agronomy

Article

Humic Acid and Selenium Supplementation Modulate the Growth and Antioxidant Potential of Chili under Cadmium Stress

Muhammad Zohaib [1], Kamran Ashraf [2], Komal Fatima [1], Khawar Sultan [1,*], Abdel-Rhman Z. Gaafar [3], Mohamed S. Hodhod [4] and Qamar uz Zaman [1,*]

[1] Department of Environmental Sciences, The University of Lahore, Lahore 54590, Pakistan
[2] Department of Bioengineering and Biotechnology, School of Biotechnology, East China University of Science and Technology, Xuhui, Shanghai 200237, China
[3] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[4] Faculty of Biotechnology, October University for Modern Sciences & Arts, 6th October City 12566, Egypt
* Correspondence: khawar.sultan@envs.uol.edu.pk (K.S.); qamar.zaman1@envs.uol.edu.pk (Q.u.Z.)

Abstract: Maximizing food production under adverse conditions is a major challenge to food security and sustainability in the face of population growth and climatic change. The use of amendments applied as a supplement under adverse conditions may play a significant role in the mitigation of biotic and abiotic stress. This study aimed to explore the morpho-physio-biochemical changes in chili in response to cadmium (Cd) stress. The present study investigated the effects of foliar-applied selenium (Na_2SeO_4) (3 µM) and soil-applied humic acid (250 mg kg^{-1} w/w soil) in the sole and synergistic form under varying levels of cadmium stress (0, 2, and 4 mM using $CdCl_2$) in chili. Results revealed that a linear decrease was noticed in the growth, biomass, and phenological attributes of chili plants by increasing the Cd stress. More reduction was noticed at the higher levels of Cd stress as compared to control due lowering plant dry weight (18.15 and 39.67%), relative water content (RWC) (10.73 and 24.17%), total chlorophyll concentrations (16.01 and 31.44%) and increased electrolyte leakage (49.44 and 129.35%) and malonaldehyde contents (MDA) (68.41 and 104.04%). Dry biomass significantly increased with humic acid and selenium treatments, regardless of cadmium level. The reduced enzymatic activities associated with reactive oxygen species (ROS) detoxification, underscores the pivotal role of Se and humic acid in maintaining redox homeostasis. The combined effect of selenium and humic acid proved better results as compared to the sole application in minimizing Cd uptake in roots and fruit. This study demonstrates that the application of humic acid and selenium activates physio-biochemical defense responses against cadmium stress in chili plants and provides significant pavement for the cultivation of chili in cadmium-containing soils with a target of high-yielding and quality.

Keywords: cadmium accumulation; chili; combined; enzymatic activities; reactive oxygen species

check for updates

Citation: Zohaib, M.; Ashraf, K.; Fatima, K.; Sultan, K.; Gaafar, A.-R.Z.; Hodhod, M.S.; Zaman, Q.u. Humic Acid and Selenium Supplementation Modulate the Growth and Antioxidant Potential of Chili under Cadmium Stress. *Agronomy* **2023**, *13*, 2554. https://doi.org/10.3390/agronomy13102554

Academic Editor: Diego Pizzeghello

Received: 1 September 2023
Revised: 25 September 2023
Accepted: 28 September 2023
Published: 4 October 2023

1. Introduction

Heavy metal contamination is a serious threat to organisms, human health, and the ecosystem, as well as causing widespread environmental problems [1,2]. Soil is a medium to support the growth of plants and provide nutrients to plants [3,4]. Cadmium (Cd) is a high-cytotoxic industrial and environmental pollutant. Mining, Cd-containing fertilizers, industrial sewage sludge, and atmospheric deposition are the primary causes of the rapid increase of Cd accumulation in the soil [5,6]. The toxicity of Cd can affect the normal physiological and biochemical processes of plants and by consumption of foods with a high Cd content may indicate a potential risk to human health because cadmium can accumulate in the body over the course of many years to a chronic level [7].

Chili (*Capsicum annuum*) is an important crop in Pakistan both for consumption and export. It is commonly known as "red chili" in Pakistan and is a significant ingredient in the country's cuisine. Chili cultivation contributes to the livelihoods of many farmers in rural areas. It is often grown as a cash crop due to its demand in both domestic and international markets. Like many crops, chili cultivation in Pakistan faces challenges such as pests, diseases, irrigation with wastewater, and changing climate patterns [8]. Chili plants, like other crops, can take up cadmium from contaminated soil or water, leading to a range of physiological, biochemical, and morphological changes that can impact their growth and development [9]. It disrupts cellular structures and functions, including cell membrane integrity. It can also impact enzymatic and metabolic processes within the plant, leading to oxidative stress [10].

Using both organic and inorganic amendments can be effective in managing cadmium pollution in soil [11]. These amendments work by modifying the physical, chemical, and biological properties of the soil, thereby reducing the availability and mobility of cadmium [12]. Humic acid is known to be the black gold of agriculture having properties to be soluble in water and serve as an organic manure [13]. It acts as a growth promoter and helps in the nutrient uptake of crops as well as increased yield of vegetables [14,15]. It can increase cell membrane permeability, respiration, and photosynthesis [16], root cell elongation, and phosphate uptake in plants under abiotic stress [17,18]. Similarly, Selenium (Se) is a trace mineral that animals and humans need as a micronutrient [19]. It helps in the detoxification of heavy metals. It can form complexes with cadmium ions, reducing their availability for plant uptake [20]. Selenium supplementation under cadmium stress has been reported to promote plant growth by reducing the negative impact of cadmium on nutrient uptake, photosynthesis, and overall plant metabolism [21]. It helps to mitigate these nutrient imbalances by enhancing the uptake and translocation of essential minerals [22].

This combined approach holds the potential to mitigate the adverse impacts of Cd stress on plant growth and health, thereby contributing to sustainable agricultural practices and environmental protection [23]. Additionally, humic acid's capacity to enhance nutrient uptake and promote root development could synergistically support selenium's role in bolstering antioxidant defense mechanisms and detoxification pathways within plants [24]. However, there are a few reports about the participation of the combined effect of humic acid and Se in the mitigation of Cd stress in terms of biochemical traits in chili plants. We hypothesized that the synergistic effect of Se and humic acid can modulate Cd stress by improving plant growth, limiting membrane damage, and minimizing Cd uptake. Hence, the objectives of the current study were: (1) to explore the effect of various levels of Cd on the growth, biomass, and tissue health of chili and (2) to quantify the sole and synergistic effect of Se and humic acid on the physio-biochemical variables and Cd accumulation of chili plants in the presence and absence of Cd. These results will be helpful in discovering the interactions between selenium, and humic acid for alleviating the detrimental effects of cadmium contributing to sustainable agricultural practices and environmental protection.

2. Materials and Methods

2.1. Study Site, Experimental Design and Treatments

This comprehensive experimental approach aimed to elucidate the interactive effects of selenium and humic acid on reducing Cd stress in chili at the Department of Environmental Sciences, University of Lahore, Pakistan. A completely randomized design (CRD) under factorial arrangement was applied with triplicates. The total number of experimental units were ($2 \times 4 \times 3 = 24$). Experimental treatments were comprised of two factors cadmium stress control (0, 2, and 4 mM using $CdCl_2$) and selenium and vermicompost treatments viz., T_0 = control (Ck); T_1 = selenium (3 μM, using Na_2SeO_4 by Sigma-Aldrich, USA, with $\geq$98.0% purity) [25]; T_2 = humic acid (250 mg kg^{-1} w/w) [26], and T_3 = combination of selenium and humic acid treatments.

2.2. Crop Management

The soil used in this study was sampled to a depth of 15 cm from nearby agricultural fields. It was dried indoors until it could be crumbled to pass through a 4 mm-sieve and a 2 mm-sieve, for pot experiments. The composition of the soil was 30% sand, 35% silt, 35% clay, and 0.79% organic matter. For heavy metal stress treatments, cadmium ($CdCl_2$) was mixed with the medium at three different concentrations (0, 2, and 4 mM), incubated for 3 weeks, and filled in the plastic pots (23 cm height & 25 cm top diameter). The seeds of chili were germinated in a peat at 25 °C for 10 days. Three plants with uniform sizes were selected and transplanted into pots. After 10 days of transplanting, the humic acid was applied according to treatment, and manual hoeing was carried out for three days for the complete homogenization of the humic acid. To meet the nutritional requirements of the plants, Hoagland solution (50 percent) was applied at a rate of 1 L per week per pot. During the experiment, the necessary agronomic practices like weeding and irrigation were carried out on a regular basis in accordance with the physical observations of the plants in pots. The foliar application of Se was applied to plants 15 days after the application of humic acid. foliar applications of Se (as prescribed) were used, using 500 mL of the solution per pot using a handheld sprayer in each of two sprays at a 10-day interval [27]. Surfactant (commercial surf; at 1.00 mg L^{-1}) was added in each to desired levels of Se solution to improve the adhesion of the spray substance to the aerial parts of chili by following the protocols of Naz et al. [28]. A plant set without Cd, Se, and humic acid treatments was worked as a mock. After harvesting, data on biochemical morphological and physiological attributes was recorded.

2.3. Morphological, Phonological and Biomass Variables

After harvesting, plant samples were randomly selected from each replication. After counting the leaves, plant samples were cleaned with distilled water to remove any remaining dirt, and the plant's height, as well as the length of its shoots, roots, leaf length, and leaf width, were measured using a meter rod. To estimate the fresh weight and dry weight, one plant from each treatment was selected randomly. Root and shoot samples were separated by cutting the plants at the root-shoot junction, and the fresh weight was measured immediately between root and shoot portions and the collective weight was considered as plant fresh weight. For dry weight, samples were placed in a hot air oven at 65 °C for 48 h. The measurements were conducted in an electric balance. The leaf area of a plant was measured by multiplying the length and width of the chili plant [29]. The stem diameter was measured using the vernier caliper.

2.4. Physio-Biochemical and Water-Related Variables

A portable photosynthesis infrared gas analyzer (IRGA, Analytical Development Company, London, UK) was used to measure the photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), on a fully sunny day from upper leaves at 9 AM. For the purpose of extracting pigment, a crushed leaf sample (about 5 g) from the second replication was introduced to a test tube that contained 85% acetone (v/v) and then left for 24 h. The samples were centrifuged at $4000\times g$ and 4 °C for ten minutes. The chlorophyll contents of the supernatant were determined using a spectrophotometer (Halo DB-20/DB-20S; Dynamica Scientific, Bain Square, Livingston, UK) between wavelengths of 470, 647, and 664.5 nm, in accordance with Lichtenthaler's protocols [30]. The total chlorophyll contents were measured by adding the chlorophyll a and b. The relative water content (RWC) of a leaf of constant size from each treatment was determined using the equation below: RWC% was estimated using the formula: (fresh weight oven-dried weight/fully turgid weight oven-dried weight) 100, and electrolyte leakage (EL) was determined using the formula [31]: EL% = [EC1/EC2] 100, where EC1 = EC of the solution containing leaves in test tubes, and EC2 = EC of the solution in test tubes after autoclaving for 20 min at 121 °C

2.5. Enzymatic Antioxidant and Lipid Peroxidation Variables

Fresh chili leaves (1.0 g) were extracted in 50 mM phosphate buffer (pH 7.8), homogenized, and centrifuged at 15,000× *g* for 10 min to determine the enzyme activity. The supernatant was used for the experiment. Measurements of the activities of peroxidase (POD), catalase (CAT), or superoxide dismutase (SOD) were made in accordance with the methods published by Velikova et al. [32], Aebi [33], and Beauchamp and Fridovich [34], respectively. The malondialdehyde (MDA) as an indicator of cellular lipid peroxidation of chili leaves was measured by following the protocols of Heath and Packer [35] and Ali et al. [36]. The leaf sample (0.2 g) was homogenized in 1.5 mL of 0.1% Trichloroacetic acid followed by centrifugation at 11,500× *g* at 4 °C for 15 min. The collected supernatant (0.4 mL) was then added to 1 mL of the reaction mixture. For blank samples only 0.4 mL of 0.1% TCA was mixed with 1 mL of reaction mixture. The tube was kept for 30 min at 95 °C in a boiling water bath. After that tube was incubated in an icebox to terminate the reaction, and centrifugation was conducted for 30 min again at 10,000× *g*. The absorbance of the collected supernatant was measured at 532 and 600 nm.

2.6. Cadmium Contents in Plant Tissues

A blend of di-acid (HNO_3: $HClO_4$ proportion of 2:1) was used for the wet digestion of chili shoot samples. An atomic absorption spectrophotometer (Perkin-Elmer, Waltham, MA, USA, Model 3300) was used to examine the concentration of cadmium content in the roots and fruit samples. Quality assurance for Cd internal standards (CPAchem, Bogomilovo, Bulgaria) were used while standardizing the equipment with reference material as explained by Salama et al. [37]. The calibration curve was prepared from the cadmium atomic absorption standard solution of 1000 µg mL^{-1} Cd in 1% HNO_3 (Sigma Aldrich, St. Louis, MO, USA). The instrument detection limit for Cd was (0.03 mg g^{-1} DW).

2.7. Quality Assurance

Merck-Germany analytical grade chemicals were utilized for all the procedures. To avoid contamination, high-quality glassware (Merck-Germany, Darmstadt, Germany) was used after washing with diluted HNO_3. To improve the accuracy of the data, the analysis was performed three times for each sample using the usual reference approach.

2.8. Data Analysis

Statistical analysis of data was performed by applying Fisher's analysis of variance (ANOVA) and the means of all treatments were compared by the highest significant differences (HSD) test using Statistix 8.1 software. Regression, correlation, Principal component analysis, and heat map were performed using Minitab Mtb EXE (2) software (Minitab Inc., State College, PA, USA).

3. Results

3.1. Growth and Phenological Variables

Induced cadmium stress caused a significant reduction in plant height (21.49 and 54.75%), root length (28.32 and 61.97%), shoot length (20.83 and 51.79%), number of leaves (28.77 and 38.43%), leaf length (6.95 and 41.71%), leaf width (26.52 and 42.65%), leaf area (24.10 and 61.16%) and stem diameter (14.03 and 26.47%) of at 2 and 4 mM, respectively in chili plants. Whereas, soil-applied humic acid and foliage-applied Se enhance growth attributes and phenology both in control and Cd-amended treatments (Figure 1(1A–1H)). Maximum growth attributes were observed where the combined application of humic acid and Se was applied both in control and Cd-contaminated treatments.

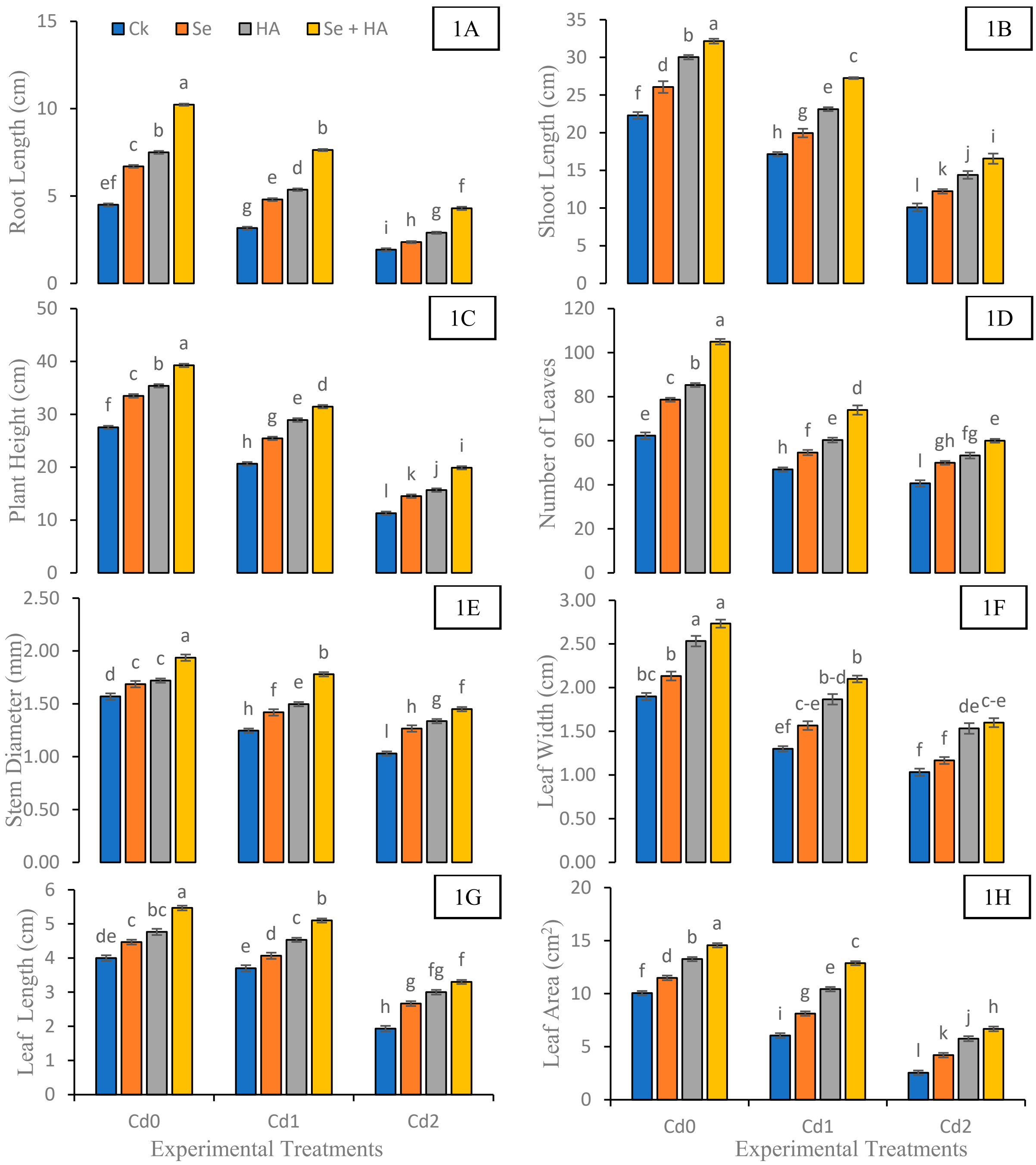

Figure 1. Effect of different treatments of selenium and humic acid on the growth variables of chili grown under various levels of cadmium stress (Cd_0 = control; Cd_1 = 2 mM; Cd_2 = 4 mM). The lowercase letters in the bar graph show the significant variations across treatment means at $p < 0.05$. Ck = Control; Se = Selenium (3 µM, using Na_2SeO_4); HA = Humic acid (250 mg kg^{-1} of soil *w*/*w*); Se + HA = Selenium + Humic acid. 1A = Root length; 1B = Shoot length; 1C = Plant height; 1D = Number of leaves; 1E = Stem diameter; 1F = Leaf width; 1G = Leaf length; 1H = Leaf area.

3.2. Fresh and Dry Biomass Variables

Data analysis showed that the combined effect of humic acid and Se was significant for the fresh and dry biomass of chili shoots, roots, and plants (Figure 2(2A–2F)). Escalation of humic acid and Se dosage progressively enhanced the fresh and dry biomass of shoot, roots, and plant for T_3 treatments both in Cd-regimes and under control. Rather this treatment (T_2) slightly increased the fresh and dry biomass more as compared to T_1. The Cd-affected plants exhibited decreased root fresh weight (14.45 and 21.60%), shoot fresh weight (16.38 and 38.24%), plant fresh weight (16.24 and 36.87%), root dry weight (38.74 and 57.66%), shoot dry weight (16.32 and 38.12%), and plant dry weight (18.15 and 39.67%) at 2 and 4 mM, respectively.

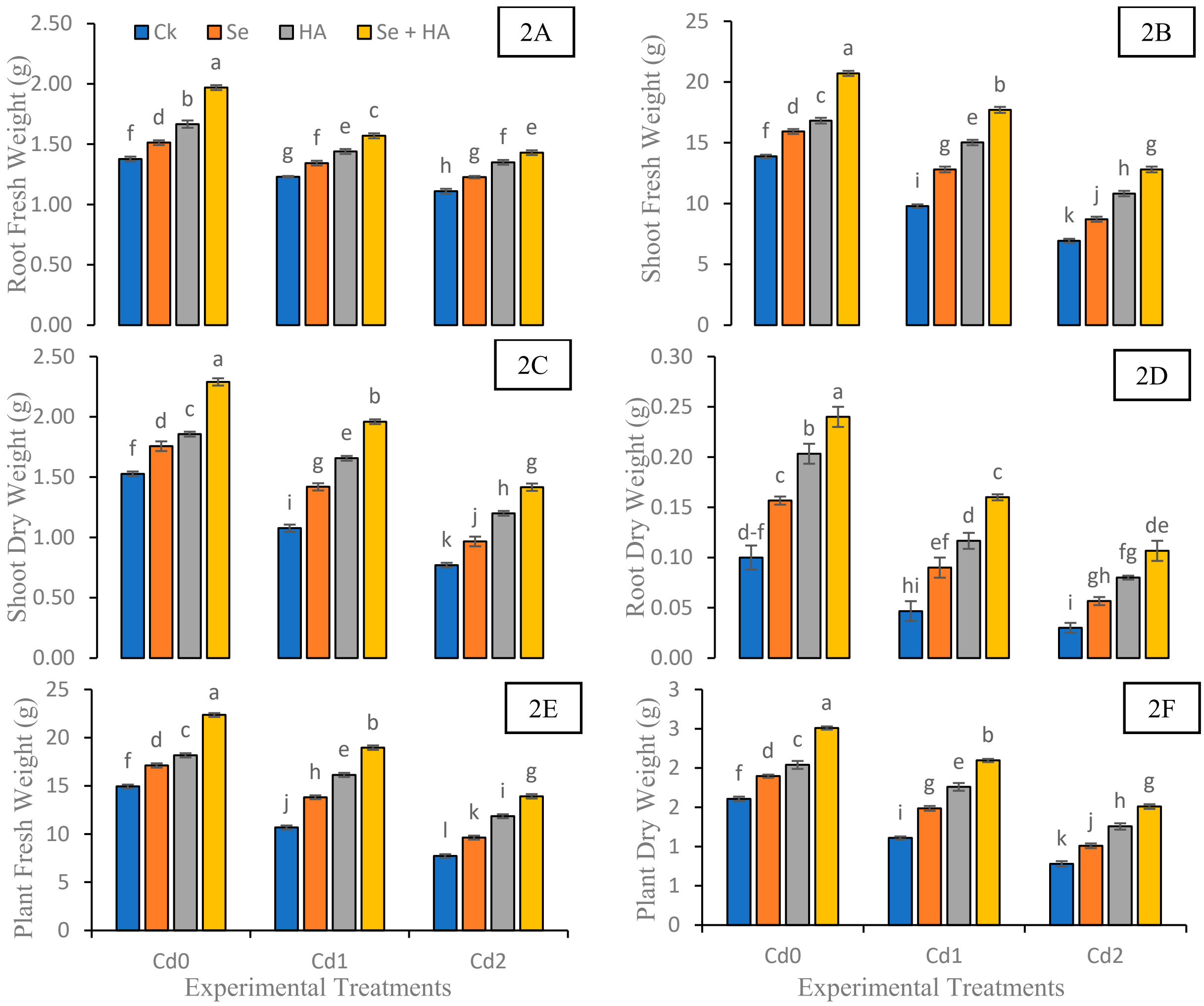

Figure 2. Effect of different treatments of selenium and humic acid on the biomass (fresh and dry) variables of chili grown under various levels of cadmium stress (Cd_0 = control; Cd_1 = 2 mM; Cd_2 = 4 mM). The lowercase letters in the bar graph show the significant variations across treatment means at $p < 0.05$. Ck = Control; Se = Selenium (3 µM, using Na_2SeO_4); HA = Humic acid (250 mg kg^{-1} of soil *w*/*w*); Se + HA = Selenium + Humic acid. 2A = Root fresh weight; 2B = Shoot fresh weight; 2C = Shoot dry weight; 2D = Root dry weight; 2E = Plant fresh weight; 2F = Plant dry weight.

3.3. Physiological and Photosynthetic Variables

The Cd-induced toxicity reduced chlorophyll *a* (18.02 and 30.97%), chlorophyll *b* (11.88 and 32.18%), total chlorophyll contents (16.01 and 31.44%), carotenoids contents (20.14 and 43.20%), photosynthetic rate (21.49 and 54.28%), transpiration rate (22.39 and 46.77%), and stomatal conductance (28.05 and 52.48%) at 2 and 4 mM, respectively as compared to control (no Cd stress). A maximum of all the photosynthetic and physiological attributes was observed in the case of T_3 where the combined application of humic acid and Se was applied to plants growing in control and Cd-stressed conditions (Figure 3(3A–3G)).

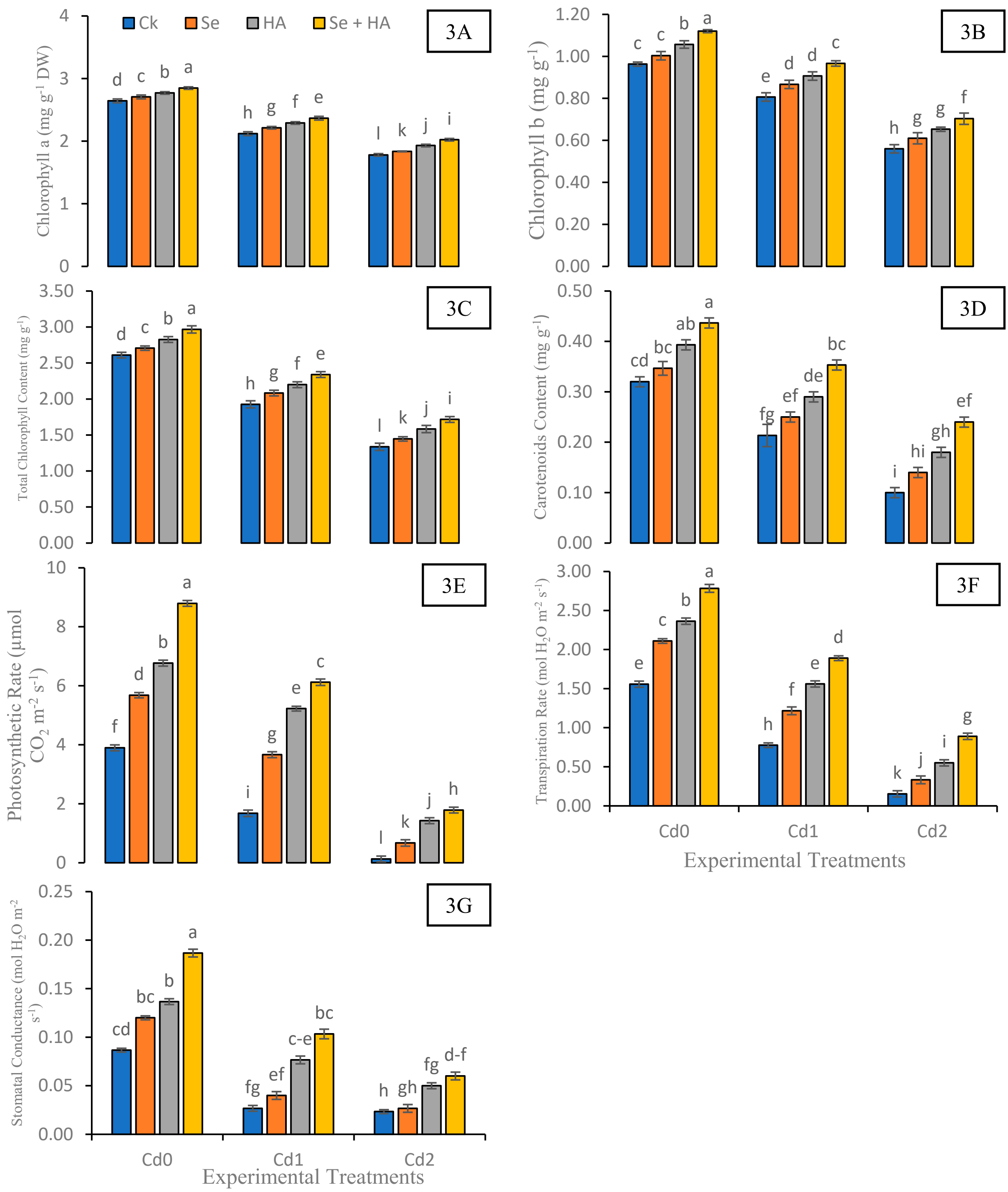

Figure 3. Effect of different treatments of selenium and humic acid on the physiological and photosynthetic variables of chili grown under various levels of cadmium stress (Cd_0 = control; Cd_1 = 2 mM; Cd_2 = 4 mM). The lowercase letters in the bar graph show the significant variations across treatment means at $p < 0.05$. Ck = Control; Se = Selenium (3 µM, using Na_2SeO_4); HA = Humic acid (250 mg kg^{-1} of soil w/w); Se + HA = Selenium + Humic acid. 3A = Chlorophyll *a* contents; 3B = Chlorophyll *b* contents; 3C = Total chlorophyll contents; 3D = Carotenoids contents; 3E = Photosynthetic rate; 3F = Transpiration rate; 3G = Stomatal conductance.

3.4. Water-Related and Biochemical Variables

The findings exhibited that relative water contents and electrolyte leakage were significantly affected in Cd-affected chili plants as compared to control. Cd stress decreased the relative water contents (10.73 and 24.17%) and increased electrolyte leakage (49.44 and 129.35%) and MDA (68.41 and 104.04%) activities as compared to control. Likewise, plants grown by the combined application of humic acid and Se further enhanced relative water contents and decreased the membrane leakage in chili plants. Maximum relative water contents and minimum membrane damage were revealed by Cd-affected and control plants supplemented with humic acid and Se. The Cd-affected plants treated with only humic acid proved a better response as compared to the sole application of Se (Table 1).

Table 1. Effect of different treatments of selenium and humic acid on the water-related, biochemical, and lipid per oxidation variables of chili grown under various levels of cadmium stress.

Cd Stress	Treatments	RWC (%)	EL (%)	MDA (μ mole g^{-1} FW)
Control	Ck	84.56 d	11.45 g	6.55 a
	Se	85.54 c	8.56 j	5.67 b
	HA	87.34 b	7.54 k	5.12 d
	Se + HA	91.23 a	5.67 l	4.34 g
2 mM	Ck	73.23 h	15.56 d	10.89 c
	Se	77.45 g	13.45 f	9.23 e
	HA	79.54 f	11.23 h	8.97 f
	Se + HA	81.23 e	9.41 i	7.42 h
4 mM	Ck	61.23 l	22.34 a	14.23 i
	Se	64.56 k	20.21 b	11.22 j
	HA	67.54 j	18.45 c	10.43 k
	Se + HA	71.23 i	15.21 d	8.34 l

RWC = Relative water contents; EL = Electrolyte leakage; MDA = Malonaldehyde contents; The lowercase letters in the bar graph show the significant variations across treatments means at $p < 0.05$. Ck = Control; Se = Selenium (3 μM, using Na_2SeO_4); HA = Humic acid (250 mg kg^{-1} of soil w/w); Se + HA = Selenium + Humic acid.

3.5. Enzymatic Antioxidant Variables

The activity of enzymatic antioxidants in chili plants were altered by the application of humic acid and Se under induced cadmium stress (Table 2). Increased cadmium stress from 2 to 4 mM caused the increased SOD (29.35 to 47.84%), POD (136.63 to 298.02%), and CAT (56.33 to 126.58%) significantly. In terms of the activities of antioxidant enzymes, cadmium stress was shown to have a decreasing pattern of 4 mM > 2 mM > control conditions, whereas humic acid and selenium treatments were observed to have a decreasing pattern of $T_0 > T_1 > T_2 > T_3$.

3.6. Cadmium Contents in Plant Tissues

Cadmium stress and various treatments of soil-applied humic acid and foliar-applied selenium significantly ($p \leq 0.01$) affected the root and fruit Cd accumulation in chili plants that are grown under Cd stressed as well as normal conditions. Cadmium stress increased the root and fruit Cd contents as compared to control (normal conditions). Maximum root Cd contents of 6.65 and 11.18 mg g^{-1} in roots and 3.30 and 6.90 mg g^{-1} in fruit under control conditions were observed where no addition of humic acid and foliar application of distilled water was carried out. Minimum root and fruit Cd accumulation was observed where foliar application of selenium and soil-applied humic acid was applied synergistically (Figure 4(4A and 4B)).

Table 2. Effect of different treatments of selenium and humic acid on the enzymatic antioxidant variables of chili grown under various levels of cadmium stress.

Cd Stress	Treatments	SOD (U mg^{-1} Protein)	POD (min^{-1} mg^{-1} Protein)	CAT (min^{-1} mg^{-1} Protein)
Control	Ck	2.93 g	0.13 g	0.18 ef
	Se	2.85 h	0.10 gh	0.15 fg
	HA	2.71 i	0.07 hi	0.11 hi
	Se + HA	2.56 j	0.04 i	0.09 i
2 mM	Ck	3.91 c	0.29 cd	0.27 c
	Se	3.67 d	0.21 e	0.23 d
	HA	3.53 e	0.17 f	0.19 de
	Se + HA	3.21 f	0.13 fg	0.14 gh
4 mM	Ck	4.67 a	0.41 a	0.38 a
	Se	4.23 b	0.37 b	0.31 b
	HA	3.91 c	0.31 c	0.28 bc
	Se + HA	3.54 e	0.25 d	0.23 d

SOD = Superoxide dismutase activity; POD = Peroxidase activity; CAT = Catalase activity; The lowercase letters in the bar graph show the significant variations across treatment means at $p < 0.05$. Ck = Control; Se = Selenium (3 µM, using Na_2SeO_4); HA = Humic acid (250 mg kg^{-1} of soil *w*/*w*); Se + HA = Selenium + Humic acid.

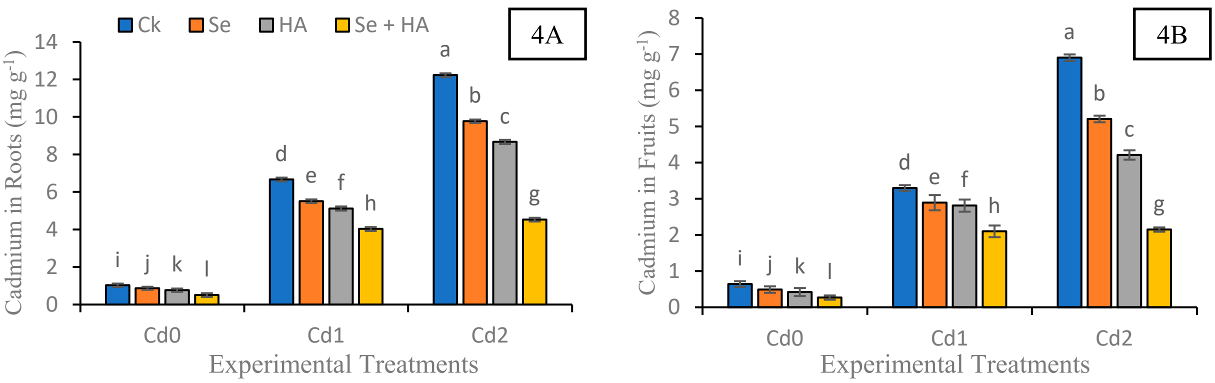

Figure 4. Effect of different treatments of selenium and humic acid on the Cd accumulation in roots and fruit of chili grown under various levels of cadmium stress (Cd_0 = control; Cd_1 = 2 mM; Cd_2 = 4 mM). The lowercase letters in the bar graph show the significant variations across treatment means at $p < 0.05$. Ck = Control; Se = Selenium (3 µM, using Na_2SeO_4); HA = Humic acid (250 mg kg^{-1} of soil *w*/*w*); Se + HA = Selenium + Humic acid. 4A = Cadmium in roots; 4B = Cadmium in fruits.

3.7. Pearson Correlation Matrix

Growth, enzymatic, water-related, and biochemical and Cd accumulation characteristics of chili plants were connected with one another in order to support the veracity of the conclusions above (Figure 5). All the enzymatic attributes (SOD, CAT, and POD) were negatively correlated with chlorophyll contents, growth, and biomass as well as relative water contents, however, a significant positive correlation was noticed for MDA and electrolyte leakage contents. The relative water contents and dry weight of the root shoot and plant were favorably associated with the chlorophyll contents.

3.8. Principle Component Analysis

Chili cultivated on cadmium-contaminated soil with exogenously applied Se and soil-applied humic acid treatments was shown to have a connection between Cd, growth, biomass, physio-biochemical, and tissue Cd contents using loading plots of principal component analysis (Figure 6). The first two primary components, PC1 and PC2, make up the largest percentage of all components and account for more than 94% of the entire database, whereas PC2 makes up 3% of it. The first set of variables that PC1 has a positive correlation with are: Cd in the root, MDA, and leaf area were positively correlated. The factors related to SOD, CAT, and POD are slightly negative with Cd in root. The factors related to growth parameters, leaf fresh weight, root fresh weight, and leaf dry weight were found to have a strong positive relationship with PC1 variables.

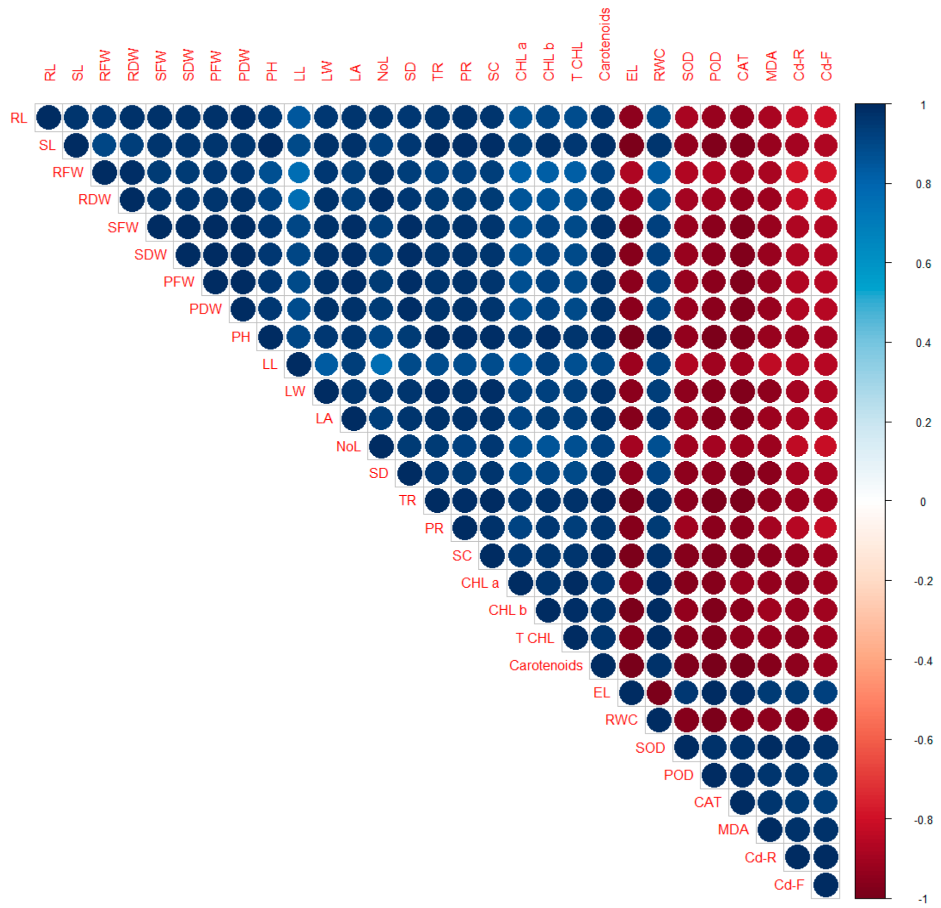

Figure 5. Correlation coefficients of various attributes of chili by different treatments of selenium and humic acid under various levels of cadmium stress (Cd_0 = control; Cd_1 = 2 mM; Cd_2 = 4 mM). RL = Root length; SL = Shoot length; RFW = Root fresh weight; RDW = Root dry weight; SFW = Shoot fresh weight; SDW = Shoot dry weight; PFW = Plant fresh weight; PDW = Plant dry weight; PH = Plant height; LW = leaf width; LL = Leaf length; LA = Leaf area; NOL = Number of leaves; SD = Stem diameter; TR = Transpiration rate; PR = Photosynthetic rate; SC = Stomatal conductance; CHL a = Chlorophyll a; CHL b = Chlorophyll b; TCHL = Total chlorophyll contents; EL = Electrolyte leakage; RWC = Relative water contents; SOD = Superoxide dismutase activity; POD = Peroxidase activity; CAT = Catalase activity; MDA = Malonaldehyde contents; Cd-R; Cd contents in roots; Cd-F = Cd contents in fruits.

3.9. Heat Map Analysis

The interaction between the many examined characteristics of the chili grown in soil that had been spiked with Cd was evaluated using the heat-map histogram (Figure 7). There is a negative link between the MDA concentration and relative water content; there is a positive correlation between the MDA concentration and Cd in the root and shoot of chili. Electrolyte leaks have a significant correlation with Cd levels in plant roots. A significant positive association between root length, shoot length, and leaf area was found where a combined application of Se and humic acid was applied. With all three antioxidant enzymes (SOD, CAT, and POD), Cd uptake showed a negative connection.

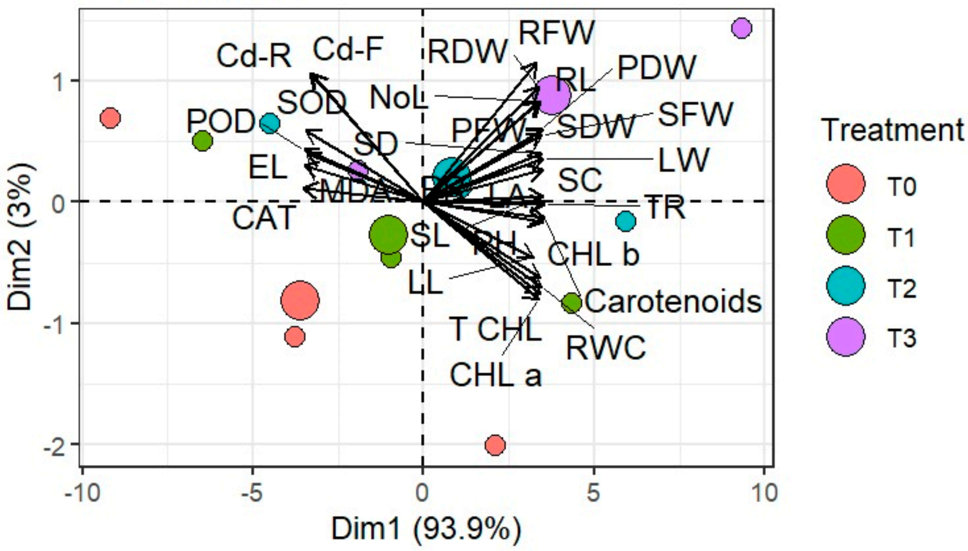

Figure 6. Loading plot of principal component analysis (PCA) on various attributes of chili under various Se and humic treatments grown under the induced Cd stress; RL = Root length; SL = Shoot length; RFW = Root fresh weight; RDW = Root dry weight; SFW = Shoot fresh weight; SDW = Shoot dry weight; PFW = Plant fresh weight; PDW = Plant dry weight; PH = Plant height; LW = leaf width; LL = Leaf length; LA = Leaf area; NOL = Number of leaves; SD = Stem diameter; TR = Transpiration rate; PR = Photosynthetic rate; SC = Stomatal conductance; CHL a = Chlorophyll a; CHL b = Chlorophyll b; TCHL = Total chlorophyll contents; EL = Electrolyte leakage; RWC = Relative water contents; SOD = Superoxide dismutase activity; POD = Peroxidase activity; CAT = Catalase activity; MDA = Malonaldehyde contents; Cd-R; Cd contents in roots; Cd-F = Cd contents in fruits.

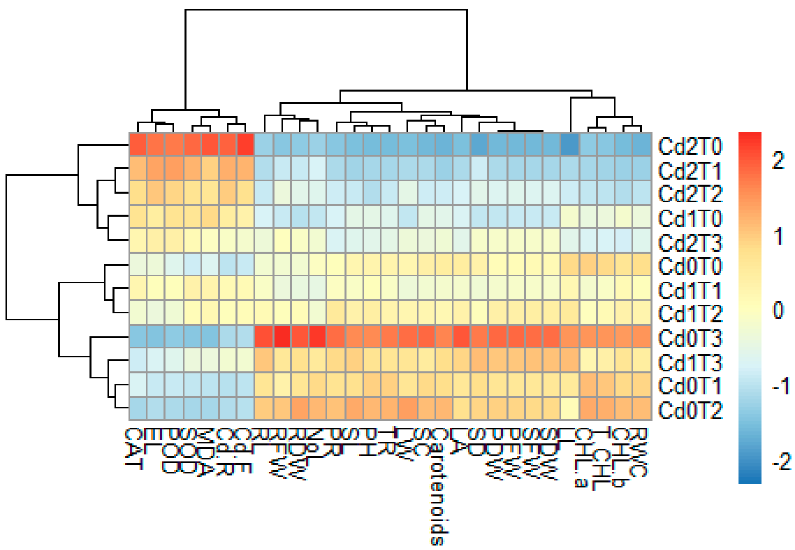

Figure 7. Heat map plot of various attributes of chili grown under various levels of cadmium stress (Cd_0 = control; Cd_1 = 2 mM; Cd_2 = 4 mM) under selenium and humic acid treatments; RL = Root length; SL = Shoot length; RFW = Root fresh weight; RDW = Root dry weight; SFW = Shoot fresh weight; SDW = Shoot dry weight; PFW = Plant fresh weight; PDW = Plant dry weight; PH = Plant height; LW = leaf width; LL = Leaf length; LA = Leaf area; NOL = Number of leaves; SD = Stem diameter; TR = Transpiration rate; PR = Photosynthetic rate; SC = Stomatal conductance; CHL a = Chlorophyll a; CHL b = Chlorophyll b; TCHL = Total chlorophyll contents; EL = Electrolyte leakage; RWC = Relative water contents; SOD = Superoxide dismutase activity; POD = Peroxidase activity; CAT = Catalase activity; MDA = Malonaldehyde contents; Cd-R; Cd contents in roots; Cd-F = Cd contents in fruits.

4. Discussion

Globally, plant health, growth, and productivity significantly decreased as a result of agricultural soils with elevated hazardous metal levels, such as Cd [38]. The results of this study demonstrate that Cd-induced stress decreased the growth, phenological, and biomass (fresh and dry) associated parameters significantly. Humic acid and selenium treatments improved the plant growth variables under control and stress conditions in a variety of ways (Figure 8).

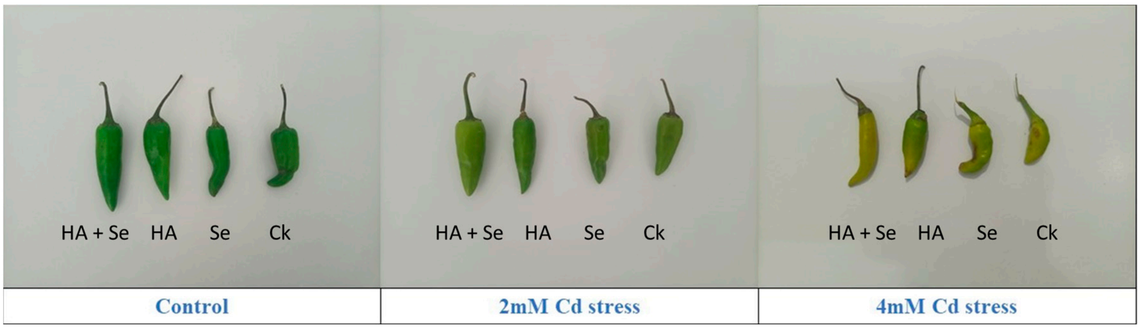

Figure 8. Pictorial view of chili fruit under various treatments of selenium and humic acid grown in cadmium-stressed soil. Ck = Control; Se = Selenium (3 µM, using Na_2SeO_4); HA = Humic acid (250 mg kg^{-1} of soil w/w); Se + HA = Selenium + Humic acid.

The improvement in the growth and biomass attributes might be due to boosting their chlorophyll levels and cell division rates, increase in root volume, distribution of nutrients, release of organic acids, and division [39–41]. The enhancement in the chili plant growth and biomass might be due to the reduction of ROS production and the enhancement of the antioxidant defense mechanism, they promote plant development [42,43]. Humic acid and selenium, however, have a favorable impact on the structural changes imposed by Cd-content-related stress in chilies in terms of maintaining plant integrity [44,45]. By enhancing the environment for the growth of chili plants, humic acid, and selenium addition plays a crucial part in reducing the stress conditions caused by Cd in plants. Combined application of humic acid and selenium, interact chemically to improve the absorption of several important elements in plant tissues [46]. A rise in biomass may result from humic acid and selenium treatment plants might be due to the increased mineral nutrient content [47].

Cadmium stress decreases cellular activity, physiological, and photosynthetic processes due to an imbalance in dietary requirements, metallic toxicity, osmotic, and oxidative stressors, as well as the photosynthetic process [48,49]. This study has demonstrated that the application of humic acid with selenium improves the reduction of abiotic stress (i.e., stress brought on by Cd) by keeping nutritional balance and osmotic stress in chili plants. Humic acid and selenium were added to the photosynthetic process, according to Qianqian et al. [50], which improved the process, which is possibly connected to the biological mechanisms used to reduce stress-related harm to plants. The increase in antioxidant defense capacity is lessened by the humic acid and selenium addition causes oxidative damage to enzymes, which aids in plant photosynthesis [51,52].

The synergistic effect of humic acid and selenium resulted in decreased electrolyte leakage and increased MDA activity in chili tissues. This demonstrates the fact that exogenously applied humic acid and selenium under Cd-stressed conditions likely protect and increase membrane stability in chili plants [52,53]. According to Zulfiqar et al. [54], linolenic acid increases during times of stress causing higher levels of electrolyte leakage in plants, whereas these membrane-associated fatty acids are much less of a concern when humic acid and selenium are present [55]. In this study, electrolyte leakage was found to be greater at increased Cd levels [56,57], whereas humic acid and selenium application considerably mitigated this effect.

Under the Cd stress, all the enzymatic variables increased in the chili plants, a linear increase was noticed with increasing the Cd stress, and similar observations were also noticed in previous studies [58]. The co-application of Se and humic acid led to a noteworthy decrease in enzymatic activities associated with oxidative stress in chili plants. Superoxide dismutase (SOD), an enzyme responsible for scavenging superoxide radicals, exhibited reduced activity under the Se and humic acid treatment [59]. This phenomenon suggests that the synergistic action of Se and humic acid helped regulate ROS accumulation, thereby mitigating the need for excessive SOD activity. This intriguing outcome suggests that the combined treatments helped maintain a balanced ROS level, which in turn lessened the reliance on these enzymatic activities for ROS detoxification [56]. The intricate interplay between Se and humic acid likely facilitated a more efficient antioxidant defense system, leading to reduced oxidative stress and cellular damage in the chili plants [57].

According to our findings, humic acid and selenium treatment under Cd stress caused a considerable decrease in Cd accumulation in chili plant tissues. This proves that Cd^{2+} uptake by chili plant roots and transport into plant leaves were reduced by humic acid and selenium [60,61]. Similar results in maize [62], wheat [63], and rice [7] were also noticed. A decrease in Cd^{2+} absorption and transport as well as improvements in plant resilience may be the causes of the increase in plant resistance to Cd^{2+} [64]. Similar results were also seen in garlic plants, where humic acid and selenium addition reduced Cd^{2+} accumulation in various plant parts. These findings highlight the potential of Se and humic acid to jointly modulate enzymatic activities in chili plants under Cd stress, promoting a more effective and balanced response to oxidative stress. The reduced enzymatic activities, rather than indicating compromised antioxidant defense, suggest an optimized and harmonized ROS-scavenging mechanism. This study sheds light on the multifaceted interactions between these treatments and their ability to fine-tune the plant's stress responses, offering valuable insights into potential strategies for enhancing plant tolerance to heavy metal stress and advancing sustainable agricultural practices.

5. Conclusions

Humic acid and Se-treated plants performed better than control plants under Cd stress in terms of RWC, enzymatic activity, photosynthetic pigment levels, and biomass (dry and fresh). Overall, this study provides compelling evidence that the combined application of Se and humic acid holds a significant strategic tool for alleviating Cd stress in chili plants. Integrating humic acid and Se can offer several benefits, pointing to the amelioration of Cd-induced development, and physiological and osmolytes adjustment of the chili crop. Their synergistic actions in promoting plant growth, limiting Cd uptake, and enhancing antioxidant defense mechanisms collectively contribute to an integrated approach for enhancing plant tolerance to heavy metal stress. Moreover, a reduction in Cd accumulation in the fruits ensures chili quality. These findings are linked to the main sustainable development goals (SDGs) for good health and zero hunger. These observations unveil a promising avenue for advancing research, wherein we can delve deeper into the interactions between plant-organic amendments and essential elements. These findings have implications not only for sustainable agriculture but also for the broader goals of environmental remediation and food safety in regions affected by heavy metal contamination. Additionally, combining humic acid usage with other soil and irrigation management practices can further enhance the resilience of chili crops to metal stress. Prior to making a commercial recommendation, economic factors must be taken into account.

Author Contributions: Conceptualization, Q.u.Z. and K.S.; methodology, M.Z. and K.F.; software, A.-R.Z.G. and K.A.; formal analysis, M.S.H., M.Z. and K.F.; investigation, Q.u.Z.; resources, M.Z. and K.F.; data curation, M.Z.; writing—original draft preparation, M.Z., K.S. and A.-R.Z.G.; writing—review and editing, Q.u.Z., K.A., K.S., M.S.H. and A.-R.Z.G.; supervision, Q.u.Z.; project administration, Q.u.Z.; funding acquisition, M.S.H. and A.-R.Z.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: The authors extend their appreciation to the Deputyship for Research and Innovation, "Ministry of Education" in Saudi Arabia for funding this research (IFKSUOR3-486-2).

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

References

1. Li, C.; Zhou, K.; Qin, W.; Tian, C.; Qi, M.; Yan, X.; Han, W. A review on heavy metals contamination in soil: Effects, sources, and remediation techniques. *Soil Sediment Contam.* **2019**, *28*, 380–394. [CrossRef]
2. Muhammad, S.; Ullah, R.; Jadoon, I.A. Heavy metals contamination in soil and food and their evaluation for risk assessment in the Zhob and Loralai valleys, Baluchistan province, Pakistan. *Microchem. J.* **2019**, *149*, 103971. [CrossRef]
3. Edogbo, B.; Okolocha, E.; Maikai, B.; Aluwong, T.; Uchendu, C. Risk analysis of heavy metal contamination in soil, vegetables and fish around Challawa area in Kano State, Nigeria. *Sci. Afr.* **2020**, *7*, e00281. [CrossRef]
4. Gupta, N.; Yadav, K.K.; Kumar, V.; Krishnan, S.; Kumar, S.; Nejad, Z.D.; Khan, M.A.M.; Alam, J. Evaluating heavy metals contamination in soil and vegetables in the region of North India: Levels, transfer and potential human health risk analysis. *Environ. Toxicol. Pharmacol.* **2021**, *82*, 103563. [CrossRef] [PubMed]
5. Coskun, D.; Deshmukh, R.; Sonah, H.; Menzies, J.G.; Reynolds, O.; Ma, J.F.; Kronzucker, H.J.; Bélanger, R.R. The controversies of silicon's role in plant biology. *New Phytol.* **2019**, *221*, 67–85. [CrossRef] [PubMed]
6. Thind, S.; Hussain, I.; Rasheed, R.; Ashraf, M.A.; Perveen, A.; Ditta, A.; Hussain, S.; Khalil, N.; Ullah, Z.; Mahmood, Q. Alleviation of cadmium stress by silicon nanoparticles during different phenological stages of Ujala wheat variety. *Arabian J. Geosci.* **2021**, *14*, 1028. [CrossRef]
7. Zaman, Q.; Rashid, M.; Nawaz, R.; Hussain, A.; Ashraf, K.; Latif, M.; Heile, A.O.; Mehmood, F.; Salahuddin, S.; Chen, Y. Silicon fertilization a step towards cadmium free fragrant rice. *Plants* **2021**, *10*, 2440. [CrossRef]
8. Dawid, M.; Grzegorz, K. Microwave-assisted hydrotropic pretreatment as a new and highly efficient way to cellulosic ethanol production from maize distillery stillage. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 3381–3392. [CrossRef]
9. Ngugi, M.M.; Gitari, H.I.; Muii, C.; Gweyi-Onyango, J.P. Cadmium mobility, uptake, and accumulation in spinach, kale, and amaranths vegetables as influenced by silicon fertilization. *Bioremediat. J.* **2022**, *26*, 113–127. [CrossRef]
10. Xu, L.; Zhang, F.; Tang, M.; Wang, Y.; Dong, J.; Ying, J.; Chen, Y.; Hu, B.; Li, C.; Liu, L. Melatonin confers cadmium tolerance by modulating critical heavy metal chelators and transporters in radish plants. *J. Pineal Res.* **2020**, *69*, e12659. [CrossRef]
11. Rehman, S.; Abbas, G.; Shahid, M.; Saqib, M.; Farooq, A.B.U.; Hussain, M.; Murtaza, B.; Amjad, M.; Naeem, M.A.; Farooq, A. Effect of salinity on cadmium tolerance, ionic homeostasis and oxidative stress responses in conocarpus exposed to cadmium stress: Implications for phytoremediation. *Ecotoxicol. Environ. Safety* **2019**, *171*, 146–153. [CrossRef] [PubMed]
12. Zhao, F.J.; Wang, P. Arsenic and cadmium accumulation in rice and mitigation strategies. *Plant Soil* **2020**, *446*, 1–21. [CrossRef]
13. Izhar Shafi, M.; Adnan, M.; Fahad, S.; Wahid, F.; Khan, A.; Yue, Z.; Danish, S.; Zafar-ul-Hye, M.; Brtnicky, M.; Datta, R. Application of single superphosphate with humic acid improves the growth, yield and phosphorus uptake of wheat (*Triticum aestivum* L.) in calcareous soil. *Agronomy* **2020**, *10*, 1224. [CrossRef]
14. Al-Fraihat, A.H.; Al-Tabbal, J.A.; Abu-Darwish, M.S.; Alhrout, H.H.; Hasan, H.S. Response of onion (Allium cepa) crop to foliar application of humic acid under rain-fed conditions. *Int. J. Agri. Biol.* **2018**, *20*, 1235–1241.
15. Al-Shareef, A.R.; El-Nakhlawy, F.S.; Ismail, S.M. Enhanced mungbean and water productivity under full irrigation and stress using humic acid in arid regions. *Legume Res. An Int. J.* **2018**, *41*, 428–431. [CrossRef]
16. Ali, I.; Khan, A.A.; Khan, A.; Asim, M.; Ali, I.; Zib, B.; Khan, I.; Rab, A.; Sadiq, G.; Ahmad, N.; et al. Humic acid and nitrogen levels optimizing productivity of green gram (*Vigna radiate* L.). *Russ. Agric. Sci.* **2019**, *45*, 43–47.
17. Zaremanesh, H.; Akbari, N.; Eisvand, H.R.; Ismaili, A.; Feizian, M. The Effect of humic acid on soil physicochemical and biological properties under salinity stress conditions in pot culture of Satureja khuzistanica Jamzad. *Ecopersia* **2020**, *8*, 147–154.
18. Skowrońska, M.; Bielińska, E.J.; Szymański, K.; Futa, B.; Antonkiewicz, J.; Kołodziej, B. An integrated assessment of the long-term impact of municipal sewage sludge on the chemical and biological properties of soil. *Catena* **2020**, *189*, 104484. [CrossRef]
19. Yin, H.; Qi, Z.; Li, M.; Ahammed, G.J.; Chu, X.; Zhou, J. Selenium forms and methods of application differentially modulate plant growth, photosynthesis, stress tolerance, selenium content and speciation in *Oryza sativa* L. *Ecotoxicol. Environ. Safety.* **2019**, *169*, 911–917. [CrossRef]
20. Mimmo, T.; Tiziani, R.; Valentinuzzi, F.; Lucini, L.; Nicoletto, C.; Sambo, P.; Scampicchio, M.; Pii, Y.; Cesco, S. Selenium biofortification in Fragaria × ananassa: Implications on strawberry fruits quality, content of bioactive health beneficial compounds and metabolomic profile. *Front. Plant Sci.* **2017**, *8*, 1887. [CrossRef]
21. De Feudis, M.; D'Amato, R.; Businelli, D.; Guiducci, M. Fate of selenium in soil: A case study in a maize (*Zea mays* L.) field under two irrigation regimes and fertilized with sodium selenite. *Sci. Total Environ.* **2019**, *659*, 131–139. [CrossRef] [PubMed]
22. Preciado-Rangel, P.; Hernández-Montiel, L.G.; Valdez-Cepeda, R.D.; Cruz-Lázaro, E.D.L.; Lara-Capistrán, L.; Morales-Morales, B.; Gaucin-Delgado, J.M. Biofortification with selenium increases bioactive compounds and antioxidant capacity in tomato fruits. *Terra Latinoam.* **2021**, *39*, 1–10. [CrossRef]

23. Leite, J.M.; Arachchige, P.S.P.; Ciampitti, I.A.; Hettiarachchi, G.M.; Maurmann, L.; Trivelin, P.C.; Prasad, P.V.; Sunoj, S.J. Co-addition of humic substances and humic acids with urea enhances foliar nitrogen use efficiency in sugarcane (*Saccharum officinarum* L.). *Heliyon* **2020**, *6*, e05100. [CrossRef] [PubMed]
24. López, E.A.T.; Sandoval-Rangel, A.; Mendoza, A.B.; Ortiz, H.O.; Pliego, G.C.; de la Fuente, M.C. Nanopartículas de selenio absorbidas en hidrogeles de quitosán-polivinil alcohol en la producción de pepino injertado. *Rev. Mexicana Cienc. Agric.* **2021**, 159–169. [CrossRef]
25. Mozafariyan, M.; Shekari, L.; Hawrylak-Nowak, B.; Kamelmanesh, M.M. Protective role of selenium on pepper exposed to cadmium stress during reproductive stage. *Biol. Trace Elem. Res.* **2014**, *160*, 97–107. [CrossRef]
26. Aminifard, M.H.; Aroiee, H.; Azizi, M.; Nemati, H.; Jaafar, H.Z. Effect of humic acid on antioxidant activities and fruit quality of hot pepper (*Capsicum annuum* L.). *J. Herbs Spices Medi. Plants* **2012**, *18*, 360–369. [CrossRef]
27. Khosa, Q.; Zaman, Q.; An, T.; Ashraf, K.; Abbasi, A.; Nazir, S.; Chen, Y. Silicon-mediated improvement of biomass yield and physio-biochemical attributes in heat-stressed spinach (*Spinacia oleracea*). *Crop Pasture Sci.* **2022**, *74*, 230–243. [CrossRef]
28. Naz, T.; Mazhar Iqbal, M.; Tahir, M.; Hassan, M.M.; Rehmani, M.I.A.; Zafar, M.I.; Ghafoor, U.; Qazi, M.A.; Sabagh, A.E.; Sakran, M.I. Foliar application of potassium mitigates salinity stress conditions in spinach (*Spinacia oleracea* L.) through reducing NaCl toxicity and enhancing the activity of antioxidant enzymes. *Horticulturae* **2021**, *7*, 566. [CrossRef]
29. Rico-Garcia, E.; Hernandez-Hernandez, F.; Soto-Zarazua, G.M.; Herrera-Ruiz, G. Two new methods for the estimation of leaf area using digital photography. *Int. J. Agric. Biol.* **2009**, *11*, 97–400.
30. Lichtenthaler, H.K. Chlorophylls and carotenoids pigments of photosynthetic bio membranes in Methods in Enzymology. *Plants* **1987**, *148*, 183–350.
31. Lutts, S.; Guerrier, G. Peroxidase activities of two rice cultivars differing in salinity tolerance as affected by proline and NaCl. *Biol. Plant* **1995**, *37*, 577–586. [CrossRef]
32. Velikova, V.; Yordanov, I.; Edreva, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci.* **2000**, *151*, 59–66. [CrossRef]
33. Aebi, H. Catalase. In *Methods of Enzymatic Analysis*; Academic Press: Cambridge, MA, USA, 1974; pp. 673–684.
34. Beauchamp, C.; Fridovich, I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analyt. Biochem.* **1971**, *44*, 276–287. [CrossRef]
35. Heath, R.L.; Packer, L. Photoperoxidation in Isolated Chloroplasts. I. Kinetics and Stoichiometry of Fatty Acid Peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [CrossRef] [PubMed]
36. Ali, M.B.; Hahn, E.J.; Paek, K.Y. Effects of Light Intensities on Antioxidant Enzymes and Malondialdehyde Content during Short-Term Acclimatization on Micropropagated *Phalaenopsis Plantlet*. *Environ. Exp. Bot.* **2005**, *54*, 109–120. [CrossRef]
37. Salama, F.M.; Al-Huqail, A.A.; Ali, M.; Abeed, A.H. Cd Phytoextraction potential in halophyte *Salicornia fruticosa*: Salinity impact. *Plants* **2022**, *11*, 2556. [CrossRef] [PubMed]
38. Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R.; Wang, M.Q. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics* **2021**, *9*, 42. [CrossRef]
39. El-Gohary, A.E.; Amer, H.M.; Salem, S.H.; Hussein, M.S. Foliar application of selenium and humic acid changes yield, essential oil, and chemical composition of *Plectranthus amboinicus* (Lour.) plant and its antimicrobial effects. *Egypt. Pharm. J.* **2019**, *18*, 356.
40. Newman, R.; Waterland, N.; Moon, Y.; Tou, J.C. Selenium biofortification of agricultural crops and effects on plant nutrients and bioactive compounds important for human health and disease prevention–a review. *Plant Foods Hum. Nutr.* **2019**, *74*, 449–460. [CrossRef]
41. Ampong, K.; Thilakaranthna, M.S.; Gorim, L.Y. Understanding the role of humic acids on crop performance and soil health. *Front. Agron.* **2022**, *4*, 848621. [CrossRef]
42. Samavatipour, P.; Abdossi, V.; Salehi, R.; Samavat, S.; Moghadam, A.L. Investigation of morphological, phytochemical, and enzymatic characteristics of Anethum graveolens L. using selenium in combination with humic acid and fulvic acid. *J. Appl. Biol. Biotechnol.* **2019**, *7*, 69–74.
43. Amerian, M.; Khoramivafa, M.; Rabani, B.A. Effect of selenium and humic acid on germination and some morphological characteristics of quinoa under drought and salinity stress. *J. Vegetables Sci.* **2023**, *6*, 1–16.
44. Gaucin-Delgado, J.M.; Preciado-Rangel, P.; González-Salas, U.; Sifuentes-Ibarra, E.; Núñez-Ramírez, F.; Vidal, J.A.O. Biofortification with selenium improves bioactive compounds and antioxidant activity in jalapeño pepper. *Rev. Mexicana Cienc. Agric.* **2021**, *12*, 1339–1349.
45. Ichwan, B.; Eliyanti, E.; Irianto, I.; Zulkarnain, Z. Combining humic acid with NPK fertilizer improved growth and yield of chili pepper in dry season. *Adv. Hortic. Sci.* **2022**, *36*, 275–281. [CrossRef]
46. Elkhatib, H.; Gabr, S.M.; Elazomy, A.A. Salt stress relief and growth-promoting effect of sweet pepper plants (*Capsicum annuum* L.) by glutathione, selenium, and humic acid application. *Alex. Sci. Exch. J.* **2021**, *42*, 583–608. [CrossRef]
47. Qassem, M.E.; Bardisi, A.; Nawar, D.; Ibraheem, S.K. Effect of some stimulants as foliar application on growth, yield and fruit quality of cucumber under plastic house conditions. *Zagazig. J. Agric. Res.* **2022**, *49*, 9–22. [CrossRef]
48. Dogan, M.; Bolat, I.; Karakas, S.; Dikilitas, M.; Gutiérrez-Gamboa, G.; Kaya, O. Remediation of Cadmium Stress in Strawberry Plants Using Humic Acid and Silicon Applications. *Life* **2022**, *12*, 1962. [CrossRef]
49. Ouyang, X.; Ma, J.; Liu, Y.; Li, P.; Wei, R.; Chen, Q.; Weng, L.; Chen, Y.; Li, Y. Foliar cadmium uptake, transfer, and redistribution in Chili: A comparison of foliar and root uptake, metabolomic, and contribution. *J. Hazard. Mater.* **2023**, *453*, 131421. [CrossRef]

50. Qianqian, M.; Haider, F.U.; Farooq, M.; Adeel, M.; Shakoor, N.; Jun, W.; Cai, L. Selenium treated Foliage and biochar treated soil for improved lettuce (*Lactuca sativa* L.) growth in Cd-polluted soil. *J. Clean. Prod.* **2022**, *335*, 130267. [CrossRef]
51. Huang, W.; Liu, Z.; Zhang, Y.; Zhu, Y.; Wang, D. Effects of soil nano-conditioning agent on selenium content, yield and quality of pepper. *Integr. Ferroelectr.* **2021**, *216*, 94–107. [CrossRef]
52. Zhang, Y.; Liu, G.; Gao, S.; Zhang, Z.; Huang, L. Effect of humic acid on phytoremediation of heavy metal contaminated sediment. *J. Hazard. Mater.* **2023**, *9*, 100235. [CrossRef]
53. Garza-García, J.J.; Hernández-Díaz, J.A.; Zamudio-Ojeda, A.; León-Morales, J.M.; Guerrero-Guzmán, A.; Sánchez-Chiprés, D.R.; López-Velázquez, J.C.; García-Morales, S. The role of selenium nanoparticles in agriculture and food technology. *Bio. Trace Elem. Res.* **2021**, *200*, 2528–2548. [CrossRef]
54. Zulfiqar, U.; Jiang, W.; Xiukang, W.; Hussain, S.; Ahmad, M.; Maqsood, M.F.; Ali, N.; Ishfaq, M.; Kaleem, M.; Haider, F.U.; et al. Cadmium phytotoxicity, tolerance, and advanced remediation approaches in agricultural soils; a comprehensive review. *Front. Plant Sci.* **2022**, *13*, 773815. [CrossRef]
55. Zhang, H.; Xie, S.; Bao, Z.; Carranza, E.J.M.; Tian, H.; Wei, C. Synergistic inhibitory effect of selenium, iron, and humic acid on cadmium uptake in rice (*Oryza sativa* L.) seedlings in hydroponic culture. *Environ. Sci. Pollut. Res.* **2021**, *28*, 64652–64665. [CrossRef]
56. Rehman, S.; Chattha, M.U.; Khan, I.; Mahmood, A.; Hassan, M.U.; Al-Huqail, A.A.; Salem, M.Z.M.; Ali, H.M.; Hano, C.; El-Esawi, M.A. Exogenously applied trehalose augments cadmium stress tolerance and yield of mung bean (*Vigna radiata* L.) grown in soil and hydroponic systems through reducing cd uptake and enhancing photosynthetic efficiency and antioxidant defense systems. *Plants* **2022**, *11*, 822. [CrossRef]
57. Hassan, M.J.; Raza, M.A.; Ur Rehman, S.; Ansar, M.; Gitari, H.; Khan, I.; Wajid, M.; Ahmed, M.; Shah, G.A.; Li, Z. Effect of cadmium toxicity on growth, oxidative damage, antioxidant defense system and cadmium accumulation in two sorghum cultivars. *Plants* **2020**, *9*, 1575.
58. Batool, T.; Javied, S.; Ashraf, K.; Sultan, K.; Zaman, Q.U.; Haider, F.U. Alleviation of cadmium stress by silicon supplementation in peas by the modulation of morpho-physio-biochemical variables and health risk assessment. *Life* **2022**, *12*, 1479. [CrossRef]
59. Zhu, Y.; Wang, H.; Lv, X.; Zhang, Y.; Wang, W. Effects of biochar and biofertilizer on cadmium-contaminated cotton growth and the antioxidative defense system. *Sci. Rep.* **2020**, *10*, 20112. [CrossRef]
60. Ismael, M.A.; Elyamine, A.M.; Moussa, M.G.; Cai, M.; Zhao, X.; Hu, C. Cadmium in plants: Uptake, toxicity, and its interactions with selenium fertilizers. *Metallomics* **2019**, *11*, 255–277. [CrossRef]
61. Qu, C.; Chen, J.; Mortimer, M.; Wu, Y.; Cai, P.; Huang, Q. Humic acids restrict the transformation and the stabilization of Cd by iron (hydr) oxides. *J. Hazard. Mater.* **2022**, *430*, 128365. [CrossRef]
62. An, T.; Gao, Y.; Kuang, Q.; Wu, Y.; Zaman, Q.U.; Zhang, Y.; Xu, B.; Chen, Y. Effect of silicon on morpho-physiological attributes, yield and cadmium accumulation in two maize genotypes with contrasting root system size and health risk assessment. *Plant Soil* **2022**, *477*, 117–134. [CrossRef]
63. Heile, A.O.; Aslam, Z.; Hussain, A.; Aslam, M.; Saleem, M.H.; Abualreesh, M.H.; Alataawi, A.; Ali, S. Alleviation of cadmium phytotoxicity using silicon fertilization in wheat by altering antioxidant metabolism and osmotic adjustment. *Sustainability* **2021**, *13*, 11317. [CrossRef]
64. Rizwan, M.; Ali, S.; Rehman, M.Z.U.; Maqbool, A. A critical review on the effects of zinc at toxic levels of cadmium in plants. *Environ. Sci. Pollut. Res.* **2019**, *26*, 6279–6289. [CrossRef]