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Recycling Agricultural Waste into Plant Protectants: Mechanisms of Wood Vinegar in Alleviating Salt Stress in *Triticum aestivum* L.

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Abstract

Soil salinity severely impairs crop productivity by inducing osmotic stress, ionic toxicity, and oxidative damage. This study investigated the mechanisms by which foliar-applied wood vinegar (WV), a biomass pyrolysis byproduct rich in organic acids and minerals, alleviates salt stress (100 mM NaCl) in hydroponically grown wheat (*Triticum aestivum* L.). Three WV dilutions (100×, 300×, 500×) were tested to evaluate their effects on growth, antioxidant systems, chlorophyll metabolism, and ion homeostasis. The results demonstrated that 300×-diluted WV (WV3) most effectively mitigated salt stress, increasing shoot biomass by 81% and root length by 75% compared to salt-stressed controls. WV3 restored antioxidant enzyme activities to non-stressed levels, reduced lipid peroxidation, and normalized chlorophyll overaccumulation induced by salinity. Elemental profiling revealed that WV3 enhanced shoot K^+ and Ca^{2+} uptake while reducing Na^+ accumulation, thereby improving ion homeostasis. Additionally, WV3 promoted Fe translocation to shoots, supporting chlorophyll synthesis. However, 100× WV (WV1) exhibited phytotoxicity due to excessive organic acids, while 500× (WV5) showed limited efficacy. These findings highlight a 300-fold diluted solution of WV as an optimal dilution for enhancing wheat salt tolerance through coordinated ROS scavenging, photosynthetic protection, and ion regulation. This study provides a scientific basis for integrating WV into sustainable strategies to combat salinity in wheat cultivation.

Keywords: salt stress; wood vinegar; wheat (*Triticum aestivum* L.); antioxidant enzymes; ion homeostasis; foliar application

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1. Introduction

Soil salinity is a global agricultural challenge; the global area of saline–alkali soil amounts to 1.381 billion hectares, accounting for 10.7% of the total global land area. Additionally, approximately 10% of irrigated farmland and 10% of rain-fed farmland are affected by salinization (FAO, www.fao.org, accessed on 5 May 2025). Excessive sodium chloride (NaCl) in soils imposes osmotic stress, ionic toxicity, and nutritional imbalance on plants, leading to reduced photosynthesis, oxidative damage, and stunted growth, which severely threaten crop productivity [1–3].

Wheat (*Triticum aestivum* L.), as the staple food crop that sustains billions of people worldwide, plays a crucial role in maintaining global food security and social stability [4,5]. However, soil salinity has emerged as a major threat to wheat, severely impacting growth,

yield, and quality, particularly during the highly sensitive seedling stage [6], which is critical for establishing subsequent development and final yield [7]. During this rapid-growth phase, characterized by active cell division and high environmental sensitivity, excess soluble salts like NaCl impose multiple stresses on seedlings. High NaCl concentrations cause osmotic stress by increasing soil osmotic pressure, restricting water uptake and inhibiting root growth, thereby limiting nutrient acquisition [8–12]. Furthermore, they cause ion toxicity, particularly from Na^+ interfering with essential K^+ uptake and Cl^- accumulation damaging cellular structures; both disrupt physiological functions and significantly impair photosynthesis, leading to leaf chlorosis, stunted growth, and potentially plant death [13–15]. Salt stress also disrupts ion homeostasis, leading to cellular Na^+ accumulation and K^+/Ca^{2+} depletion, compromising membrane stability and enzyme activity [16–18]. Concurrently, excess reactive oxygen species (ROS) accumulation causes oxidative damage to lipids, proteins, and DNA [19,20]. While plants activate antioxidant defenses to counter ROS, key components like photosynthetic pigments are often compromised, further reducing photosynthetic efficiency under salt stress [21,22].

Plant growth-promoting substances, such as biochar, humic acids, and plant extracts, have gained attention for their potential to alleviate abiotic stresses [23–25]. Wood vinegar (WV, also known as wood distillate under EU regulatory frameworks such as Regulation (EU) 2017/419), a byproduct of biomass pyrolysis typically produced from agricultural and forestry wastes such as crop straw, branches, and fruit tree residues, is a complex aqueous solution containing organic acids (e.g., acetic acid, formic acid), phenolic compounds, and mineral elements [26–28]. Previous studies have demonstrated that WV can enhance plant growth, improve stress tolerance, and regulate soil microbial communities [29,30]. The organic acids in WV, such as acetic acid and phenolic compounds, may act as antioxidants to scavenge reactive oxygen species (ROS) and modulate ion transport, while mineral elements like potassium (K^+) and calcium (Ca^{2+}) can restore ionic homeostasis by competing with sodium (Na^+) uptake [31]. WV's multifunctional components suggest that it may address multiple aspects of salt stress. For example, acetic acid in WV can chelate Na^+ ions, reducing their availability for uptake, while phenolic compounds may enhance the expression of antioxidant enzymes [32–34]. Mineral elements in WV, such as Fe, K, and Ca, could supplement nutrient requirements and promote osmoregulation [35]. Treatment with an appropriate concentration of WV enhances photosynthetic efficiency by increasing chlorophyll content in plant leaves, thereby improving light energy capture and conversion [36–38]. Additionally, components in WV regulate stomatal conductance and elevate the intercellular CO_2 concentration, providing more substrates for photosynthetic carbon assimilation [39,40]. However, the efficacy of WV is highly concentration-dependent; low dilutions may exert phytotoxic effects due to high organic acid content, while excessive dilution might reduce bioactive component availability [41,42]. Previous studies on WV and salt stress have primarily focused on soil application or limited physiological parameters. However, in this study, foliar spraying and hydroponic systems were selected for several potential advantages. Foliar application allows for a more direct and rapid uptake of active components by the plant, bypassing the soil or root system limitations; for example, the organic acids and trace elements in the wood vinegar can be rapidly absorbed through the leaf surface, and the phenolic substances regulate the opening and closing of stomata [42,43]. In hydroponic systems where roots are directly exposed to stress, foliar application provides timely protection and supplementation to the shoot, thereby enhancing the plant's overall stress resistance. Compared to soil-based cultivation, hydroponics offers a more direct assessment of WV 's effects on the plant itself (rather than on the soil), since substantial evidence already demonstrates WV's efficacy in ameliorating saline–alkali soils.

This study aims to investigate the physiological and biochemical mechanisms by which foliar-applied WV alleviates salt stress in hydroponically grown wheat seedlings. Specifically, we hypothesized that WV modulates antioxidant enzyme activities, chlorophyll metabolism, and mineral element uptake to mitigate salt-induced damage. Based on preliminary studies by our research group and previous work by Afsharipour et al. [35,42,44] on WV-enhanced salt tolerance, we selected a dilution range of 100× to 500× for assessment. Specifically, three WV dilutions (100×, 300×, 500×) were tested under 100 mM NaCl stress, with evaluations encompassing growth parameters, chlorophyll content, lipid peroxidation, antioxidant enzyme activities, total protein content, and the elemental composition in shoots and roots. By identifying the optimal WV concentration and underlying mechanisms, this research seeks to provide a scientific basis for integrating WV into salt-stress management strategies in wheat cultivation.

2. Materials and Methods

2.1. Characterization of Wood Vinegar

The WV used in this study, sourced from the raw solution of Tangshan Jinhai New Materials Co., Ltd. (Tangshan, China), complies with the Chinese National Standard for wood vinegar (T/CNFPIA 3024-2022 [45]). The concentrations of elements in the WV were determined using ICP-MS (DRCII, PerkinElmer and Norwalk, Waltham, MA, USA). The composition and content of the WV were determined by using a gas chromatography–mass spectrometry instrument (GC-MS 6800, Skyray Instrument, Kunshan, China). The composition and element contents of the WV solution are shown in Table 1. Functionally, acetic acid may enhance osmotic adjustment and ion chelation under stress; phenols typically act as antioxidants and signaling modulators; Fe is essential for chlorophyll synthesis and redox catalysis [46–49]. These components collectively contribute to WV's bioactivity, though their individual roles require further isolation and validation.

Table 1. The composition and element contents of the wood vinegar solution.

Component	Component Content	Element	Concentration (mg/L)
Water	89.0–91.0%	Fe	205.19
Formic Acid	0.1–0.2%	Si	63.387
Acetic Acid	5.5–6.5%	K	40.085
Succinic Acid	0.05–0.07%	Ca	8.8788
Propionic Acid	0.8–1.0%	Na	23.609
Methanol	1.0–2.0%	Mg	3.637
Acetone	0.0156%	Cr	1.9426
Methyl Acetate	0.0954%	Mn	1.8255
Methyl Propionate	0.0225%	Zn	1.6998
1-Hydroxy-2-Butanone	0.0200%	Ni	0.9202
Phenol	0.0506%	Cu	0.4978

2.2. Experimental Design

A hydroponic experiment was conducted to evaluate the alleviating effects of WV on salt stress in wheat seedlings (*Triticum aestivum* L., var. 'AiKang58', obtained from TaoBao). Prior to the experiment, seeds underwent the following pre-treatment procedures:

- The seeds were surface-sterilized by soaking them in 10% (v/v) H_2O_2 for 10 min, followed by thorough rinsing with deionized water.
- The seeds were placed in a constant-temperature and -humidity incubator and kept in darkness at a temperature of 25 ± 1 °C for 2 days to germinate.

- The seedlings were placed in the seedling trays and allowed to grow for 5 days (25 ± 1 °C, 18 h light, 8 h darkness).

Wheat seedlings with similar growth conditions (the plant height and weight were similar) were selected for the hydroponic experiment. The experiment was conducted in a constant-temperature and -humidity incubator (25 ± 1 °C, 18 h light, 8 h darkness). The experiment comprised five treatments with four replicate containers per treatment, each container holding five seedlings, resulting in 20 plants per treatment group. The groups were designed as follows: CK: Control (no NaCl, no WV). CK2: Salt-stressed control (100 mM NaCl, no WV). WV1, WV3, and WV5: Salt-stressed seedlings (100 mM NaCl) treated with WV diluted 100-, 300-, and 500-fold, respectively. The pH and electrical conductivity (EC) values of the diluted wood vinegar are presented in Table 2. A nutrient solution prepared according to Table 3 was used. Initially, the seedlings were grown in a half-strength nutrient solution for 7 days, and then, they were grown in the full-concentration solution until harvest (a total of 20 days). The nutrient solution was changed every 4 days. To prevent osmotic shock, salinity stress was gradually induced in the nutrient solution through incremental additions of NaCl: Starting from Day 7, we increased the NaCl concentrations in the nutrient solution by 25 mM daily until they reached 100 mM. WV was applied via foliar spraying at specified dilutions (100×, 300×, 500×) on days 7 and 14 of the salt stress treatment until the leaves were fully wetted. WV was applied via foliar spraying at specified dilutions (100×, 300×, 500×) until the leaves were fully wetted on day 7. After harvesting, the fresh weight of plant shoots and roots, along with their lengths, were determined immediately. Shoot and root tissues (0.2 g) were excised from plants, immediately flash-frozen in liquid nitrogen, pulverized, and suspended in 1.8 mL of phosphate-buffered saline (PBS) to prepare tissue homogenates for subsequent analyses.

Table 2. The character of Wood Vinegar.

	Raw Solution	**100×**	**300×**	**500×**
pH	2.83	3.05	3.23	3.33
EC (mS/cm)	3.30	0.20	0.13	0.08

Table 3. The composition of the nutrient solution.

Chemical	**Concentration**	**Chemical**	**Concentration**
$Ca(NO_3)_2 \cdot 4H_2O$	5 mmol L^{-1}	$MnSO_4 \cdot H_2O$	10 μmol L^{-1}
KNO_3	5 mmol L^{-1}	$ZnSO_4 \cdot 7H_2O$	1 μmol L^{-1}
KH_2PO_4	1 mmol L^{-1}	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.05 μmol L^{-1}
$MgSO_4 \cdot 7H_2O$	2 mmol L^{-1}	$CuSO_4 \cdot 5H_2O$	0.95 μmol L^{-1}
H_3BO_3	29.6 μmol L^{-1}	Fe (III)-EDTA	50 μmol L^{-1}

2.3. Chlorophyll Content

The chlorophyll content was quantified using a commercial assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Briefly, 0.1 g of powdered plant tissue was homogenized in 1 mL of distilled water and 50 mg of extractant under dark conditions. The homogenate was thoroughly mixed with acetone and incubated in the dark for 3 h (until the tissue residue at the bottom of the tube turned completely white). The absorbance of the extract was measured at 645 nm and 663 nm using a microplate reader (EPOCH-SN, BioTek, Winooski, VT, USA) [50].

2.4. Lipid Peroxidation Analysis and Antioxidant Enzyme Activities

Following centrifugation of the homogenates at 8000× *g* for 10 min at 4 °C, the supernatants were assayed to determine the malondialdehyde (MDA) levels and the enzymatic activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) [51,52].

The SOD activity was determined using a xanthine/xanthine oxidase system (kit protocol), with the absorbance recorded at 560 nm to quantify nitroblue tetrazolium (NBT) reduction [53].

The POD activity was assayed by monitoring the oxidation of guaiacol to tetraguaiacol at 470 nm. Absorbance measurements were taken at 0 and 10 min to calculate reaction rates [54].

The CAT activity was measured based on the decomposition of H_2O_2, where residual peroxide formed a yellow complex with ammonium molybdate. The absorbance at 405 nm was inversely correlated with enzyme activity [55].

The MDA content was determined via the thiobarbituric acid (TBA) reaction. The absorbance at 532 nm (corrected at 600 nm for background interference) was used to quantify the MDA concentration, calculated using a molar extinction coefficient [56].

2.5. Total Protein Content

The total protein content was quantified using the Coomassie Brilliant Blue (CBB) G-250 method with rigorous quality controls. Homogenates were centrifuged (8000× *g*, 10 min, 4 °C), and the supernatants were mixed with CBB reagent for 10 min incubation at 25 ± 1 °C. The absorbance was measured at 595 nm (EPOCH-SN microplate reader, BioTek, Winooski, VT, USA). Technical precision was confirmed by a <3% inter-assay RSD (n = 6 replicates per plate), while accuracy (95–102% recovery) was validated through spiked BSA samples. A BSA standard curve (0–1.0 mg/mL, $R^2 > 0.998$) enabled concentration calculation [57].

2.6. Elemental Composition Analysis

The elemental composition of the wheat shoot and root was analyzed following the protocol of Zhou [58]. Dried plant samples were subjected to a two-stage dehydration process: initial drying at 105 °C for 30 min, followed by 75 °C until they reached a constant weight. Approximately 0.2 g of the dried sample was transferred to a digestion tube containing 8 mL of high-purity concentrated HNO_3, and dark-incubated overnight with a vented stopper. Microwave-assisted digestion (MARS6, CEM, Matthews, NC, USA) was then performed in three phases: Phase 1: 120 °C for 30 min; Phase 2: 140 °C for 3 h; Phase 3: 170 °C until acid evaporation reduced the volume to 1 mL. The digestate was diluted to 50 mL with ultrapure water, filtered through a 0.25 µm PTFE membrane, and further diluted prior to analysis. The elemental concentrations were quantified using inductively coupled plasma mass spectrometry (ICP-MS; Elan DRC-e, Perkin Elmer, Waltham, MA, USA).

2.7. Statistical Analysis

Values represent mean ± SD (n = 4). Differences among groups were analyzed using one-way ANOVA (SPSS 27.0), followed by Tukey's test. Asterisks (*) indicate statistically significant differences compared to the control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3. Results and Discussion

3.1. Growth Modulation

To evaluate the alleviating effects of WV on salt stress in wheat, this study systematically analyzed morphological and biomass variations in both shoot and root tissues under different WV dilution treatments. The WV5 and WV3 treatments significantly alleviated the salt stress-induced inhibition of shoot growth in wheat seedlings (Figure 1A,C), with the WV3 group showing the most pronounced effects. Compared to the salt-stressed control (CK2), the shoot length increased by 18% and 35% in the WV5 and WV3, showing milder improvements, and the shoot biomass rose by 52% and 81% in the WV5 and WV3, respectively. However, both values remained lower than those in the unstressed control (CK), indicating partial but incomplete recovery. Notably, the WV3 treatment also mitigated root growth suppression under salt stress (Figure 1B,D). Relative to CK2, the root length and biomass in the WV3 group increased by 75% and 51%. Despite these improvements, the root parameters in WV3 were extremely significantly lower than the CK values, suggesting persistent salt-induced damage to the root architecture. Foliar application of WV significantly alleviated the salt stress-induced inhibition of shoot growth, but exhibited limited efficacy in restoring root morphology and biomass. This disparity suggests that foliar spraying preferentially protects aerial tissues, while roots remain vulnerable to direct ion toxicity and osmotic stress in the rhizosphere.

WV1, contrary to expectations, failed to alleviate salt stress. The shoot and root biomass in this group showed no significant difference from CK2, likely due to phytotoxicity from excessive organic acids or phenolic compounds in undiluted WV. Only moderate dilutions (e.g., WV3) balance bioactive molecule efficacy and toxicity.

3.2. Impact of Wood Vinegar on Chlorophyll Content

The salt-stressed group (CK2) exhibited significantly higher chlorophyll a and b contents compared to the plants subjected to other treatments (Figure 2), likely due to the activation of protective mechanisms in plants to sustain photosynthetic efficiency under stress. Such mechanisms may involve the transient enhancement of chlorophyll biosynthesis or delayed degradation as an adaptive strategy to counteract salinity-induced damage [59–61].

In contrast, WV treatments significantly reduced the chlorophyll content relative to CK2 (salt-stressed control). This reduction indicates that foliar-applied WV mitigates the stress-induced dysregulation of chlorophyll metabolism, potentially through phenolic compounds and organic acids that stabilize photosynthetic complexes and attenuate oxidative damage. Among WV treatments, the 300× dilution (WV3) demonstrated optimal efficacy—effectively restoring chlorophyll homeostasis while maximizing shoot biomass. This concentration balances stress alleviation with minimal phytotoxicity, avoiding the growth inhibition observed at higher WV doses.

3.3. Impact of Wood Vinegar on Antioxidant System

Salt stress disrupts cellular ion homeostasis, leading to reactive oxygen species (ROS) accumulation. Excess ROS cause lipid peroxidation, protein denaturation, and membrane damage, reflected in increased MDA content [62,63]. To counteract this, plants activate their antioxidant system, including POD, SOD, and CAT, which scavenge ROS and alleviate oxidative damage [64–66].

The application of WV at varying dilutions (WV1-100×, WV3-300×, WV5-500×) exhibited distinct regulatory effects on antioxidant enzyme systems and membrane lipid peroxidation in salt-stressed wheat seedlings (Figure 3). Compared with the non-stressed control (CK), salt stress (CK2) significantly induced oxidative damage as evidenced by extremely

significantly elevated MDA levels (Figure 3D,H) in both shoots and roots, accompanied by the differential modulation of antioxidant enzymes: the SOD and POD activities in the shoots significantly increased, but the SOD activity in the roots significantly decreased.

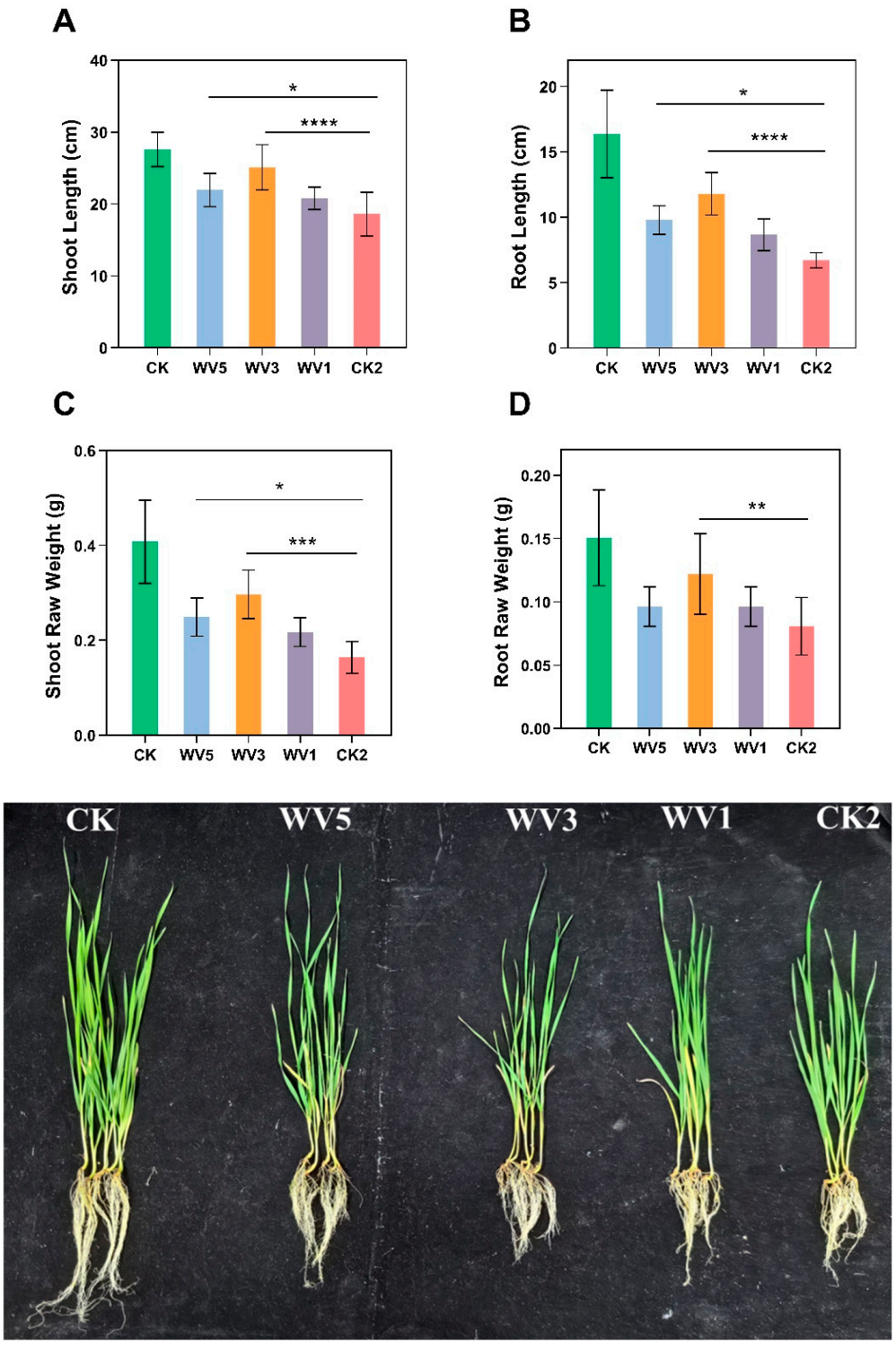

Figure 1. Wheat shoot length (**A**). Wheat root length (**B**). Wheat shoot raw weight (**C**). Wheat root raw weight (**D**). Asterisks (*) indicate statistically significant differences compared to the control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

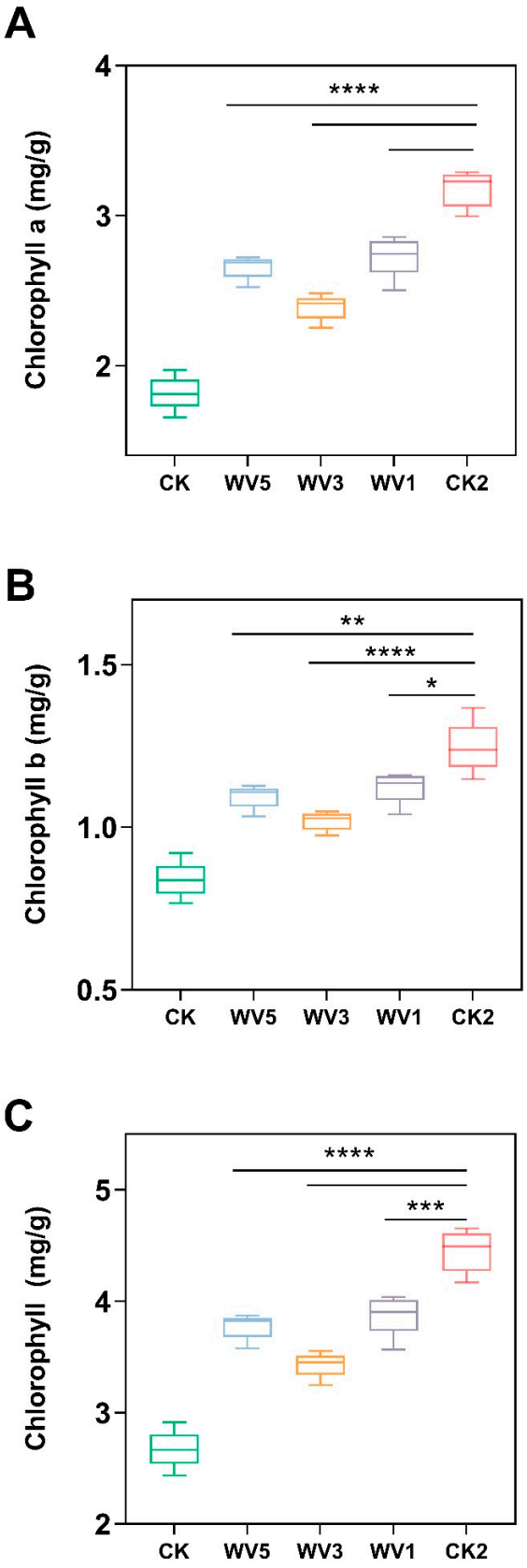

Figure 2. Chlorophyll content of wheat. Chlorophyll a (**A**). Chlorophyll b (**B**). Total chlorophyll (**C**). Asterisks (*) indicate statistically significant differences compared to the control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

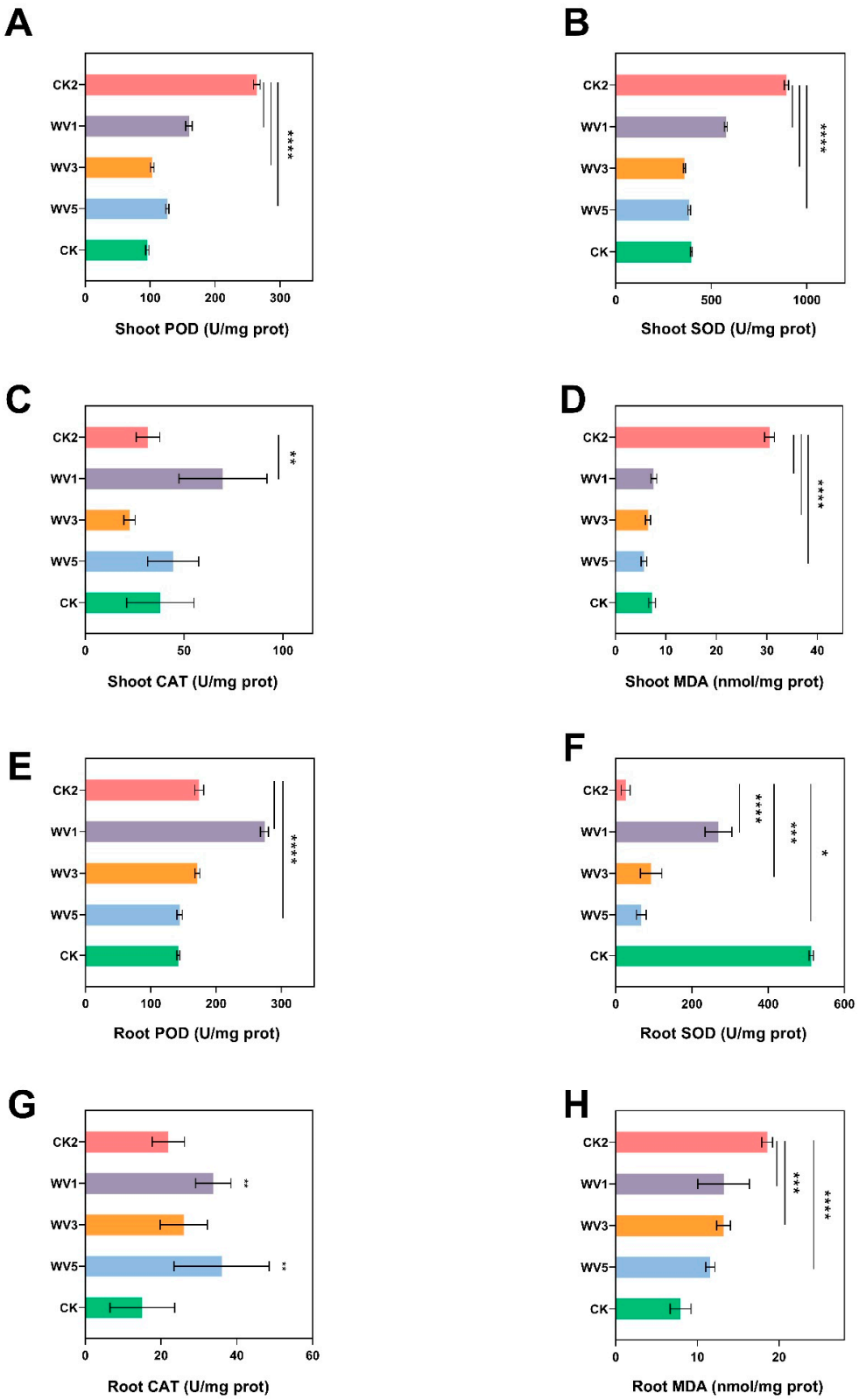

Figure 3. Impact of wood vinegar on wheat seedlings antioxidant enzyme system. Shoot POD activity (**A**). Shoot SOD activity (**B**). Shoot CAT activity (**C**). Shoot MDA content (**D**). Root POD activity (**E**). Root SOD activity (**F**). Root CAT activity (**G**). Root MDA content (**H**). Asterisks (*) indicate statistically significant differences compared to the control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Notably, applying different concentrations of vinegar solution significantly reduced the MDA content in the shoots. WV3 and WV5 demonstrated the most pronounced alleviation effect. They restored the shoot POD and SOD activity to the same level of CK. Although WV1 significantly reduced the MDA content in the shoots, its restorative effects on peroxidase POD and superoxide dismutase SOD activities were less pronounced compared to those of WV3 and WV5. In contrast, WV1 treatment even significantly enhanced shoot CAT activity relative to CK2. The application of WV at different dilutions exhibited significant mitigation effects on salt-stressed wheat roots (Figure 3E–H). Compared with the salt-stressed control (CK2), all the WV treatments (WV1–WV5) demonstrated dose-dependent regulation of antioxidant enzyme activities and lipid peroxidation levels. The roots appeared to be more responsive to WV treatment in terms of CAT activity, showing a more pronounced increase compared to the shoots. The differential responses of shoots and roots to salt stress and WV treatment suggests that the antioxidant systems in these two plant parts may be regulated differently. Roots, being in direct contact with salt stress, may have a more active or sensitive antioxidant response.

The inverse correlation between antioxidant enzyme activities and MDA levels indicates that WV enhances ROS-scavenging capacity. Notably, shoot and root tissues exhibited differential antioxidant responses to WV dilutions under salt stress. In shoots, the intermediate dilution (WV3, 300×) optimally restored POD and CAT activities (Figure 3A,C), correlating with reduced photooxidative damage. In roots, higher dilutions (WV5, 500×) sustained SOD activity and attenuated lipid peroxidation (Figure 3F,H). These tissue-specific adaptation patterns suggest distinct ROS management strategies in aerial versus subterranean organs. The differential dilution efficacy between shoots (300×) and roots (500×) informs precision biostimulant design. For instance, integrating WV with root-targeting amendments like biochar could synergistically optimize whole-plant stress resilience [67–69]. As a pyrolysis byproduct from agricultural waste (e.g., crop straw), WV exemplifies circular agriculture by converting residues into value-added agro-inputs [70–72], simultaneously mitigating crop stress and open-field burning pollution.

3.4. Impact of Wood Vinegar on Total Protein

Salt stress can lead to dynamic changes in protein metabolism within wheat, and it is a sensitive indicator reflecting the physiological damage caused by salt stress [10,73,74]. Violin plots (Figure 4) revealed a dose-dependent restoration of the total protein content in salt-stressed wheat seedlings treated with WV. The foliar application of wood vinegar significantly increased the total protein content in the shoots relative to the salt-stressed control (CK2), with the 300× dilution (WV3) demonstrating optimal restoration efficacy under salinity. The 500× dilution (WV5) led to the strongest recovery in the roots, while higher concentrations (WV1, 100×) showed diminished efficacy, likely due to phytotoxicity from excessive organic acids/phenolics, as evidenced by unimodal distributions reflecting enhanced physiological stability compared to CK2. Physiologically, this restoration may occur through attenuated protein degradation, mediated by phenolic-induced protease inhibition in WV, thereby preserving cellular integrity.

3.5. Effects of Wood Vinegar on Nutrient Elements

This study utilized inductively coupled plasma mass spectrometry (ICP-MS) to quantify elemental profiles, thereby assessing the regulatory effects of WV on mineral nutrient uptake and spatial distribution in wheat under salt stress. Raw ICP-MS concentrations (provided in Supplementary Table S1) were normalized using z-score transformation to eliminate batch effects, enabling comparative analysis of relative elemental abundance trends across treatment groups.

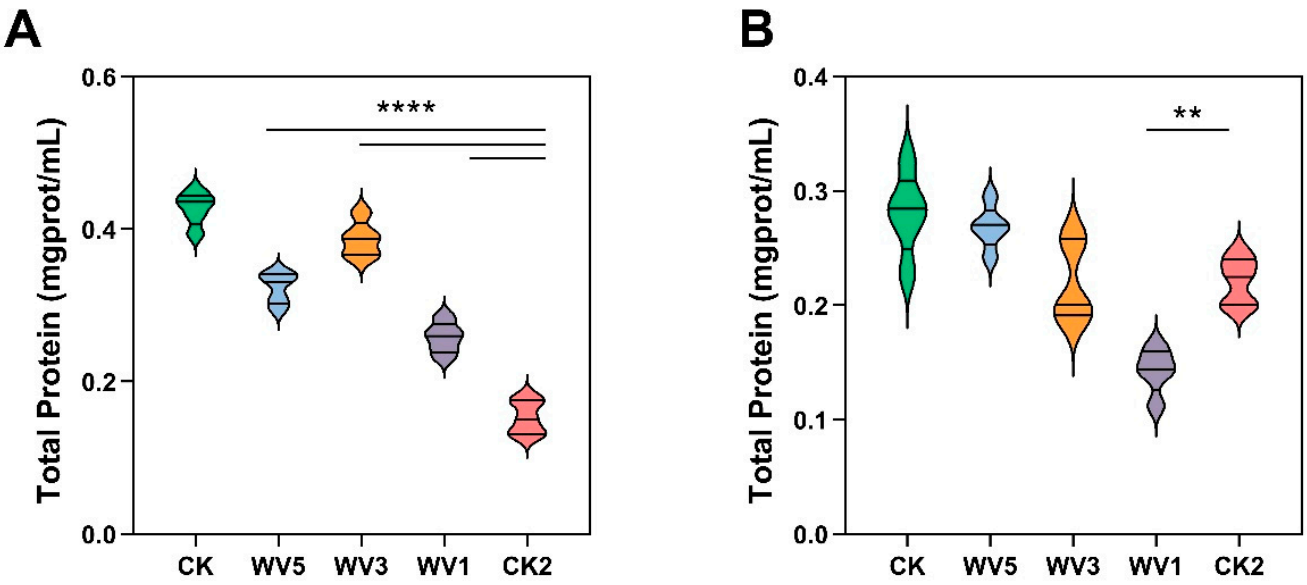

Figure 4. Salt stress affects the total protein content of wheat seedlings. Shoot (**A**). Root (**B**). Asterisks (*) indicate statistically significant differences compared to the control group: ** $p < 0.01$, **** $p < 0.0001$.

The standardized mineral element profiles in wheat shoots and roots (Figure 5A,B) revealed distinct organ-specific responses to WV treatments under salt stress. Compared to the salt-stressed control (CK2), the 100× diluted WV treatment (WV1) exhibited the most significant mitigation effect, particularly in shoot tissues. In shoots, WV1 enhanced K^+ and Ca^{2+} absorption, two cations critical for counteracting Na^+ toxicity through competitive uptake and membrane stabilization. Root Zn^{2+} absorption, however, displayed a dose-dependent suppression. The 300× diluted WV (WV3) induced Fe accumulation in shoots, potentially enhancing chlorophyll synthesis, while root Fe decreased, suggesting improved root-to-shoot translocation efficiency. Phosphorus partitioning also varied: WV1 increased shoot P but reduced root P, implying that WV modulates phloem-mediated redistribution under stress. Higher dilutions (WV5, 500×) showed limited efficacy, with shoot Mg and Cu remaining below CK2 levels, indicating a threshold for organic acid-mediated chelation. The boron dynamics diverged between organs: shoot B increased with WV concentration, whereas root B peaked with WV3.

The alleviation of salt stress by WV may stem from multifactorial interactions, driven by the bioactive properties of wood vinegar components. Organic acids (e.g., acetic acid) could potentially chelate Na^+ or compete for root absorption sites, which might reduce ionic toxicity and facilitate K^+/Ca^{2+} uptake. Phenolic compounds are postulated to regulate ion transporter gene expression, possibly enhancing Na^+ efflux and vacuolar compartmentalization. Acidification of the rhizosphere by WV organic components could further solubilize micronutrients, enhancing their bioavailability. These mechanisms collectively explain the dose- and organ-dependent efficacy of WV, with higher concentrations balancing chelation capacity and phytotoxicity. Future studies integrating transcriptomics and rhizosphere metagenomics could elucidate WV-regulated transporter networks and microbial synergies, advancing precision strategies for salinity mitigation.

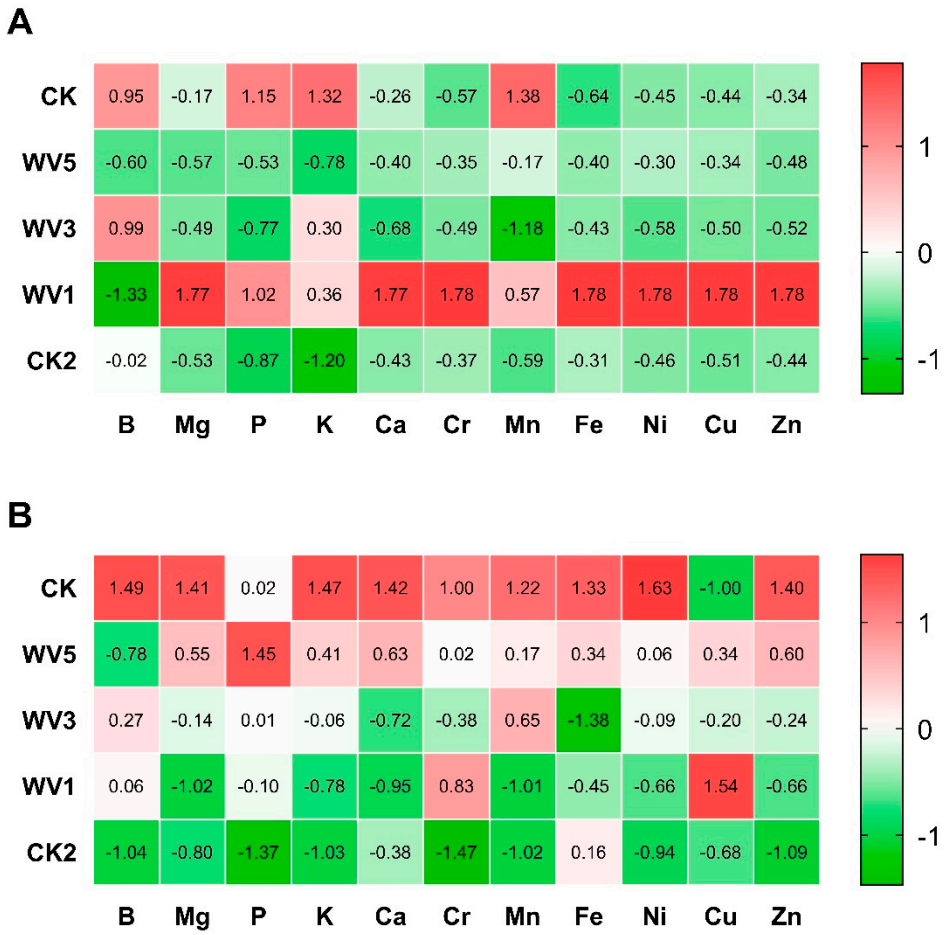

Figure 5. The distribution of standardized contents of major elements in seedlings under different treatments. Shoot (**A**). Root (**B**).

3.6. Agricultural Practice of Wood Vinegar

The field deployment of WV could leverage existing precision agriculture infrastructure—foliar spraying via drone/boom systems enables uniform delivery while minimizing labor costs. For saline soils, drip irrigation with WV may concurrently improve rhizosphere microenvironments and nutrient availability; this indicates the various effects of the WV. As agricultural waste, WV can be mixed with organic fertilizers or nano-materials (such as ferrophosphorus). Research shows that the combined use of FeP-NMs and WV can reduce the amount of phosphate fertilizer by 80%, while increasing soybean yield [35]. Synergistic integration with organic amendments (e.g., biochar for cation exchange) could further reduce WV dosage requirements while enhancing soil health [72]. The development and utilization of wood vinegar liquid align with the goals of sustainable development, offering significant potential for economic, social, and environmental benefits.

4. Conclusions

This study reveals that foliar-applied WV mitigates salt stress (100 mM NaCl) in hydroponic wheat through dose-dependent physiological and biochemical mechanisms. Moderate dilutions (WV3: 300×, WV5: 500×) significantly alleviated salt-induced growth inhibition, with WV3 extremely significantly increasing shoot biomass and root length compared to salt-stressed controls. In contrast, concentrated WV (WV1: 100×) induced phytotoxicity, emphasizing the need for optimal dilution. WV3 balanced antioxidant responses by restoring shoot SOD/POD activities to non-stressed levels while reducing MDA, whereas

roots exhibited CAT-dependent H_2O_2 detoxification. Notably, WV treatments normalized stress-induced chlorophyll overaccumulation, likely through the phenolic-mediated stabilization of photosynthetic machinery, and restored protein synthesis efficiency.

Elemental profiling demonstrated WV3's dual role in ion homeostasis: reducing shoot Na^+ while enhancing K^+ and Ca^{2+} uptake. It also improved Fe translocation to shoots, supporting chlorophyll synthesis, and modulated boron/zinc partitioning between organs. These effects stem from WV's organic acids (e.g., acetic acid) chelating toxic ions and its phenolics enhancing antioxidant capacity. The tissue-specific responses—shoots prioritizing ROS scavenging and roots optimizing nutrient uptake—highlight WV's ability to coordinate whole-plant stress adaptation.

These findings suggest the potential of 300×-diluted WV as an eco-friendly biostimulant for salt-affected agriculture, demonstrating improved wheat resilience through integrated antioxidant activation, ion homeostasis, and photosynthetic protection under controlled conditions. Future research must prioritize elucidating molecular mechanisms (e.g., ion transporter gene regulation) and field validation to assess WV's efficacy in sustainable crop management. This work contributes exploratory evidence for employing plant-derived amendments to mitigate salinity challenges in intensive agriculture, though their large-scale applicability requires further verification.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy15092078/s1. Table S1: Raw ICP-MS concentrations.

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