soil systems

MDPI

Article

Selenium Uptake by Lettuce Plants and Se Distribution in Soil Chemical Phases Affected by the Application Rate and the Presence of a Seaweed Extract-Based Biostimulant

Ioannis Zafeiriou, Dionisios Gasparatos *, Dafni Ioannou and Ioannis Massas *

Laboratory of Soil Science and Agricultural Chemistry, Department of Natural Resources Management & Agricultural Engineering, School of Environment & Agricultural Engineering, Agricultural University of Athens, 11855 Athens, Greece; j.zafeiriou@gmail.com (I.Z.); dioannou@aua.gr (D.I.)
* Correspondence: gasparatos@aua.gr (D.G.); massas@aua.gr (I.M.)

Abstract: To tackle selenium (Se) malnutrition, biofortification is among the proposed strategies. A biostimulant application in soils is thought to support a plant's growth and productivity. Biofortification with Se(VI) may lead to a leaching hazard due to the high mobility of Se(VI) in the soil environment. In this study, the effect of the application of two Se(VI) rates—5 and 10 mg kg^{-1} soil—and a biostimulant on the Se uptake by lettuce plants and on the Se(VI) distribution in soil fractions following the plants harvest, was investigated. Phosphorus (P) and sulfur (S) concentrations in plants were also determined. A high Se(VI) rate suppressed plant growth, leading to a significant fresh weight decrease from 12.28 to 7.55 g and from 14.6 to 2.43 g for the control and high Se(VI) without and with biostimulants, respectively. Impaired plant growth was verified by the SPAD, NDVI and NDRE measurements. The significantly highest Se concentration in plants, 325 mg kg^{-1}, was recorded for the high Se(VI) rate in the presence of the biostimulant. Compared to controls, the low Se(VI) rate significantly decreased P and increased the S concentrations in plants. The post-harvest soil fractionation revealed that, in the presence of the biostimulant, the Se(VI) soluble fraction increased from 0.992 to 1.3 mg kg^{-1} at a low Se(VI) rate, and decreased from 3.T85 to 3.13 mg kg^{-1} at a high Se(VI) rate. Nevertheless, at a low Se(VI) rate, 3.6 and 3.1 mg kg^{-1} of the added Se(VI) remained in the soil in less mobile forms, in the presence or absence of the biostimulant, respectively. This study indicated that the exogenous application of Se in soil exerted dual effects on lettuce growth and Se availability, depending on the level of selenate applied.

Keywords: Se uptake; Se fractions; biofortification; lettuce; biostimulant

check for updates

Citation: Zafeiriou, I.; Gasparatos, D.; Ioannou, D.; Massas, I. Selenium Uptake by Lettuce Plants and Se Distribution in Soil Chemical Phases Affected by the Application Rate and the Presence of a Seaweed Extract-Based Biostimulant. *Soil Syst.* **2022**, *6*, 56. https://doi.org/10.3390/soilsystems6020056

Academic Editor: Sokrat Sinaj

Received: 15 May 2022
Accepted: 13 June 2022
Published: 15 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Selenium (Se) is an element that can, within a very narrow concentration range, be converted from an essential trace element for humans and animals to a toxic agent [1,2]. Its deficiency in the human body has been linked in the literature to many serious diseases, such as Keshan disease, autoimmune thyroid disease and Kashin–Beck disease [3,4]. For plants, the role of Se is still controversial, as, on the one hand, it has shown positive effects on their growth traits and also increased plant resistance to biotic and abiotic stresses in many studies, however, on the other hand, there are multiple reports connecting its existence to the occurrence of toxicity [2,5]. The World Health Organization (WHO) recommended a daily Se intake for humans of 55 μg day^{-1}, and it is estimated that more than 1 billion people are selenium-undernourished worldwide [6,7]. The main source of Se intake is through the consumption of agri-food products, whose Se concentration is directly dependent on the concentration of the element in soils. The total Se concentration in soils worldwide ranges between 0.01–2.0 mg kg^{-1}, with a relatively low average value of 0.4 mg kg^{-1} [8,9]. Soils found in Europe have low Se concentrations, particularly in Germany, Finland, Scotland and the Balkan countries, and according to Gupta and Gupta [2],

Greece is one of them. Selenium is present in soil environments, mainly as selenate and selenite oxyanions, with the former being the form with the highest efficiency for plant uptake and thus, is recommended when inorganic fertilization is applied [10–12]. In addition to the total Se concentration in soils, its fractionation and chemical form also affect the bioavailability of the element [13,14]. Therefore, the chemical behavior of Se, in combination with the physicochemical properties of the soil, such as the pH and Eh, the clay content, Fe/Mn oxides, organic matter and microbial activity, should be taken into account. The ability to predict the bioavailability of Se in the rhizosphere environment requires an understanding of adsorption–desorption phenomena, oxidation-reduction reactions, complexation and precipitation. The ability of plants to take up Se is directly related to their botanical characteristics and, consequently, differs between species [15]. Plants accumulating more than 1 g of Se kg^{-1} dry weight are classified as hyperaccumulators, while plant species that accumulate 100–1000 mg kg^{-1} of dry weight are classified as secondary accumulators [16]. Since Se occurs mainly in the protein fraction, fruits and vegetables, which usually contain a small amount of protein, are a poor Se source [17]. Considering that plants are the first link in the food chain, their ability to accumulate Se is vital for human nutrition and health. Agronomic biofortification (fertilizer application) is a common practice for the Se fortification of crops grown in Se-deficient soils [8,18]. Crop biofortification, through fertilization, is a safe enrichment method for increasing Se in human and animal foods, as the chances of an overdosage consumed through plant foods are extremely limited [8].

However, the application of biostimulants as innovative plant nutrition products regarding selenium availability and Se uptake by plants is an understudied research topic. Since lettuce (*Lactuca sativa* L.) is the most widely grown vegetable crop worldwide, its selection as a target for experimental study and the attempt to enrich its nutritional value with trace elements is expected. Motivated by this consideration, the objectives of the study were to investigate: (a) the uptake of Se added to the soil in the form of sodium selenate by lettuce plants; (b) the effect of a biostimulant on the uptake of Se; (c) the effect of Se and a biostimulant addition on the uptake of S and P by plants; (d) the redistribution of Se-chemical phases in the soil after the harvest of plants, through the application of a sequential extraction protocol; and (e) the consistency of the SPAD (chlorophyll estimation), NDVI (normalized difference vegetation index) and NDRE (red-edge index) measurements when compared to analytical data.

2. Materials and Methods

2.1. Soil Selection and Characterization of Soil Physicochemical Properties

A calcareous loam soil, typical for vegetable cultivation in Attica, Greece, was collected from the agricultural area of Markopoulo, East Attica, and transferred in sterile 25 L plastic bags to the Laboratory of Soil Science and Agricultural Chemistry of the Agricultural University of Athens, where it was air-dried, grounded, and after passing through a 2 mm sieve, was stored for further analysis. A full set of soil analyses was performed on the homogenized soil in three replicates and the results are presented as mean values in Table 1. The soil pH and electrical conductivity (EC) were measured in a slurry of a 1:1 *w*/*w* (soil/water) ratio [19] and were determined by the J.P. SELECTA s.a 2005 pH and CD meters. The soil particle size distribution was determined using the Bouyoucos hydrometer method, while the percentage of organic matter in the soil samples was determined by using Walkley–Black's method [20,21]. The percentages of the calcium carbonate equivalent and active calcium carbonate were determined by a Bernard calcimeter [22] and the ammonium oxalate method, as modified by Loeppert and Suarez [23], respectively. The concentration of the available P (Olsen-P) was determined at a wavelength of 882 nm on a Shimadzu UV-1700 spectrophotometer after the color development had been achieved by the addition of the Murphy–Riley reagent [24]. The concentrations of amorphous iron (Feo), manganese (Mno) and aluminium (Alo) oxides were calculated using the ammonium oxalate method [25], while the free oxides of iron (Fed), manganese (Mnd) and aluminium

(Ald) were determined by the sodium dithionite (CDB) method, as proposed by Mehra and Jackson [26]. Varian-spectra A-300 atomic absorption spectrophotometry was used to determine the concentrations of iron (Fe), manganese (Mn) and aluminium (Al). The total Se concentration was extracted with aqua regia [27] and a Varian-model VGA77 hydride generator was used for its determination.

Table 1. Physical and chemical characteristics of the experimental soil.

Parameter	Value
Clay (g kg^{-1})	180
Silt (g kg^{-1})	420
Sand (g kg^{-1})	400
pH (1:1)	7.40
EC (mS cm^{-1})	0.575
$CaCO_3$ (g kg^{-1})	184
Act. $CaCO_3$ (g kg^{-1})	47.5
Org. matter (g kg^{-1})	13.0
P-Olsen (mg kg^{-1})	15.40
CEC ($cmol_c$ kg^{-1})	22.40
Fed (g kg^{-1})	11.6
Feo (g kg^{-1})	1.4
Mnd (g kg^{-1})	0.7
Mno (g kg^{-1})	0.4
Ald (g kg^{-1})	0.5
Alo (g kg^{-1})	0.6
Total Se (μg kg^{-1})	156

2.2. Experimental Design

After determining the physicochemical properties of the studied soil, a two-factor experiment of growing lettuce in a greenhouse with five replications was applied. The first factor was the presence or absence of a biostimulant, while the second was the rate of Se added to the soil. More specifically, in the first control treatment, T1, there was no biostimulant or Se added; in treatment T2, Se was added at a rate of 5 mg kg^{-1} without the biostimulant; in treatment T3, Se was added at a rate of 10 mg kg^{-1} without the biostimulant; in treatment T4, the biostimulant was added without a Se addition, thus, this treatment served as a second control; in treatment T5, Se was added at a rate of 5 mg kg^{-1} with the addition of the biostimulant, and finally, in treatment T6, Se was added at a rate of 10 mg kg^{-1} with the addition of the biostimulant. Both the addition of the Se rates and the addition of the biostimulant were done through the irrigation water in two consecutive irrigation applications. The final amount of biostimulant per pot was 1.5 mL of the product (Actiwave). The composition of Actiwave is 3% total N (38.7 g L^{-1}), 1.096% organic N (12.9 g L^{-1}), 2% urea N (25.5 g L^{-1}), 7% water-soluble K_2O (90.3 g L^{-1}), 20.6% total organic matter, 0.5% water-soluble Fe (6.45 g L^{-1}), 0.5% chelated Fe (6.45 g L^{-1}), 0.08% water-soluble Zn (1.03 g L^{-1}) and 0.08% chelated Zn (1.03 g L^{-1}). Under the specific experimental conditions, selenium resulted from the dissolution of the sodium selenate (Na_2SeO_4) present in the soil environment as Se(VI) (selenate, SeO_4^{-2}). Afterwards, plants were spatially randomized within the greenhouse and their position was changed at least once a week.

The experiment lasted 10 weeks, from September 2020 to November 2020. For the experiment, 50 lettuce seedlings, aged 8–10 days, were selected and transplanted into plastic pots (one plant per pot), filled with 1 kg of soil, which was then passed through a 1 cm sieve to remove the gravel and stones. Transplanting was followed by a 10-day period of monitoring of the successful establishment of the plants, during which the cultivation practices that followed were common to all plants. Irrigation was carried out daily or every second day, with the aim of maintaining the soil's moisture at 60% of its water capacity throughout the plant's growth. The recommended macronutrient N–P–K fertilization was

applied to all plants with fertigation. At the end of the 10-day period, the 30 healthiest plants were selected and finally participated in the greenhouse experiment. The selection was done by a visual observation/evaluation of the plants and the homogeneity among the 30 selected plants was confirmed using the NDVI (normalized difference vegetation index), NDRE (normalized difference red-edge) and SPAD measurements.

After 10 weeks, the lettuce plants were taken inside the laboratory for the NDVI, NDRE and SPAD measurements and immediately afterwards, harvesting followed. After washing the plants with deionized water to remove any residues and dust, the plants were divided into above-ground and below-ground parts (heads/roots) and the fresh biomass of each part was immediately determined using a three-decimal precision balance, as well as the height of the above-ground part.

The NDVI and NDRE measurements were made using a Rapid SCAN CS-45 sensor from Holland Scientific, which provides measurements in three channels/spectra (red, red-edge and infrared of the electromagnetic spectrum). It then automatically calculates the indices as presented in Equations (1) and (2) [28].

$$\text{NDVI} = (\text{R\_NIR-R\_RED})/(\text{R\_NIR} + \text{R\_RED}) \quad (1)$$

$$\text{NDRE} = (\text{R\_NIR-R\_(RED EDGE)})/(\text{R\_NIR} + \text{R\_(RED EDGE)}) \quad (2)$$

Reflectance data from the Rapid SCAN CS-45 were collected just before harvest, with a plant-to-plant measurement, where the device was 20 cm vertically above the center of the highest point of the leaves of each plant. The SPAD measurements were made using a KONICA MINOLTA SPAD-502Plus portable chlorophyll meter, which provides a non-destructive and rapid estimate of the chlorophyll content in leaves.

2.3. Plant Tissue Analysis

Plant samples were dried in an oven (DHG-9203A) at 60 °C until they reached a constant weight (complete dehydration) and were then ground in a mill (Retsch, ZM 1000, Labexchange, Burladingen, Germany) to a particle diameter of <0.5 mm. Se, P and S were determined by the liquid digestion method, as described by Jones et al. [29]. In detail, 1 g of plant tissue from each sample was dissolved in concentrated nitric acid (HNO_3) while adding the required amount of 30 % (v/w) H_2O_2 to completely decolorize the solution. The procedure was carried out on a hot plate at 80 °C. The solution was then filtered and made up to 25 mL with the addition of deionized water and analyzed to determine the concentration of the three elements. The determination of the elements was performed on an inductively coupled plasma atomic mass spectrophotometer (ICP-MS) (model Thermo iCAP Qc, Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Selenium Fractionation

After the plants were harvested, the soil from each pot of every treatment was homogenized and a composite soil sample was transferred to the laboratory for the determination of the Se fractions using the five-step sequential extraction procedure, as described by Wang et al. [13] (Table 2). A total of four soil samples in two replicates were subjected to sequential extraction, as in, the soil of the control treatments that did not receive Se, the Se concentration was extremely low. One gram of soil was treated in a 50 mL polyethylene centrifuge tube, following the fractionation scheme presented in Table 2. After each extraction step, centrifugation for 10 min at 4000 rpm was conducted to separate the extractant from the residue, and the supernatant was collected after the filtration for further analysis. Before and after each extraction step, the centrifuge tube was weighted to correct for the remaining amount of extractant by the residue. Selenium concentrations in the extracting solutions were determined in the ICP-MS instrument mentioned in Section 2.3.

2.5. Statistical Analysis

Tukey's HSD test at a $p < 0.05$ was used for the detection of differences between the treatment means, while correlation analysis was performed to reveal any significant

relations between the studied parameters. The STATISTICA software, Hamburg, Germany (version) was run for the statistical analysis.

Table 2. Sequential extraction procedure of soil Se.

Step	Fraction	Reagents	Procedure
1	Soluble	10 mL 0.25 mol L^{-1} KCl	1 h shaking 200 rpm T = 25 °C
2	Exchangeable and carbonate bound	10 mL 0.7 mol L^{-1} KH_2PO_4 (pH = 5)	4 h shaking 200 rpm T = 25 °C
3	Fe/Mn-oxide bound	10 mL 2.5 mol L^{-1} HCl	50 min heating in a water bath shaking intermittently T = 90 °C
4	Organic matter bound and elemental	8 mL 5% $K_2S_2O_8$ 2 mL conc. HNO_3	3 h heating in a water bath Capped vials Shaking intermittently T = 90 °C
5	Residual	8 mL conc. HNO_3 2 mL conc. $HClO_4$	Transferring into Teflon crucibles with the reagents Heating in a sand bath until the soil turns white or gray in color. Covered crucibles T = 170 °C. Transfer remaining solution in 25 mL volumetric flask with DI water

3. Results

3.1. Plant Tissues

The results of the plant analyses (mean values) is summarized in Table 3. The fresh plant biomasses ranged between 2.43 and 21.16 g. The significantly higher mean value of the fresh biomass was shown by plants receiving 5 mg Se kg^{-1} soil in the presence of the biostimulant, while the significantly lower mean value of the dry biomass was shown by plants receiving 10 mg Se kg^{-1} soil in the presence of the biostimulant. Regarding the plants of the treatments grown in the absence of the biostimulant, there was a trend towards an increase in the fresh biomass for the 5 mg Se kg^{-1} soil application rate compared to the plants of the T1 treatment and a subsequent decrease in the biomass for the plants of the T3 treatment, without any significant differences between the three treatments. A similar pattern was also observed in the case of treatments where plants were grown in the presence of the biostimulant, except that in this case, there was a significant increase in the fresh biomass with treatment T5 compared to the plants of treatment T4, as well as a significant decrease in the biomass with treatment T6 compared to the fresh biomass regarding treatments T5 and T4.

Table 3. Se, S and P concentrations in plant tissues, biometric and sensor measurements. Comparisons between means are valid within rows. Different letter indicates significant differences.

Treatment	T1	T2	T3	T4	T5	T6
mg Se kg^{-1} Soil	**0**	**5**	**10**	**0**	**5**	**10**
		No Biostimulant			**Biostimulant**	
Se (μg g^{-1})	0.14 a	196.74 c	110.9 b	0.15 a	147.6 bc	325.42 d
P (mg g^{-1})	2.41 c	1.5 ab	1.27 a	2.48 c	1.8 b	2.04 b
S (mg g^{-1})	1.47 a	2.58 b	1.24 a	1.66 a	3.77 c	2.5 b
F.W. (g) [1]	12.28 b	13.32 b	7.55 ab	14.6 b	21.16 c	2.43 a
D.W. (g) [2]	1.38 c	1.31 c	1.12 b	1.57 c	1.81 c	0.65 a
SPAD	24.36 b	20.04 ab	15.26 a	26.3 b	36.16 c	16 a
NDRE	0.342 b	0.253 ab	0.329 b	0.351 b	0.324 b	0.188 a
NDVI	0.9118 b	0.8218 b	0.791 b	0.899 b	0.912 b	0.561 a

[1] Fresh Weight. [2] Dry Weight.

The dry biomasses of the plants ranged between 0.65 and 1.81 g. The highest mean dry biomass value was obtained by plants receiving 5 mg Se kg^{-1} soil in the presence of the biostimulant, while the lowest mean dry biomass value with a significant difference among all treatments was obtained by plants receiving 10 mg Se kg^{-1} soil in the presence of the biostimulant. Regarding the treatments without the Se addition, there was no significant difference in the dry biomass of plant tissues, however, plants grown in the presence of the biostimulant showed a tendency to increase their dry biomass compared to plants grown in the absence of the biostimulant. The application of 10 mg Se kg^{-1} soil also resulted in a significant decrease in the dry biomass of plants in the absence of a biostimulant compared with plants that received 5 mg Se kg^{-1} soil and plants that did not receive the selenium addition.

The Se concentration in plant tissues ranged between 0.14 μg g^{-1} dry biomass and 325.42 μg g^{-1}, with the minimum recorded for treatment T1, in which no selenium was added, and the maximum for treatment T6, in which a rate of 10 mg Se kg^{-1} soil was applied (Figure 1).

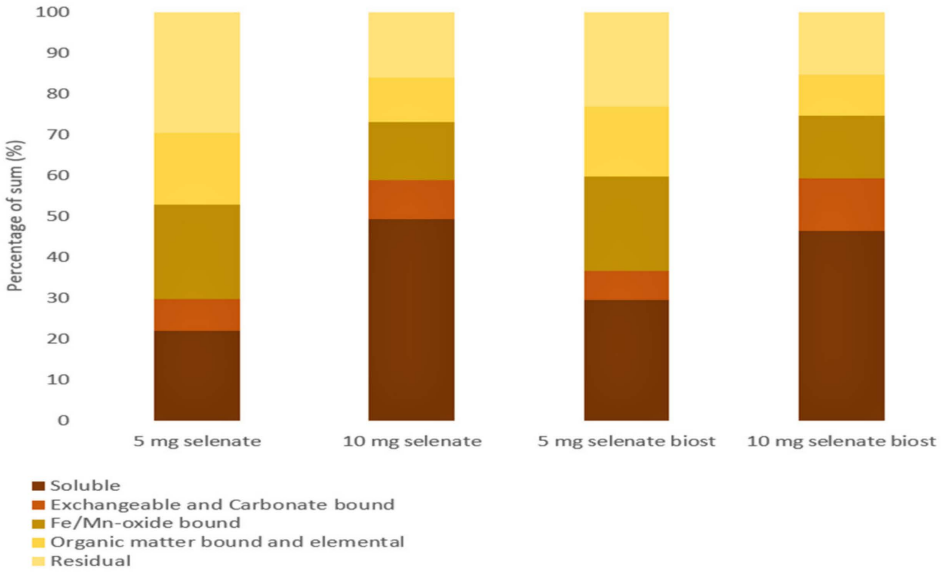

Figure 1. Percentage participation of Se fractions in soil samples.

The T1 treatment showed no significant difference only with the T4 treatment, while compared to all other treatments, it showed a significantly reduced Se concentration in plant tissues. In the case of treatments T2 and T3, where plants were grown in the absence of the biostimulant, the Se concentration in plant tissues was significantly increased for plants receiving a rate of 5 mg Se kg^{-1} soil compared to plants receiving the rate of 10 mg Se kg^{-1} soil, while the exact opposite pattern was observed for plants grown in the presence of the biostimulant, with plants in the T6 treatment showing a significantly increased Se concentration in plant tissues compared to plants in the T5 treatment.

The sulfur (S) concentration ranged between 1.24 mg g^{-1} of dry plant tissue biomass and 3.77 mg g^{-1}, with the maximum value occurring in plants of the treatment receiving the 5 mg Se kg^{-1} soil rate in the presence of the biostimulant and the minimum in plants of the treatment in which 10 mg Se kg^{-1} was applied in the absence of the biostimulant. As shown in Table 3, with respect to the plants grown in the absence of the biostimulant, the sulfur concentration was significantly increased for treatment T2, while there was no significant difference recorded between treatments T1 and T3. The obtained pattern was different for plants of treatments grown in the presence of the biostimulant, with the concentration of S being significantly increased in plant tissues for treatments T5 and T6 compared with the concentration of the element recorded for plants of treatment T4.

The phosphorus (P) concentration ranged between 1.5 mg g^{-1} of dry plant tissue biomass and 2.48 mg g^{-1} of dry plant tissue biomass. More specifically, the highest P concentration values were recorded for plants of the two treatments grown without the Se addition, i.e., treatments T1 and T4. Both in the case of plants grown in the presence of the biostimulant and in the case of plants grown in the absence of the biostimulant, the P concentration did not show significant differences between the application rates of 5 and 10 mg Se kg^{-1} soil.

The SPAD values ranged between 15.26 and 36.16, with the minimum mean value corresponding to the plants receiving 10 mg Se kg^{-1} soil in the absence of the biostimulant and the maximum for the plants receiving 5 mg Se kg^{-1} soil in the presence of the biostimulant. Regarding the plants grown in the absence of the biostimulant, the SPAD values showed a decreasing trend from the lowest to the highest Se application rate, which also led to a significant difference in values between the treatments T1 and T3. On the contrary, in the case of the plants grown in the presence of the biostimulant, the mean value of SPAD was significantly increased compared to the plants of treatment T4, while again, the significantly lower values were recorded for the plants of the treatment where the high Se rate was applied. The NDVI values ranged between 0.561 and 0.912, while the NDRE values ranged between 0.188 and 0.351, with the statistical analysis showing no particular differences between treatments' means, but mainly trends, with the only exception being the values of the T6 treatment, which for both indices, were the lowest.

3.2. Se Fractions

The results obtained from the application of the sequential extraction protocol are presented in Table 4. The procedure was carried out only for the Se-treated soils, as the concentration of the element in the soils from the control treatments was already too low and, therefore, the application of the sequential extraction was not possible. The recovery factor (RF) was calculated as the sum of the selenium concentration in the five stages of the extraction protocol divided by the total Se concentration in the soil samples that were obtained by HNO_3 + $HCLO_4$ dissolution, as described for the final stage of the sequential extraction scheme.

Table 4. Concentration of Se (μg kg^{-1}) in soil fractions according to the sequential extraction scheme of Wang et al. [13].

Treatment	Soluble (Sol-Se) (μg kg^{-1})	Exchangeable and Carbonate Bound (EXC-Se) (μg kg^{-1})	Fe/Mn-Oxide Bound (Fe/Mn-Se) (μg kg^{-1})	Organic Matter Bound and Elemental (OM-Se) (μg kg^{-1})	Residual (Res-Se) (μg kg^{-1})	Recovery Factor (%)
T2	992	361	1050	794	1350	114
T3	3850	760	1100	860	1260	112
T5	1300	310	1020	750	1020	111
T6	3130	870	1040	670	1050	109

In the present study, the Sol-Se content in low-Se and high-Se level soils without the biostimulant application were 992 μg kg^{-1} and 3850 μg kg^{-1}, respectively (Table 4), and its corresponding proportion in the total Se was 21.8%, and 49.2%, respectively (Figure 1). The EXC-Se content was 361 μg kg^{-1}, and 760 μg kg^{-1}, respectively (Table 4), and its corresponding proportion was 7.9% and 9.7%, respectively (Figure 1). Fe/Mn-Se, OM-Se, and Res-Se are strongly bound to the solid phase, and it is difficult to be absorbed by plants. These three soil-Se fractions, with extremely low bioavailability, accounted for nearly 70% of the total-soil Se for the low-Se level but only 41% for the high-Se rate.

The soluble-Se content in the low-Se and high-Se level soils with a biostimulant application were 1300 μg kg^{-1} and 3130 μg kg^{-1}, respectively (Table 4), and its corresponding proportion in the total Se was 29.5%, and 46.3%, respectively (Figure 1). The EXC-Se content

was 310 μg kg^{-1}, and 870 μg kg^{-1}, respectively, and its corresponding proportion was 7.0% and 12.9%, respectively (Figure 1). Fe/Mn-Se, OM-Se, and Res-Se accounted for nearly 63.4% of the total-soil Se for the low-Se level but only 41% for the high-Se level, as in the case for the treatments without the biostimulant.

Interestingly, the present results indicated that the application of a biostimulant increased the available Se (Soluble-Se + EXC-Se) under a low level of selenate (5 mg kg^{-1}), while the contrary pattern was recorded under a high level of selenate (10 mg kg^{-1}). Regardless of the level of selenate spiked in soil, the biostimulant application decreased the concentration of Se bound in the organic matter and residual fractions.

4. Discussion

As expected, the addition of Se in the form of sodium selenate (Na_2SeO_4) increased the concentration of the element in plants, regardless of the presence or absence of the biostimulant, as SeO_4^{-2} ions are the most available Se form due to their increased mobility in the soil solution [1,2]. Under conditions similar to those of the present experiment, i.e., oxidizing and a neutral-alkaline environment, Se is found in the form Se(VI) (selenate), while its reduction to Se(IV) (selenite), a form which is significantly less available in the soil environment due to the high tendency of adsorption by soil colloids, is not favored [8,10]. The addition of the low rates of Se led to an increase in fresh/dry biomasses when accompanied by a simultaneous application of biostimulant, while it did not affect the fresh/dry biomass in the case where there was no biostimulant applied. Similar results of a positive effect of a low-rate Se application on a plant's characteristics have been frequently reported in the literature [30–32], with the explanation being attributed to several mechanisms in which Se plays an essential role, such as a reduction in metal toxicity, an improvement in photosynthetic capacity and the maintenance of cell structures and functions [2]. However, the necessity of Se for plants is still a controversial issue for the scientific community, even though its presence improves the plant characteristics, whether they are under biotic or abiotic stress or not [2]. On the contrary, the addition of Se at high rates may cause toxicity in plants [2,5,8]; in fact, this was observed in the present study, since fresh/dry biomasses were significantly reduced in treatments where 10 mg Se kg^{-1} soil was applied, and a correspondingly significant reduction in the NDVI/NDRE and SPAD values was recorded. The biostimulant seems to have had a significant effect on the above phenomenon, as the plants that received the high rate in combination with a biostimulant application continued to increase the Se concentration levels in their tissue, resulting in further degradation of their weight and physiological characteristics (Table 3). Key components of the Actiwave biostimulant, beyond nutrients, are vitamin K1 derivatives, betaines and polysaccharides, which enhance the uptake of nutrients by plants through direct and indirect mechanisms [33,34]. The increased uptake, due to biostimulant application, was also shown in the case of the P and S macronutrients, with an observed increasing trend in their concentrations in the plants of T4, T5 and T6 treatments (Figure 2). Regarding the two macronutrients, in addition to the effect of the biostimulant on their uptake, the effect of the Se application on the fluctuation of their concentrations in plant tissues was also observed. More specifically, a Se application to plants decreased the P content, while it increased the S content as indicated by the significantly negative and significantly positive correlations, respectively (Table 3). Similar results are reported in the literature [8,18], with the interpretation given firstly by the fact that Se(VI), as a species with a similar chemical behavior to S, is taken up and moved in the plant using the S transporters (sulfate transporters) and, secondly, it competes with phosphate and sulfate anions in the soil environment, thus affecting their availability [8,32]. Of particular interest, are the significant correlations that emerged between the NDVI/NDRE and SPAD measurements with the plant measurements (Table 5). Other studies report similar correlations between precision-agriculture-instrument measurements and physiological traits [5,32,35] while emphasizing the importance of calibrating these non-destructive instrument measurements with the laboratory results of a plant's nutrient

status, with the aim of using such techniques/instrumentation in modern sustainable agriculture models.

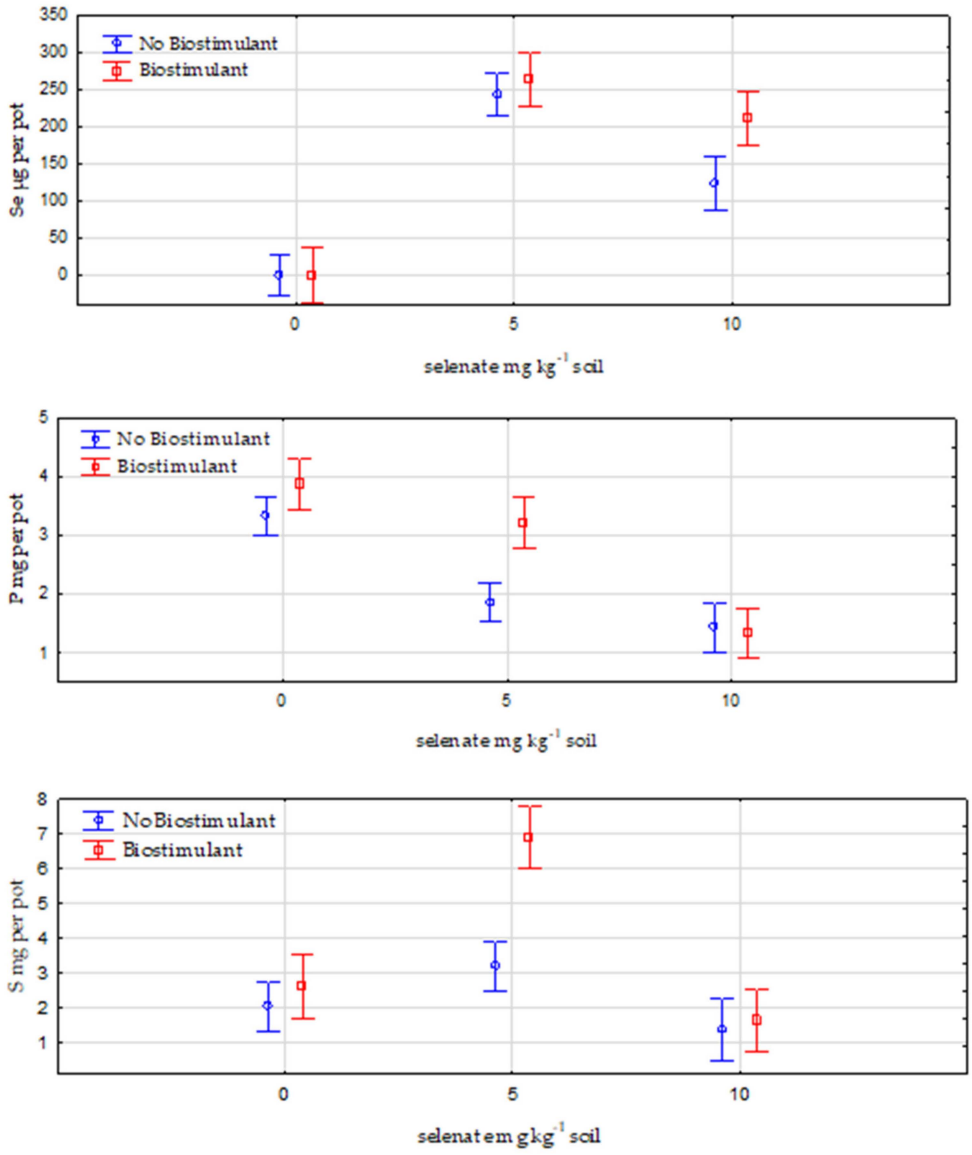

Figure 2. Effects of Se(VI) rates and application of biostimulant on plants' Se, S and P contents (µg Se pot^{-1}, mg P pot^{-1} and mg S pot^{-1}). The bar represents the standard error of the mean.

Table 5. Significant correlations between studied parameters in selenate treatments ($n = 30$, $p < 0.05$, + and − indicate a positive and negative correlation, respectively).

	Se µg kg^{-1}	**P mg kg^{-1}**	**S mg kg^{-1}**	**D.W.**	**SPAD**	**NDVI**	**NDRE**
Se µg kg^{-1}		−0.48	+0.55	−0.57		−0.82	−0.73
P mg kg^{-1}	−0.48						
S mg kg^{-1}	+0.55				+0.46		
D.W.	−0.57				+0.82	+0.76	+0.53
SPAD			+0.46	+0.82		+0.62	
NDVI	−0.82			+0.76	+0.62		+0.57
NDRE	−0.73			+0.53		+0.57	

Total Selenium, Phosphorus and Sulfur Uptake by Lettuce Plants

To better evaluate the results of the biofortification experiment, the total amounts of Se, P, and S, accumulated in lettuce plants, were determined using the following formula:

element concentration (mg g^{-1} D.W. plant for P and S or μg g^{-1} D.W. plant for Se) × (plant D.W.g pot^{-1}) and is expressed as the mg/pot for P and S and as μg/pot for Se. In our study, mg and μg pot^{-1} were equal to mg kg^{-1} and μg kg^{-1} soil, as each pot contained 1 kg of soil. The results are presented in Figure 2.

The effect of the biostimulant presence on the increasing Se uptake by plants forms a clear pattern with respect to the μg per pot values. In particular, for the low application rates where the biostimulant increased the uptake of selenium, no signs of plant toxicity were observed, leading to a maximum mean value of 263.9 μg pot^{-1}, which was recorded in the case of the application of 5 mg Se kg^{-1} soil. An increase of 7.58% compared to the corresponding average value of 243.9 μg Se pot^{-1} was recorded for the same application rate in the absence of the biostimulant. In contrast, in the application of high-application rates of selenate due to the toxicity caused by the element to plants, the mean μg pot^{-1} values recorded were lower compared to the low application rates by about 20% and 50% for the presence and absence of the biostimulant, respectively, while again, the presence of the biostimulant led to an increased uptake between the same application rates. This suggests that an application rate of around 5 mg Se kg^{-1} soil, especially when combined with a biostimulant, can indeed lead to the successful biofortification of lettuce plants with a positive effect on plant characteristics, while application rates approaching or exceeding 10 mg Se kg^{-1} soil should be avoided. Regarding the variation of the mg pot^{-1} values of macronutrients P and S, our results indicate firstly, that there is a strong interaction of the three elements at the botanical level and, secondly, that the mechanisms controlling these interactions are different. In particular, the significantly negative correlation between the Se and P concentrations in plant tissues is even more evident in Figure 2 as, regardless of the presence or absence of a biostimulant, the mg pot^{-1} values decrease with increasing μg pot^{-1} Se values. The highest mean values of 3.87 and 3.33 mg P pot^{-1} were recorded for plants grown without the Se addition to the soil in the presence and absence of a biostimulant, respectively, while the application of 10 mg Se kg^{-1} soil resulted in a decrease in the order of 65.64 % and 57.06 %, respectively. Similar results were reported in the study by Zafeiriou et al. [32], where the application of selenate to the rhizosphere environment led to a decrease in phosphorus concentrations in the above-ground part of rocket plants. On the contrary, in the case of the mg pot^{-1} S values, an increase of 164.61% and 57.42% was observed, compared to the respective controls in the presence and absence of the biostimulant, respectively, when applying 5 mg Se kg^{-1} soil. As mentioned above, the selenate species use sulfate transporters to help with the uptake by plants, which explains both the significantly positive correlation that appeared between the Se and S concentrations in the plant (Table 4) and the pattern presented in Figure 2. The biostimulant had a positive effect on S uptake, which was significant for the 5 mg kg^{-1} rate, and was pointed out by the considerably higher mean values of the mg S pot^{-1} recorded in its presence.

The major importance of sequential extraction schemes for the determination of the chemical phases of heavy metal(loid)s has been underlined repeatedly in the past by many researchers, as it provides critical information on the mobility, bioavailability and geochemical behavior of the elements. In the case of selenium, in particular, the importance of the sequential extraction schemes becomes even more important, as the binary nature of the element, ranging from an essential trace element to a cause of toxicity, makes its management a very sensitive issue in both biofortification and phytoremediation studies. Several sequential extraction protocols have been used in the literature with regard to Se, while their reliability—as in all sequential extraction protocols—depends on the selectivity of the extractants [36–38]. In the present study, the sequential extraction protocol of Wang et al. [13] was chosen to be used, which enables the determination of selenium in five chemical phases, which in order of determination are: (i) soluble, (ii) exchangeable and carbonate bound, (iii) Fe–Mn-oxide bound, (iv) organic matter bound and elemental, and (v) residual. Applying exogenous-inorganic Se to the soil in the early stages of cultivation plays an important role in the discussion about the chemical phases in which it

was found at the end of the experimental process, as the recovery factors ranged within acceptable percentages.

Various soil-Se fractions are more valuable than the soil-total Se for predicting the Se concentration in crop plants and evaluating its ecological and environmental risks [39,40]. Figure 1 shows the distribution patterns of the soil-Se fractions measured using a five-step sequential extraction after Se application, with and without the biostimulant. The soluble Se was the predominant Se fraction in the soil, except for treatment T2, while its proportion to the total Se content in soils depends on the applied Se rate. Among the five soil-Se fractions determined by sequential extraction, soluble-Se and exchangeable-Se are considered to have the highest levels of bioavailability and, therefore, their sum was generally considered as the bioavailable Se (BA-Se) for plants. The proportion of the five Se fractions in the total-soil Se after the application of the biostimulant was in the descending order of soluble-Se (29.5%) > Fe/Mn-Se (23.2%) ~Res-Se (23.0%) > OM-Se (17.0%) > EXC-Se (7.3%) and soluble-Se (46.3%) > Res-Se (15.5) ~Fe/Mn-Se (15.4%) > EXC-Se (12.9%) > OM-Se (9.9%) for the T5 and T6 treatments, respectively. In accordance with Ali et al. [41], the percentage of Fe/Mn-Se and OM-Se fractions greatly decreased with the increasing level from the 5 mg kg^{-1} to 10 mg kg^{-1} selenate-applied soil.

Generally, Se was strongly bound by soil components, and the soil's Se bioavailability was affected by multiple soil physicochemical properties, especially the soil pH and Eh [40,42,43]. For example, Li et al. [42] observed that a higher soil pH was correlated with a higher soil's Se bioavailability. According to recent findings, in soils without a selenate addition, the Fe/Mn−Se and OM−Se are the predominant fractions that support the strong immobilization and the low availability of Se. Contrary to Lyu et al. [40] and Wang et al. [44], who found that the contents of soluble- and exchangeable-Se fractions are extremely low (<10% of soil-total Se) in naturally seleniferous areas, our results showed that after the application of exogenous Se, the dominant fractions in the studied soil were the available Se fractions. This was different from the situation in naturally seleniferous soils, where our results indicated that when exogenous Se was applied to soil, the SOL-Se and EXC-Se fractions with high mobility increased. Irrespective of the biostimulant application or not, the dominant Se fraction in the soil was the available Se at the low level of selenate treatments (5.0 mg kg^{-1}), which increased with the increasing level of selenate spiked in the soil. Recently, Ali et al. [41] demonstrated that Fe/Mn-Se and OM-Se were the dominant fractions in the Se-unfertilized soil, while soluble Se was the predominant Se fraction in the selenate-applied soil.

Depending on the level of selenate applied in the soil, the addition of a biostimulant increased the concentration of the available Se (soluble Se and exch-Se) in the studied soil by 36.5% and 59.2% for the low and high levels of selenate, respectively. Similarly, Chen et al. [45], demonstrated that the application of microbial biostimulants (arbuscular mycorrhizal fungi) significantly enhanced the proportion of available Se fractions (soluble and EXC-S) in soils spiked with selenite or selenate by 21.29% and 9.74%, respectively.

Moreover, given that it takes considerable time for the exogenous-inorganic Se applied to soil to achieve equilibrium—since it is reported that there is still nearly 20% of Se present in the available fractions, even after a long time of aging [46]—the level of selenate applied to soil should receive more attention due to the possible ecological issues. Indeed, the Se application resulted in a considerably high Se presence in the soluble fraction, and considering that lettuce plants with low-Se treatments take up 20–25% of this fraction (Table 4), concerns regarding the environment can be raised. The remaining Se concentration in this readily available form may be prone to leaching, but may also be exploited by a primary or secondary Se hyperaccumulator, such as in rocket plants [32], thus minimizing the leaching hazard.

5. Conclusions

The findings of the present study showed that the two selected Se(VI) application rates influenced the growth and the Se uptake by the lettuce plants, while also verifying

the competitive and synergistic effect of Se(VI) on P and S uptake, respectively. The presence of the Actiwave biostimulant in the soil environment, when combined with the low Se(VI) application rate, enhanced the growth and the physiological characteristics of the plants, while it did not drastically affect the Se uptake. The high Se(VI) application rate suppressed the growth of lettuce plants, and toxicity symptoms were observed and recorded by using the SPAD, NDVI and NDRE measurements. Sequential extraction results, obtained after the harvest, showed that for both Se(VI) rates, a considerable portion of the added Se(VI) remained in the soluble fraction and increased at a low rate when Actiwave was present. Thus, it is evident that the high Se(VI) should be avoided for lettuce biofortification purposes, as serious leaching hazards may emerge. Under the specific experimental conditions, the 5 mg Se(VI) kg^{-1} soil can be proposed as a safe and adequate dose to achieve the biofortification of lettuce with Se. However, the outcome of the concurrent application of the low Se(VI) rate and the selected biostimulant resulted in healthy and satisfactory biofortified lettuce plants. A very promising result is that, after harvest, almost 78% and 71% of the added Se(VI) remained in the soil in less mobile forms, thus representing a pool of reserved Se that could be slowly released and made available for a plant's uptake. Since the results are encouraging, the experimental setup of this study should be tested through field trials for different soil types with the continuous growth of various vegetables, including the monitoring of the Se(VI) fractionation and soil depth.

Author Contributions: Conceptualization, I.Z., D.G., D.I. and I.M.; methodology, I.Z., D.G., D.I. and I.M.; validation, I.Z., D.G., D.I. and I.M.; formal analysis, I.Z., D.G., D.I. and I.M.; investigation, I.Z., D.G., D.I. and I.M.; resources, I.Z., D.G. and I.M.; data curation, I.Z., D.G., D.I. and I.M.; writing—original draft preparation, I.Z., D.G. and D.I.; writing—review and editing, I.Z., D.G., D.I. and I.M.; supervision, I.M.; funding acquisition, D.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are available upon request.

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

References

1. Winkel, L.H.E.; Vriens, B.; Jones, G.D.; Schneider, L.S.; Pilon-Smits, E.; Bañuelos, G.S. Selenium Cycling across Soil-Plant-Atmosphere Interfaces: A Critical Review. *Nutrients* **2015**, *7*, 4199–4239. [CrossRef] [PubMed]
2. Gupta, M.; Gupta, S. An Overview of Selenium Uptake, Metabolism, and Toxicity in Plants. *Front. Plant Sci.* **2017**, *7*, 2074. [CrossRef] [PubMed]
3. Smith, L.D.; Garg, U. Chapter 17—Disorders of trace metals. In *Clinical Aspects and Laboratory Determination, Biomarkers in Inborn Errors of Metabolism*; Garg, U., Smith, D.L., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 399–426, ISBN 9780128028964. [CrossRef]
4. Rayman, M.P. Selenium and human health. *Lancet* **2012**, *379*, 1256–1268. [CrossRef]
5. Zafeiriou, I.; Gasparatos, D.; Ioannou, D.; Kalderis, D.; Massas, I. Selenium Biofortification of Lettuce Plants (*Lactuca sativa* L.) as Affected by Se Species, Se Rate, and a Biochar Co-Application in a Calcareous Soil. *Agronomy* **2022**, *12*, 131. [CrossRef]
6. World Health Organization (WHO). *Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks*; WHO: Geneva, Switzerland, 2009.
7. Wu, Z.; Bañuelos, G.S.; Lin, Z.Q.; Liu, Y.; Yuan, L.; Yin, X.; Li, M. Biofortification and phytoremediation of selenium in China. *Front. Plant Sci.* **2015**, *6*, 136. [CrossRef]
8. Natasha Shahid, M.; Niazi, N.K.; Khalid, S.; Murtaza, B.; Bibi, I.; Rashid, M.I. A critical review of selenium biogeochemical behavior in soil-plant system with an inference to human health. *Environ. Pollut.* **2018**, *234*, 915–934. [CrossRef]
9. Schiavon, M.; Nardi, S.; dalla Vecchia, F.; Ertani, A. Selenium biofortification in the 21st century: Status and challenges for healthy human nutrition. *Plant Soil* **2020**, *453*, 245–270. [CrossRef]
10. Zafeiriou, I.; Gasparatos, D.; Massas, I. Adsorption/Desorption Patterns of Selenium for Acid and Alkaline Soils of Xerothermic Environments. *Environments* **2020**, *7*, 72. [CrossRef]

11. Hossain, A.; Skalicky, M.; Brestic, M.; Maitra, S.; Sarkar, S.; Ahmad, Z.; Vemuri, H.; Garai, S.; Mondal, M.; Bhatt, R.; et al. Selenium Biofortification: Roles, Mechanisms, Responses and Prospects. *Molecules* **2021**, *26*, 881. [CrossRef]
12. Alfthan, G.; Eurola, M.; Ekholm, P.; Venäläinen, E.; Root, T.; Korkalainen, K.; Hartikainen, H.; Salminen, P.; Hietaniemi, V.; Aspila, P.; et al. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. *J. Trace Elem. Med. Biol.* **2015**, *31*, 142–147. [CrossRef]
13. Wang, S.; Liang, D.; Wang, D.; Wei, W.; Fu, D.; Lin, Z. Selenium fractionation and speciation in agriculture soils and accumulation in corn (*Zea mays* L.) under field conditions in Shaanxi Province, China. *Sci. Total Environ.* **2012**, *427–428*, 159–164. [CrossRef]
14. Dhillon, K.; Dhillon, S.; Pareek, N. Distribution and bioavailability of selenium fractions in some seleniferous soils of Punjab, India. *Arch. Agron. Soil Sci.* **2005**, *51*, 633–643. [CrossRef]
15. De Temmerman, L.; Waegeneers, N.; Thiry, C.; Du Laing, G.; Tack, F.; Ruttens, A. Selenium content of Belgian cultivated soils and its uptake by field crops and vegetables. *Sci. Total Environ.* **2014**, *468–469*, 77–82. [CrossRef]
16. Ellis, D.R.; Salt, D.E. Plants, selenium and human health. *Curr. Opin. Plant Biol.* **2003**, *6*, 273–279. [CrossRef]
17. Navarro-Alarcon, M.; Cabrera-Vique, C. Selenium in food and the human body: A review. *Sci. Total Environ.* **2008**, *400*, 115–141. [CrossRef]
18. Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Raza, A.; Hawrylak-Nowak, B.; Matraszek-Gawron, R.; Nahar, K.; Fujita, M. Selenium Toxicity in Plants and Environment: Biogeochemistry and Remediation Possibilities. *Plants* **2020**, *9*, 1711. [CrossRef]
19. Page, A.L. (Ed.) *Methods of Soil Analysis, Part. 2*, 2nd ed.; American Society of Agronomy: Madison, WI, USA, 1982.
20. Bouyoucos, G.J. A recalibration of the hydrometer method for making mechanical analysis of soils. *Agron. J.* **1951**, *43*, 434–438. [CrossRef]
21. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon and organic matter. In *Methods of Soil Analysis, Part. 2, Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; ASA-SSSA: Madison, WI, USA, 1982.
22. *NF ISO 10693*; Détermination de la Teneuren Carbonate—MéthodeVolumétrique. Qualité des Sols AFNOR: Paris, France, 1995; pp. 177–186.
23. Loeppert, R.H.; Suarez, D.L. Carbonate and gypsum. In *Methods of Soil Analysis, Part. 3, Chemical Methods*; Bigham, J.M., Bartels, J.M., Eds.; ASA-SSSA: Madison, WI, USA, 1982; pp. 437–474.
24. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; US Department of Agriculture: Washington, DC, USA, 1954; Volume 939, pp. 1–19.
25. Schwertmann, U.; Taylor, R.M. Iron oxides. In *Minerals in Soil Environments*, 2nd ed.; Dixon, J.B., Weed, S.B., Eds.; Soil Science Society of America: Madison, WI, USA, 1989; pp. 379–438.
26. Mehra, O.P.; Jackson, M.L. Iron oxide removal from soils and clay by a dithionite-citrate system buffered with sodium bicarbonate. *Clays Clay Min.* **2013**, *7*, 317–327. [CrossRef]
27. Gasparatos, D.; Haidouti, C.; Tarenidis, D. Characterization of iron oxides in Fe-rich concretions from an imperfectly-drained Greek soil: A study by selective-dissolution techniques and X-ray diffraction. *Arch. Agron. Soil Sci.* **2004**, *50*, 485–493. [CrossRef]
28. Bonfil, D.J.; Gitelson, A.A. RapidScan and CropCircle radiometers: Opportunities and limitation in assessing wheat biomass and nitrogen. In Proceedings of the 12th International Conference on Precision Agriculture, Sacramento, CA, USA, 20–23 July 2014.
29. Jones, J.B.; Case, V.W. Sampling, Handling and Analysing Plant Tissue Samples. In *Soil Testing and Plant Analysis*, 3rd ed.; Westerman, R.L., Ed.; Book Series No. 3; Soil Science Society of America: Madison, WI, USA, 1990; pp. 389–427.
30. Cartes, P.; Jara, A.; Pinilla, L.; Rosas, A.; Mora, M. Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. *Ann. Appl. Biol.* **2010**, *156*, 297–307. [CrossRef]
31. Esringu, A.; Ekinci, M.; Usta, S.; Turan, M.; Dursun, A.; Ercisli, S.; Yildirim, E. Selenium supplementation affects the growth, yield and selenium accumulation in lettuce (*Lactuca sativa* L.). *C. R. Acad. Bulg. Sci.* **2015**, *68*, 801.
32. Zafeiriou, I.; Gasparatos, D.; Ioannou, D.; Massas, I. Selenium uptake by rocket plants (*Eruca sativa*) grown in a calcareous soil as affected by Se species, Se rate and a seaweed extract-based biostimulant application. *Crop Pasture Sci.* **2022**. [CrossRef]
33. Spinelli, F.; Fiori, G.; Noferini, M.; Sprocatti, M.; Costa, G. A novel type of seaweed extract as a natural alternative to the use of iron chelates in strawberry production. *Sci. Hortic.* **2010**, *125*, 263–269. [CrossRef]
34. Karapouloutidou, S.; Gasparatos, D. Effects of Biostimulant and Organic Amendment on Soil Properties and Nutrient Status of *Lactuca Sativa* in a Calcareous Saline-Sodic Soil. *Agriculture* **2019**, *9*, 164. [CrossRef]
35. Sinka, L.; Zsembeli, J.; Ragán, P.; Duzs, L.; Hájos, M.T. Effect of different seedling growing methods on the SPAD, NDVI values and some morphological parameters of four sweet corn (*Zea mays* L.) hybrids. *Agriculture* **2021**, *67*, 177–190. [CrossRef]
36. Wang, J.; Bailey, E.H.; Sanders, H.K.; Izquierdo, M.; Crout, N.M.J.; Shaw, G.; Yang, L.; Li, H.; Wei, B.; Young, S.D. Using chemical fractionation and speciation to describe uptake of technetium, iodine and selenium by *Agrostis capillaris* and *Lolium perenne*. *J. Environ. Radioact.* **2020**, *212*, 106131. [CrossRef]
37. Shao, Y.; Xie, B.; Li, M.; Zhang, X.; Xiao, H. Selenium Fractionation and Speciation in Paddy Soils and Accumulation in Rice under Field Conditions in Jinhua Zhejinang Province, China. In Proceedings of the E3S Web of Conferences, Guilin, China, 20–22 December 2019.
38. Tokunaga, T.K.; Lipton, D.S.; Benson, S.M.; Yee, A.W.; Oldfather, J.M.; Duckart, E.C.; Johannis, P.W.; Halvorsen, K.E. Soil selenium fractionation, depth profiles and time trends in a vegetated site at Kesterson Reservoir. *Water Air Soil Pollut.* **1991**, *57–58*, 31–41. [CrossRef]

39. Bassil, J.; Naveau, A.; Bueno, M.; Tullo, P.D.; Grasset, L.; Kazpard, V.; Razack, M. Determination of the distribution and speciation of selenium in an argillaceous sample using chemical extractions and post-extractions analyses: Application to the hydrogeological experimental site of Poitiers. *Environ. Sci. Pollut. Res.* **2016**, *23*, 9598–9613. [CrossRef]
40. Lyu, C.H.; Qin, Y.J.; Zhao, Z.Q.; Liu, X.W. Characteristics of selenium enrichment and assessment of selenium bioavailability using the diffusive gradients in thin-films technique in seleniferous soils in Enshi, Central China. *Environ. Pollut.* **2021**, *273*, 116507. [CrossRef]
41. Ali, F.; Peng, Q.; Wang, D.; Cui, Z.W.; Liang, D.L. Effects of selenite and selenate application on distribution and transformation of selenium fractions in soil and its bioavailability for wheat (*Triticum aestivum* L.). *Environ. Sci. Pollut. Res.* **2017**, *24*, 8315.e8325. [CrossRef]
42. Li, Z.; Man, N.; Wang, S.S.; Liang, D.L.; Liu, J.J. Selenite adsorption and desorption in main chinese soils with their characteristics and physicochemical properties. *J. Soils Sediments* **2015**, *15*, 1150–1158. [CrossRef]
43. Dinh, Q.T.; Li, Z.; Tran, T.A.T.; Wang, D.; Liang, D.L. Role of organic acids on the bioavailability of selenium in soil: A review. *Chemosphere* **2017**, *184*, 618–663. [CrossRef]
44. Wang, M.K.; Cui, Z.W.; Xue, M.Y.; Peng, Q.; Zhou, F.; Wang, D.; Dinh, Q.T.; Liu, Y.X.; Liang, D.L. Assessing the uptake of selenium from naturally enriched soils by maize (*Zea mays* L.) using diffusive gradients in thin-films technique (DGT) and traditional extractions. *Sci. Total Environ.* **2019**, *689*, 1–9. [CrossRef]
45. Chen, X.; Zhang, Z.Y.; Gu, M.H.; Li, H.; Shohag, M.J.I.; Shen, F.K.; Wang, X.L.; Wei, Y.Y. Combined use of arbuscular mycorrhizal fungus and selenium fertilizer shapes microbial community structure and enhances organic selenium accumulation in rice grain. *Sci. Total Environ.* **2020**, *748*, 141166. [CrossRef]
46. Di Tullo, P.; Pannier, F.; Thiry, Y.; Le Hecho, I.; Bueno, M. Filed study of time-dependent selenium partitioning in soil using isotopically enriched selenite tracer. *Sci. Total Environ.* **2016**, *562*, 280.e288. [CrossRef]