F., M.D.B. and A.L.; supervision, S.B., E.G., M.D.B. and A.L.; project administration, M.D.B.; funding acquisition, E.G., M.D.B. and A.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was conducted as part of the “SECURE-FOOD2050” project, supported by the European Union’s Horizon 2020 research and innovation program under ERA-Net Cofund FOSC (Grant No. 862555). Additional funding was provided by the Italian Ministry of Universities and Research (MUR) through the “Progetto Dipartimenti di Eccellenza 2023–2027” initiative, awarded to the Department of Chemistry “Ugo Schiff” at the University of Florence. Lorenzo Bini also received support from MUR via the “Piano Operativo Nazionale (PON) Ricerca e Innovazione 2021–2027” program (Fellowship Grant No. DOT13NTSF8-1).

**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors on request.

**Conflicts of Interest:** Author Donatella Fibbi was employed by the company Gestione Impianti Depurazione Acqua (GIDA S.p.A.), Prato. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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