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Research Article

**The Effect of PGPR and Chicken Litter Ash Applications on the Development, Nutrient Element, and Heavy Metal Content of *Cephalaria syriaca* L.**

**Yusuf ARSLAN<sup>\*1</sup>, Hanife USTABAŞ<sup>2</sup>, Berfin İŞLER<sup>3</sup>, Ferit SÖNMEZ<sup>4</sup>**

<sup>1,3</sup> Bolu Aabant İzzet Baysal University, Agriculture Faculty, Field Department, 14100, Bolu, Türkiye  
<sup>2,4</sup> Bolu Aabant İzzet Baysal University, Agriculture Faculty, Seed Science and Techonolgy Department, 14100, Bolu, Türkiye

<sup>1</sup><https://orcid.org/0000-0001-8496-6037>, <sup>2</sup><https://orcid.org/0009-0004-2481-0363>, <sup>3</sup><https://orcid.org/0000-0002-4656-8879>  
<sup>4</sup><https://orcid.org/0000-0003-1437-4081>

\*Corresponding author e-mail [sonmezferit@ibu.edu.tr](mailto:sonmezferit@ibu.edu.tr)

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**Abstract:** This problem has been solved by incinerating chicken litter waste, which causes environmental pollution. The resulting chicken litter ash is a valuable source of phosphorus and potassium. This study examined the effects of increasing doses of chicken litter ash (CLA), combined with Plant Growth-Promoting Rhizobacteria (PGPR), on the growth and elemental composition of *Cephalaria syriaca* L. Chicken litter ash was applied to pots containing 2 kg of soil at 0%, 1%, 2%, and 4% rates. Microbial fertilizer was applied to some pots in combination with ash, while others were left untreated. At the end of the experiment, soil samples were analysed for soil pH, soil salinity (EC), plant growth parameters, nutrient elements, and heavy metal content of *Cephalaria syriaca* L. The results showed that CLA significantly influenced soil pH, EC, plant height, fresh weight, dry weight, and root fresh weight ( $P<0.01$ ). The application of microbial fertilizer significantly affected EC, plant fresh and dry weights, root fresh weight, and leaf count ( $P<0.01$ ), and root dry weight ( $P<0.05$ ). Interaction effects between the ash and microbial fertilizer were significant for plant height, fresh weight, root fresh weight, dry weight, and root dry weight ( $P<0.01$ ). Regarding nutrient elements and heavy metals, the CLA application, except Cu, Mn, and Ni, significantly impacted all elements at  $P<0.01$  and  $P<0.05$ . The application of microbial fertilizer had no significant effect on the elements Ca, Cu, Ni, and Pb, while it significantly affected the other elements at  $P<0.01$  and  $P<0.05$  levels. Based on these results, it is concluded that chicken litter ash applications should be based on soil analysis, and that its use in combination with microbial fertilizers would be more beneficial.

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**1. Introduction**

The *Cephalaria syriaca* L. plant, commonly known for its high resistance to cold, drought, and salinity, stands out as a valuable oil crop. Its ability to thrive in diverse climates, particularly in clayey and loamy soils, and even in eroded or sloped terrains, highlights its significance (Çiller, 1977; Arslan

et al., 2022). Recently, *Cephalaria syriaca* seeds have been utilized as a natural additive in bakery wheat flour, prolonging the shelf life of products (Baytop, 1999).

In 2022, 367 million poultry animals were raised in Türkiye, with chickens accounting for 98.5% of this figure, followed by turkeys (1.0%), geese (0.4%), and ducks (0.1%) (Anonymous, 2023). Annually, 1.5–5.7 kg of chicken litter waste is produced per animal, amounting to 551–2,091 million tons of waste (Szogi and Vanotti, 2009; Pandey et al., 2021). A relatively new method involves converting this waste into electricity through incineration, resulting in chicken litter ash. Compared to untreated chicken litter, the ash is 4 to 17 times denser in nutrients, making it advantageous for agricultural applications (Bock, 2004; Ervin et al., 2019). Additionally, this ash serves as an alternative fertilizer source due to its rich nutrient content, particularly in Ca, P, and K, and its pathogen-free nature (Codling, 2006; Cempa et al., 2022).

However, issues arise from the low water solubility of phosphorus in the ash, which affects its usability. Conversely, its high water-soluble potassium content presents unique opportunities (Demeyer et al., 2001). Factors such as the Ca/P ratio significantly influence phosphorus availability, necessitating complementary chemical or biological interventions (Shober et al., 2006; Jastrzębska et al., 2016; Bauer et al., 2019). Interestingly, the low water-soluble phosphorus content of the ash minimizes environmental pollution risks (Vance et al., 2021).

PGPR applications enhance plant phosphorus uptake, along with nitrogen and other nutrients, improving plant growth. Using PGPR with chicken litter reduces dependency on chemical fertilizers and improves soil microbial diversity (Lin et al., 2018; Calderon et al., 2021; Bhavya et al., 2022). Despite these benefits, chicken litter ash requires pretreatment before application to address its high pH and salinity, which can adversely affect both soil properties and plant growth (Hashimoto et al., 2009; Faridullah et al., 2013; Olowoboko et al., 2018).

This study aimed to evaluate the combined and individual effects of chicken litter ash and PGPR on soil properties, plant growth, nutrient composition, and heavy metal content of *Cephalaria syriaca* L.

## 2. Material and Methods

The study was conducted in the climate-controlled chambers of the Field Crops Department at Bolu Abant İzzet Baysal University's Faculty of Agriculture. *Cephalaria syriaca* L. plants were used for the experiment. Chicken litter ash (CLA) was applied at ratios of 0%, 1%, 2%, and 4% to pots containing 2 kg of soil. Half of these pots were sown with PGPR-inoculated seeds, while the other half were sown with untreated seeds. The PGPR used in the trial was the SS-SUPER PAN product of Supersol. It contains a mixture of *Pantoea agglomerans*, *Paenibacillus polymyxa*, *Bacillus megaterium* and *Pseudomonas fluorescens* microorganisms. The solution contains  $1 \times 10^7$  cfu/ml of live organisms. The study was carried out in the climate chamber according to the factorial experimental design in randomized parcels with three replications.

The climate chamber was maintained at a temperature of  $23^\circ\text{C} \pm 2$ , with 16 hours of daylight, 8 hours of darkness, 70% humidity, and appropriate light intensity for approximately two months. Plants were irrigated with distilled water throughout the study. At the end of the experiment, plant growth parameters including plant height (PH), root length (RL), plant fresh weight (PFW), plant dry weight (PDW), root fresh weight (RFW), root dry weight (RDW) and leaf number (LN) were determined.

### 2.1 Nutrient analysis in plant samples

Plant samples carefully taken from the pots were washed first with tap water and then twice with pure water in the laboratory. After being left at room temperature for a while, the samples were dried in an oven at  $70^\circ\text{C}$  in paper bags until they reached a constant weight. The dried plant samples were then ground using a plant grinder to prepare them for analysis. Elemental analyses of chicken litter ash and plant samples were performed according to Kacar and İnal (2008) using porcelain crucibles and the dry ashing method, followed by extraction. The obtained extracts were analysed for K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Cd, and Pb elements using the Shimadzu Corporation ICPE-9800 Series ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) device at the Van Yüzüncü Yıl University Central Research Laboratory. Phosphorus analysis was determined spectrophotometrically using the yellow color method (Kacar and İnal, 2008).

## 2.2 Chemical analysis of soil sample

Soil samples, collected from pots according to Kacar (1994), were placed in plastic bags and brought to the laboratory. After drying under appropriate conditions, the samples were crushed with a wooden mallet, passed through a 2 mm sieve, and stored in covered plastic containers for analysis. Soil texture, pH, salinity, lime content, organic matter, and the concentrations of N, P, K, Fe, Mn, Zn, and Cu were determined using the methods outlined by Kacar (1994). Available phosphorus was analysed using a spectrophotometer. Exchangeable K, Ca, Mg, and available Fe, Mn, and Zn were measured using the Shimadzu Corporation ICPE-9800 Series ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) device. The results of some physical and chemical analyses of the chicken litter ash (CLA) and soil used in the experiment are presented in Table 1.

Table 1. The physical and chemical properties of the soil and chicken litter ash

| Source | pH    | EC<br>mS cm <sup>-1</sup> | Lime<br>% | Texture | P<br>% | K     | Ca    | Mg    | Fe     | Mn     | Zn     | Cu     |
|--------|-------|---------------------------|-----------|---------|--------|-------|-------|-------|--------|--------|--------|--------|
| Soil   | 7.52  | 0.035                     | 2.84      | Loamy   | 0.017  | 0.198 | 0.421 | 0.241 | 0.004  | 0.009  | 0.004  | 0.011  |
| CLA    | 13.42 | 15.17                     | -         | -       | 3.85*  | 8.35* | 17.0* | 4.73* | 0.559* | 0.349* | 0.059* | 0.233* |

CLA, Chicken Litter Ash; \*, total values.

## 2.3 Statistical analysis

The data were analysed statistically using JMP software. Relationships between applications and measured parameters were further evaluated using principal component analysis (PCA) and hierarchical cluster analysis (HCA) with RStudio (Wickham, 2011).

## 3. Results

### 3.1. Effects of applications on soil properties, plant growth criteria, nutrient element and heavy metal contents

The effects of chicken litter ash (CLA) and PGPR applications on soil pH, salinity (EC), plant growth parameters, and nutrient element concentrations were analysed. Variance analysis results are presented in Table 2, while the means and Duncan multiple comparison test results for soil pH, EC, and growth parameters are given in Table 3. The averages for macronutrients, micronutrients, and heavy metals are provided in Table 4.

As seen in Table 2, chicken litter ash (CLA) applications had a significant effect at the 1% level on all elements except Cu, Mn, and Ni. PGPR (P) applications significantly influenced EC, RL, PFW, PDW, RDW, P, Fe, Zn, Pb, and Cd at the 1% level and LN, Ca, and Mg at the 5% level, but their effects on pH, PH, RFW, K, Cu, and Ni were not significant. The interaction of CLA and PGPR (C×P) significantly affected soil pH, EC, growth parameters, and macro-, micro-, and heavy metals at the 1% and 5% levels, excluding Cu.

The pH of the control soil was 8.31, which increased to 8.60 with CLA applications a rise of approximately 3.5%. The EC content of the soil also increased with CLA applications, reaching the highest value at 4% CLA. The EC value, which was 103  $\mu\text{S cm}^{-1}$  in the control, increased to 393  $\mu\text{S cm}^{-1}$ , 715  $\mu\text{S cm}^{-1}$ , and 1020  $\mu\text{S cm}^{-1}$  with 1%, 2%, and 4% CLA applications, representing increases of 281%, 594%, and 890%, respectively. PGPR application only affected EC. While the EC of soils without PGPR was measured as 527  $\mu\text{S cm}^{-1}$ , the EC of PGPR-applied soils was 589  $\mu\text{S cm}^{-1}$ , representing an 11.8% increase (Table 3).

Table 2. Variance analysis results of the effects of CLA and PGPR applications on soil pH, EC, growth parameters, and plant element contents

|     | Source   | Df | F        |    | Source   | Df | F       |
|-----|----------|----|----------|----|----------|----|---------|
| pH  | CLA (C)  | 3  | 102.26** | Ca | CLA (C)  | 3  | 7.79**  |
|     | PGPR (P) | 1  | 0.08 ns  |    | PGPR (P) | 1  | 5.10*   |
|     | CxP      | 3  | 3.38 *   |    | CxP      | 3  | 5.81**  |
| EC  | CLA (C)  | 3  | 363.97** | Mg | CLA (C)  | 3  | 12.87** |
|     | PGPR (P) | 1  | 8.68**   |    | PGPR (P) | 1  | 8.51*   |
|     | CxP      | 3  | 4.40*    |    | CxP      | 3  | 5.72**  |
| PH  | CLA (C)  | 3  | 6.37**   | P  | CLA (C)  | 3  | 12.68** |
|     | PGPR (P) | 1  | 4.22 ns  |    | PGPR (P) | 1  | 24.07** |
|     | CxP      | 3  | 18.86**  |    | CxP      | 3  | 5.87**  |
| RL  | CLA (C)  | 3  | 29.48**  | Fe | CLA (C)  | 3  | 11.37** |
|     | PGPR (P) | 1  | 110.3**  |    | PGPR (P) | 1  | 30.02** |
|     | CxP      | 3  | 6.85**   |    | CxP      | 3  | 9.07**  |
| PFW | CLA (C)  | 3  | 14.99**  | Cu | CLA (C)  | 3  | 2.26 ns |
|     | PGPR (P) | 1  | 70.28**  |    | PGPR (P) | 1  | 0.01 ns |
|     | CxP      | 3  | 54.81**  |    | CxP      | 3  | 1.86 ns |
| RFW | CLA (C)  | 3  | 7.86**   | Mn | CLA (C)  | 3  | 2.67 ns |
|     | PGPR (P) | 1  | 0.01 ns  |    | PGPR (P) | 1  | 8.27*   |
|     | CxP      | 3  | 52.25**  |    | CxP      | 3  | 15.17** |
| PDW | CLA (C)  | 3  | 7.75**   | Zn | CLA (C)  | 3  | 17.95** |
|     | PGPR (P) | 1  | 16.27**  |    | PGPR (P) | 1  | 82.79** |
|     | CxP      | 3  | 11.99**  |    | CxP      | 3  | 7.57**  |
| RDW | CLA (C)  | 3  | 11.10**  | Ni | CLA (C)  | 3  | 0.32 ns |
|     | PGPR (P) | 1  | 11.91**  |    | PGPR (P) | 1  | 0.46 ns |
|     | CxP      | 3  | 15.66**  |    | CxP      | 3  | 5.59**  |
| LN  | CLA (C)  | 3  | 9.17**   | Pb | CLA (C)  | 3  | 4.42**  |
|     | PGPR (P) | 1  | 6.05*    |    | PGPR (P) | 1  | 26.22** |
|     | CxP      | 3  | 4.08*    |    | CxP      | 3  | 21.04** |
| K   | CLA (C)  | 3  | 7.52**   | Cd | CLA (C)  | 3  | 50.29** |
|     | PGPR (P) | 1  | 3.97 ns  |    | PGPR (P) | 1  | 25.83** |
|     | CxP      | 3  | 10.27**  |    | CxP      | 3  | 7.38**  |

\*, \*\*, Significant at 5% and 1% levels, ns; non-significant, CLA; Chicken Litter Ash, PGPR; Plant Growth Promoting Rhizobacteria, PL; Plant Height, RL, Root Length; PFW, Plant Fresh Weight; RFW, Root Fresh Weight; PDW, Plant Dry Weight; RDW, Root Dry Weight; LN, Leaf Number.

Table 3. Averages and Duncan multiple comparison results of the effects of CLA and PGPR applications on soil pH and EC and plant growth criteria

| Treatments                                         | pH     | EC<br>$\mu\text{S cm}^{-1}$ | PH<br>cm | RL      | PFW<br>g plant <sup>-1</sup> | RFW     | PDW     | RDW     | LN<br>No. |
|----------------------------------------------------|--------|-----------------------------|----------|---------|------------------------------|---------|---------|---------|-----------|
| <b>Chicken Litter Ash (CLA), %</b>                 |        |                             |          |         |                              |         |         |         |           |
| 0                                                  | 8.31 b | 103 d                       | 8.84 a   | 26.83a  | 2.076 a                      | 1.137 a | 0.202 a | 0.232 a | 8.44 a    |
| 1                                                  | 8.60 a | 393 c                       | 8.25 ab  | 24.50ab | 1.742 ab                     | 1.077 a | 0.145 b | 0.077 b | 6.80 b    |
| 2                                                  | 8.59 a | 715 b                       | 7.69 bc  | 22.50ab | 1.577 b                      | 1.100 a | 0.139 b | 0.089 b | 7.93 ab   |
| 4                                                  | 8.60 a | 1020 a                      | 7.03 c   | 22.17b  | 1.280 b                      | 0.813 b | 0.130 b | 0.069 b | 7.41 ab   |
| LSD(0.05)                                          | 0.04   | 62                          | 0.92     | 3.86    | 0.260                        | 0.160   | 0.030   | 0.069   | 1.49      |
| <b>Plant Growth Promoting Rhizobacteria (PGPR)</b> |        |                             |          |         |                              |         |         |         |           |
| PGPR (-)                                           | 8.52   | 527 b                       | 7.64     | 23.33b  | 1.309 b                      | 1.034   | 0.134 b | 0.077 b | 7.35      |
| PGPR (+)                                           | 8.53   | 589 a                       | 8.27     | 24.00a  | 2.029 a                      | 1.030   | 0.174 a | 0.157 a | 7.93      |
| LSD(0.05)                                          | 0.03   | 44                          | 0.65     | 0.63    | 0.184                        | 0.113   | 0.021   | 0.049   | 1.06      |

\*, \*\*, Significant at 5% and 1% levels, ns; non-significant, CLA; Chicken Litter Ash, PGPR; Plant Growth Promoting Rhizobacteria, PL; Plant Height, RL, Root Length; PFW, Plant Fresh Weight; RFW, Root Fresh Weight; PDW, Plant Dry Weight; RDW, Root Dry Weight; LN, Leaf Number.

The increase in the amount of chicken litter ash application negatively affected plant height, fresh and dry weights of the plant, root dry weight, and leaf number. In the control group, the values were recorded as 8.84 cm, 2.076 g, 1.137 g, 0.202 g, 0.232 g, and 8.44 leaves, respectively. Under 4% chicken litter ash application, the lowest values were observed as 7.03 cm, 1.280 g, 0.813 g, 0.130 g, and 0.069 g, respectively. For leaf number, the lowest value of 6.80 leaves was noted in the 1% chicken litter ash application (Table 3).

While PGPR application resulted in increases in plant height and leaf number, these were not statistically significant. However, PGPR had a positive effect on root length, plant fresh weight, plant dry weight, and root dry weight. In plants without PGPR, the values were recorded as 23.33 cm, 1.309 g, 0.134 g, and 0.073 g, respectively. With PGPR application, these values increased to 24.00 cm, 2.029 g, 0.174 g, and 0.161 g, respectively (Table 3).

Application of CLA to the environment increased the potassium (K) content in the plant, while causing a partial decrease in phosphorus (P) content. The highest K content was recorded as 4.545%, in the 1% CLA treatment, with the most notable increase in potassium (12.5%) compared to the control. Calcium content showed irregular increases and decreases with increasing CLA doses. The calcium content of *Cephalaria syriaca* L. increased to 2.077% with the 1% CLA treatment, while the lowest value (1.752%) was observed with the 2% CLA treatment. The highest calcium content of 2.353% was recorded at the 4% CLA treatment. Magnesium content generally decreased with CLA applications compared to the control, with the lowest value of 0.493% found at 2% CLA, representing a 65.7% decrease compared to the control. Phosphorus content increased with 1% CLA compared to the control, but at 2% and 4% CLA applications, it was not significantly different from the control (Table 4).

PGPR application caused a decrease in calcium (Ca), magnesium (Mg), phosphorus (P), and iron (Fe) contents, while it caused an increase in manganese (Mn), zinc (Zn) and cadmium (Cd) contents (Table 4).

Table 4. Averages and Duncan multiple comparison results of the effects of CLA and PGPR applications on the macro and micronutrient elements and heavy metal contents of *Cephalaria syriaca* L.

| Treatments                                  | K      | Ca     | Mg     | P       | Fe                  | Cu    | Mn     | Zn   | Ni     | Pb      | Cd     |
|---------------------------------------------|--------|--------|--------|---------|---------------------|-------|--------|------|--------|---------|--------|
|                                             | %      |        |        |         | mg kg <sup>-1</sup> |       |        |      |        |         |        |
| Chicken Litter Ash (CLA), %                 |        |        |        |         |                     |       |        |      |        |         |        |
| Control                                     | 4.040b | 2.229a | 0.817a | 0.596 a | 586a                | 17.16 | 65.14  | 125c | 20.718 | 7.536 b | 0.405c |
| 1                                           | 4.545a | 2.077b | 0.689a | 0.581 a | 414b                | 20.37 | 65.61  | 161b | 21.721 | 9.135 b | 1.266a |
| 2                                           | 4.196b | 1.752c | 0.493b | 0.570 a | 441b                | 19.06 | 73.97  | 199a | 21.071 | 11.206a | 0.814b |
| 4                                           | 4.137b | 2.353a | 0.716a | 0.507 b | 375b                | 18.17 | 70.86  | 131c | 21.248 | 7.397 b | 0.662b |
| LSD(0.05)                                   | 0.240  | 0.279  | 0.114  | 0.033   | 82                  | 2.71  | 7.81   | 24   | 2.211  | 1.493   | 0.153  |
| Plant Growth Promoting Rhizobacteria (PGPR) |        |        |        |         |                     |       |        |      |        |         |        |
| PGPR (-)                                    | 4.309  | 2.208a | 0.734a | 0.590 a | 529a                | 18.65 | 65.15b | 117b | 20.940 | 8.870   | 0.657b |
| PGPR (+)                                    | 4.149  | 1.998b | 0.623b | 0.536 b | 379b                | 18.73 | 72.64a | 190a | 21.439 | 8.767   | 0.917a |
| LSD(0.05)                                   | 0.170  | 0.197  | 0.080  | 0.023   | 58                  | 1.92  | 5.53   | 17   | 1.563  | 1.056   | 0.108  |

a, b, c; there is no statistical difference between the means shown with the same letter.

The effect of CLA on the microelement content of *Cephalaria syriaca* L. showed a decrease in iron (Fe) content, while zinc (Zn) content increased, with no significant effect on manganese (Mn) and copper (Cu) contents. The iron content in the control was 586 mg kg<sup>-1</sup>, which decreased to the lowest value of 375 mg kg<sup>-1</sup> with 4% CLA application, representing a 56.3% decrease. Zinc content in the control was 125 mg kg<sup>-1</sup>, while the highest value of 199 mg kg<sup>-1</sup> was recorded with 2% CLA, showing a 59.2% increase compared to the control. PGPR application resulted in an increase in Mn and Zn contents. The Mn and Zn contents in plants without PGPR were 65.15 mg kg<sup>-1</sup> and 117 mg kg<sup>-1</sup>, respectively. In plants with PGPR, these values increased to 72.64 mg kg<sup>-1</sup> and 190 mg kg<sup>-1</sup>, representing increases of 11.5% and 62.4%, respectively (Table 4).

CLA applications caused irregular increases and decreases in the heavy metal content of *Cephalaria syriaca* L. Lead (Pb) content increased initially with CLA applications, then decreased. The cadmium (Cd) content in the control was 7.536 mg kg<sup>-1</sup>, which increased to 9.135 mg kg<sup>-1</sup> and 11.206 mg kg<sup>-1</sup> with 1% and 2% CLA applications, respectively, representing increases of 21.2% and 48.7% compared to the control. The lowest value for Pb content (7.397 mg kg<sup>-1</sup>) was observed with 4% CLA application. The cadmium content increased overall with CLA applications, with the highest value of 1% CLA application, which increased from 0.405 mg kg<sup>-1</sup> in the control to 1.266 mg kg<sup>-1</sup>, representing a 212.6% increase. Statistically, PGPR application resulted in a significant increase in cadmium content. In plants without PGPR, the cadmium content was 0.657 mg kg<sup>-1</sup>, which increased to 0.917 mg kg<sup>-1</sup> with PGPR application, representing a 39.6% increase (Table 4).

When examining the interaction effects of CLA and PGPR applications, the highest values for plant fresh weight, plant dry weight, root fresh weight, root dry weight, root length, and leaf number

were observed in PGPR-applied plants. In the control plants, the values for these parameters were 3.365 g, 0.286 g, 1.557 g, 0.409 g, 28.33 cm, and 9.82 leaves, respectively. In the 4% CLA × PGPR(+) interaction, the values decreased to 0.118 g, 0.605 g, 0.049 g, and 7.03 leaves, with the plant fresh weight in the control × PGPR(-) interaction at 0.788 g, and root length at 21.00 cm in the 4% CLA × PGPR(-) interaction. As the dose of chicken litter ash (CLA) increased, reductions in plant growth parameters were observed. However, with PGPR application, the decreases caused by CLA, particularly in plant fresh weight, root length, and leaf number, were somewhat mitigated (Figure 1).

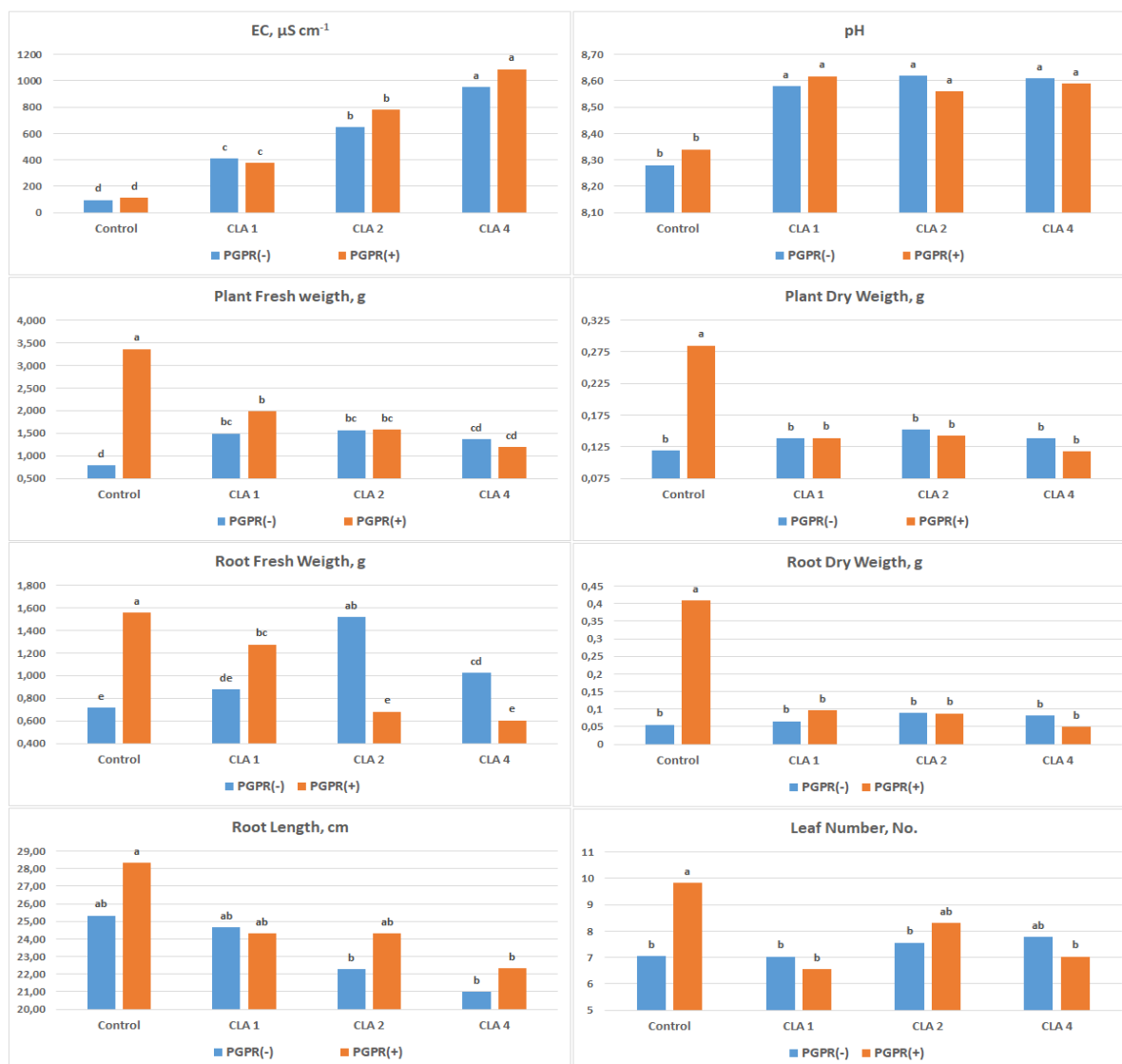


Figure 1. The Effect of CLA × PGPR interaction on soil pH, EC, and growth parameters, CLA1; 1%, CLA2; 2%, CLA4; 4%, PGPR(-); non-applied, PGPR(+); applied.

In environments without PGPR, as the dose of CLA increased, the contents of Ca, Mg, P, Fe, Mn, and Ni increased compared to the control, while the contents of K, Pb, and Cd decreased. In PGPR-treated environments, as the dose of CLA increased, the contents of K, P, and Fe decreased compared to the control, while the contents of Ca, Mg, Mn, Zn, Ni, Pb, and Cd increased (Figure 2).



Figure 2. Effect of CLA x PGPR interaction on macroelement, microelement and heavy metal contents of the plant, CLA1; 1%, CLA2; 2%, CLA4; 4%, PGPR(-); non-applied, PGPR(+); applied.

### 3.2. Statistical approaches associated with CLA and PGPR applications

To evaluate the relationships between plant growth parameters, nutrient elements, heavy metals, and soil pH and EC values, principal component analysis (PCA) was performed. The first two principal components (PC1 and PC2) accounted for 30.8% and 18.4% of the total variance, respectively. PC1 was positively correlated with root fresh weight (RFW), plant height (PH), plant fresh weight (PFW), leaf number (LN), root dry weight (RDW), plant dry weight (PDW), phosphorus (P), potassium (K), and zinc (Zn), indicating these factors contributed significantly to the variance. On the other hand, PC2 showed a positive correlation with soil pH, soil EC, lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), calcium (Ca), nickel (Ni), iron (Fe), and magnesium (Mg).

The PCA biplot also distinguished between CLA application doses: the 1% CLA dose (red ellipse), the 2% CLA dose (green ellipse), the 4% CLA dose (blue ellipse), and the control (orange ellipse) displayed different relationships among the variables. The control treatment occupied a distinct position compared to the other treatments. In environments where CLA was applied with PGPR, the relationships with RFW, PH, PFW, LN, RDW, PDW, P, K, and Zn were observed, while in the non-

PGPR environments, the correlations were with soil pH, soil EC, Pb, Cd, Cu, Mn, Ca, Ni, Fe, and Mg (Figure 3).

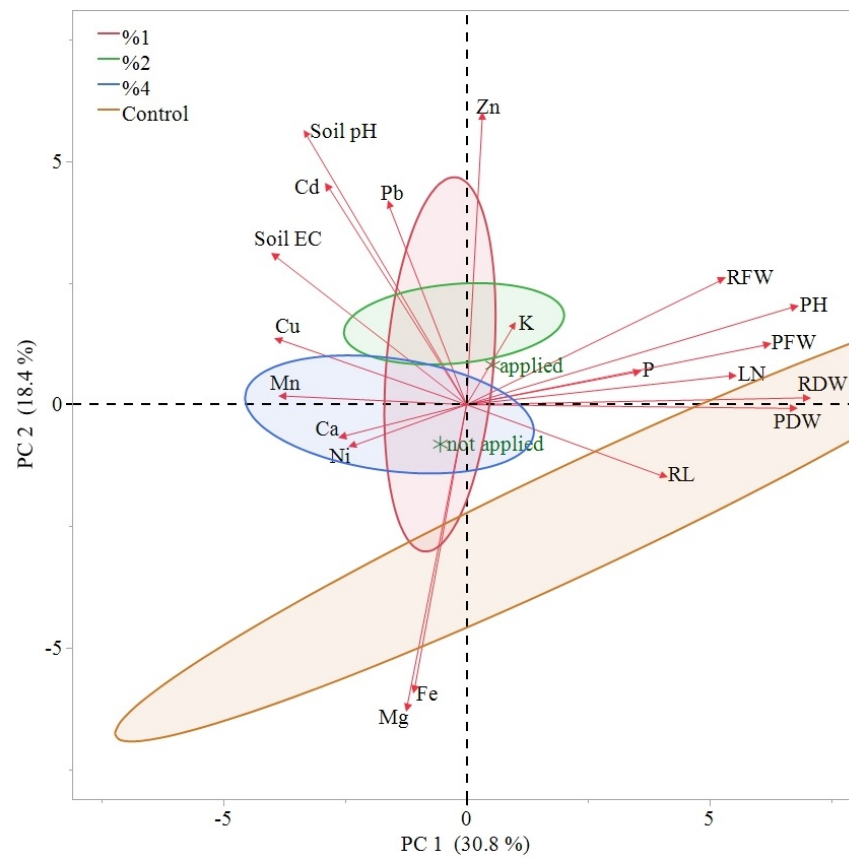


Figure 3. Biplot PCA analysis for the relationships between CLA, PGPR (P) and the studied traits. Circles are constructed according to 95% confidence intervals. In the biplot, green colored variables represent the CLA application doses from the factors, while black colors represent the studied traits.

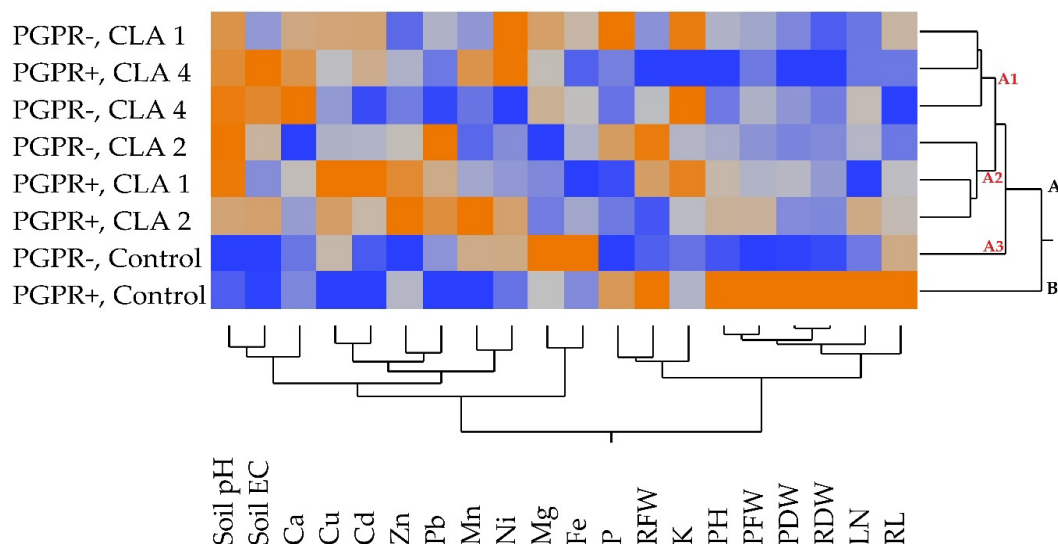


Figure 4. Heatmap analysis shows the relationship between the properties examined with CLA and PGPR applications. The color scale from blue to orange indicates values from low to high.

According to the criteria analysed with CLS and PGPR treatments, they were categorized into two main clusters, each containing two subclusters. The first cluster (A) consisted of all the treatments

except for the control x PGPR(+) treatment, while the control x PGPR(+) treatment was included in the second cluster (B). The first subcluster of A (A1) consisted of PGPR-applied and unapplied subjects of 4% and 1% doses of CLA treatments, while the second subcluster (A2) varied with PGPR-applied and unapplied subjects of 2% and 1% doses of CLA. The third subset (A3) differed only from the control treatment without PGPR (Figure 4).

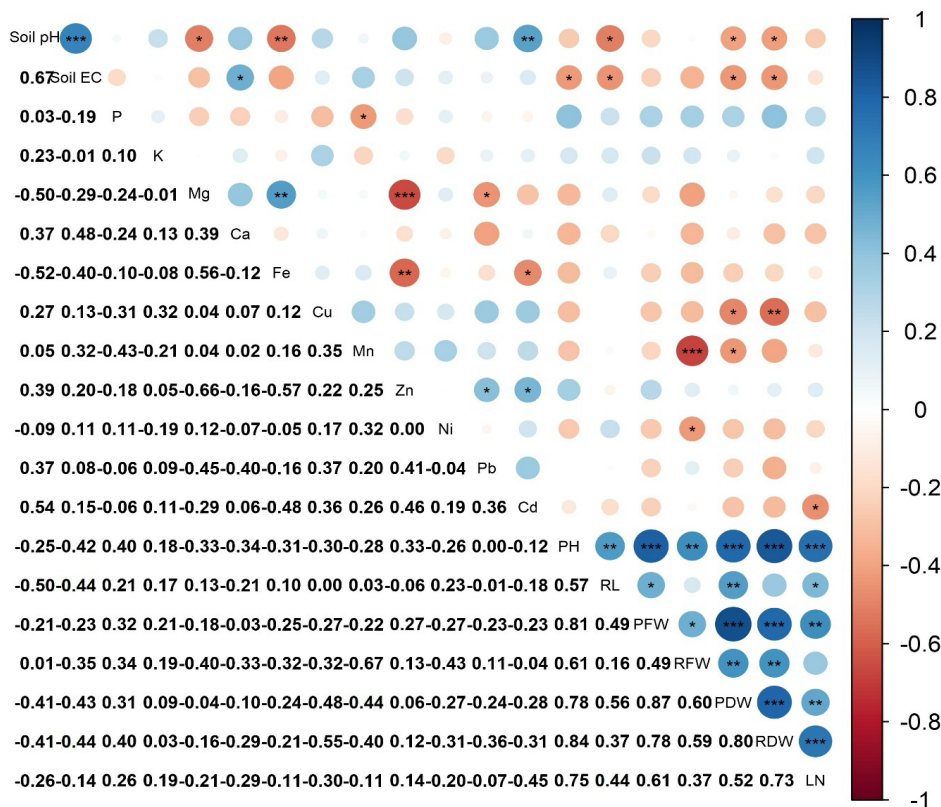


Figure 5. Correlations showing the relationship between CLA and PGPR applications and the examined traits. \*, \*\* and \*\*\* indicate significance at  $p \leq 0.05$ ,  $p \leq 0.01$  and  $p \leq 0.001$ , respectively.

In the conditions where CLA and PGPR were applied together, soil pH, soil EC, and cadmium (Cd) showed a positive correlation, while magnesium (Mg), iron (Fe), root length (RL), plant dry weight (PDW), and root dry weight (RDW) showed a negative correlation. Soil salinity was positively correlated with calcium (Ca) and negatively correlated with plant height (PH), root length (RL), plant dry weight (PDW), and root dry weight (RDW). Phosphorus (P) showed a negative correlation only with manganese (Mn). Magnesium was positively correlated with iron (Fe) and negatively correlated with zinc (Zn) and lead (Pb). Iron (Fe) showed a negative correlation with zinc (Zn) and cadmium (Cd). Copper (Cu) showed a negative correlation with plant dry weight (PDW) and root dry weight (RDW). Manganese (Mn) was negatively correlated with root fresh weight (RFW) and plant dry weight (PDW). Zinc (Zn) was positively correlated with lead (Pb) and cadmium (Cd). Nickel (Ni) was negatively correlated only with root fresh weight (RFW). Cadmium (Cd) showed a negative correlation with leaf number (LN).

Plant height (PH) showed a positive correlation with root length (RL), plant fresh weight (PFW), root fresh weight (RFW), plant dry weight (PDW), root dry weight (RDW), and leaf number (LN). Root length (RL) showed a positive correlation with plant fresh weight (PFW), plant dry weight (PDW), and leaf number (LN). Plant fresh weight (PFW) showed a positive correlation with root fresh weight (RFW), plant dry weight (PDW), root dry weight (RDW), and leaf number (LN). Root fresh weight (RFW) showed a positive correlation with plant dry weight (PDW) and root dry weight (RDW). Plant dry weight (PDW) showed a positive correlation with root dry weight (RDW) and leaf number (LN). Root dry weight (RDW) showed a positive correlation only with leaf number (LN) (Figure 5).

#### 4. Discussion

Due to the high salt content and pH values of chicken litter ash (CLA) (Table 1), an increase in EC and pH values of the applied soils was observed (Table 3). The increase rates for EC were 281%, 594%, and 890%, while the pH increase was 3.5% compared to the control. Similar studies have reported that chicken litter ash applications cause significant increases in soil pH and EC values (Yusof et al., 2015). This is believed to be due to the high levels of elements such as Ca, K, P, and Cl present in the chicken litter ash (Fahimi et al., 2020), which influence changes in pH and EC. Since chicken litter ash does not contain elements like C, N, and S (Fahimi et al., 2020) and has no positive effect on the soil's organic matter content, it does not positively impact the buffering capacity of the soil. Particularly in pot studies, the excess of elements that cause salinity from the ash may have led to the soil's buffering capacity being surpassed.

Soil salinity in environments treated with PGPR was higher than in those without PGPR. This could be due to PGPR limiting the uptake of elements that cause salinity (Rojas-Tapias et al., 2012), thereby increasing the salinity in the soil. It has been reported that in saline soils, PGPR-modulated plants show improved development in various morphological, physiological, and biochemical aspects, leading to increased resistance under stress conditions (Kumar et al., 2018; Shilev, 2020). The changes in plant growth criteria confirm this hypothesis. In PGPR-treated plants, the increase in CLA application reduced plant fresh weight, plant dry weight, root fresh weight, and root dry weight compared to untreated plants, indicating that high salinity was the cause (Figure 1).

With the increase in CLA application doses, plant height (PH), root length (RL), plant fresh weight (PFW), root fresh weight (RFW), plant dry weight (PDW), root dry weight (RDW), and leaf number (LN) were negatively affected. The decreases observed compared to the control were 25.7%, 62.2%, 39.9%, 55.4%, and 251.5%, respectively (Table 3). These declines could be a result of the increase in soil salinity. The reduction in plants' ability to absorb water, along with changes in enzyme activity, metabolic changes, and hormonal imbalances (including transport of nutrients and water across membranes), leads to growth regression (Prakash and Parthapasanen, 1990; Hasegawa et al., 2000). Salinity-induced water scarcity causes photosynthesis, oxidative and osmotic stress, ion toxicity, and a decrease in nutrient balance, resulting in the increase of certain ions to toxic levels (Zhu, 2003). Consequently, salt stress can lead to the formation of reactive oxidative species (ROS), causing DNA damage and protein metabolism disruptions (Islam et al., 2015). The observed decreases in plant growth criteria can be attributed to all these negative effects.

PGPR application led to significant increases in root length (RL), plant fresh weight (PFW), plant dry weight (PDW), and root dry weight (RDW). The increases compared to untreated plants were 2.8%, 57.3%, 29.9%, and 120.5%, respectively (Table 3). The positive effects of PGPR-treated plants on these criteria can be attributed to the supportive effects of PGPR, which help counteract the negative impacts of the increased pH and salinity caused by CLA. Soil salinity affects plant growth and development at both the physiological and biochemical levels (Munns and James, 2003; Tester and Davenport, 2003), as well as at the molecular level (Ashraf, 2004). In these environments, PGPR promote the production of indole acetic acid (IAA) and gibberellins, which enhances root elongation, increases root surface area, and boosts capillary root formation. This leads to improved nutrient uptake and enhanced plant growth under stress conditions (Egamberdieva and Kucharova, 2009). It has been reported that in saline soils, plant phosphorus content decreases due to the precipitation of phosphate ions with calcium ions (Ca) (Bano and Fatima, 2009). As seen in Figure 2, the reduction in plant phosphorus content, especially in PGPR-treated plants, confirms the hypothesis that this decrease is due to precipitation between Ca ions and phosphate ions.

Chicken litter ash applications significantly increased potassium, zinc, lead, and cadmium content compared to the control. In the control, the values for potassium (4.040%), zinc (125 mg kg<sup>-1</sup>), lead (7.536 mg kg<sup>-1</sup>), and cadmium (0.405 mg kg<sup>-1</sup>) were increased to 4.545% potassium, 199 mg kg<sup>-1</sup> zinc, 9.135 mg kg<sup>-1</sup> cadmium, and 1.266 mg kg<sup>-1</sup> cadmium with 1% CLA. The increases were 13.2%, 212.6%, 59.2%, and 48.7%, respectively. With CLA applications, calcium (Ca), magnesium (Mg), phosphorus (P), and iron (Fe) contents decreased significantly. In the control, the values were 2.229%, 0.817%, 0.596%, and 586 mg kg<sup>-1</sup>, while with 2% CLA application, calcium decreased to 1.752%, magnesium to 0.493%, phosphorus to 0.507%, and iron to 375 mg kg<sup>-1</sup>. These decreases were 27.2%, 65.7%, 17.6%, and 56.3%, respectively. Copper, manganese, and nickel content were not significantly

affected by CLA applications (Table 4). Faridullah et al. (2009) reported that with CLA applications, the phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) content of plants increased compared to the control. On the other hand, Codling et al. (2002) observed that while phosphorus content increased with CLA application compared to the control, potassium and magnesium content decreased. This situation is thought to be related to the increase in salinity and pH in the growing medium associated with the high salt content and pH of CLA (Table 1) (Table 3). Indeed, it has been reported that increased soil salinity reduces the uptake of potassium (K), calcium (Ca), and magnesium (Mg) by plants (Yaldiz et al., 2018; El Agyzy and Aboukota, 2019; Zhao et al., 2021). Studies have also reported a rapid increase in cytosolic  $\text{Ca}^{2+}$  ion levels in plants in response to soil salinity (Lynch and Läuchli, 1988). This increase in calcium ions is believed to trigger calcium signaling in the cytoplasm, leading to an increase in proteins such as calcium-dependent protein kinases (CDPKs), calcineurin B-like proteins (CBLs)/SOS3-like calcium-binding proteins (SCaBPs), and CBL-interacting protein kinases (CIPKs)/SOS2 family protein kinases (PKS) (Dodd et al., 2010). Applications of elements such as calcium and zinc in the growing medium are recommended to enhance plant resistance against salinity stress (Sönmez et al., 2013; Saeidnejad et al., 2016). In our study, the increase in calcium content in plants with 4% CLA application compared to the control is thought to be a response to the salinity in the medium (Table 4). Similarly, while the highest zinc content was observed with 2% CLA application, the sharp decline in zinc content with 4% CLA application may have been due to the increased calcium uptake and decreased root cell permeability, resulting in reduced zinc uptake.

High salinity environments cause the apoplastic pH of plants to shift from acidity to alkalinity (Geilfus, 2017). This may have reduced the apoplastic transport of micronutrients and heavy metals into the xylem. Indeed, iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), lead (Pb), and cadmium (Cd) showed significant decreases with 4% CLA application (Table 4).

PGPR application reduced the calcium (Ca), magnesium (Mg), phosphorus (P), and iron (Fe) content of plants but increased manganese (Mn), zinc (Zn), and cadmium (Cd) content. There was no significant effect on other elements (Table 4). Generally, plant growth-promoting rhizobacteria (PGPR) enhance biological nitrogen fixation or supply essential mineral nutrients such as phosphorus (P), potassium (K), zinc (Zn), and iron (Fe) to plants (Acurio Vásquez et al., 2020; Basu et al., 2021; Rehan et al., 2023). Phosphate-solubilizing bacteria (PSB) make inorganic phosphorus available to plants by producing low molecular weight organic acids such as acetic, lactic, oxalic, succinic, citric, gluconic, and ketogluconic acids. For this process to occur, the rhizosphere must have suitable conditions for microorganisms. Phosphate-solubilizing bacteria (PSB) make inorganic phosphorus available to plants by producing low molecular weight organic acids such as acetic, lactic, oxalic, succinic, citric, gluconic, and ketogluconic acids. For this process to occur, the rhizosphere must have suitable conditions for microorganisms. However, under soil salinity conditions, these bacteria can fight salinity stress by producing indole-3-acetic acid (IAA), ACC deaminase, dissolving phosphorus, producing exopolysaccharides, and producing volatile compounds (Dodd and Pérez-Alfocea, 2012). In our study, PGPR application reduced the uptake of these elements in the presence of chicken litter ash in the environment. This may have been due to the reduced activity of the PGPR under saline conditions.

In environments without CLA application, PGPR inoculation resulted in the highest values for all plant growth criteria. In environments with CLA application, PGPR inoculation had a significant effect on root length and leaf number (Figure 1). When the effect of interactions on nutrient element contents was examined, PGPR inoculation in control plants significantly increased the potassium (K), phosphorus (P), and zinc (Zn) content compared to untreated plants. When CLA was applied, PGPR inoculation caused significant increases in manganese (Mn), zinc (Zn), nickel (Ni), and cadmium (Cd) contents, while significant changes in other elements were observed in plants without PGPR (Figure 2).

## Conclusion

The findings of this study demonstrate that chicken litter ash (CLA), due to its high salt content and alkalinity, significantly alters soil chemical properties, particularly by increasing soil pH and electrical conductivity (EC). These changes negatively impacted the growth and physiological performance of *Cephalaria syriaca* L., especially at higher CLA doses, primarily due to salinity-induced stress conditions. Reductions in plant height, biomass accumulation,

and nutrient uptake were associated with increased soil salinity, ion toxicity, and impaired nutrient balance.

The application of plant growth-promoting rhizobacteria (PGPR) showed a potential to mitigate some of the adverse effects of CLA by enhancing root development and biomass production under stress conditions. PGPR inoculation also influenced nutrient dynamics by increasing the uptake of certain microelements such as Zn, Mn, and Cd, although its effectiveness appeared to diminish under elevated salinity levels, likely due to reduced microbial activity in such environments.

The application of CLA led to increased accumulation of certain nutrients and heavy metals, such as K, Zn, Pb, and Cd, while reducing the uptake of essential elements including Ca, Mg, P, and Fe. These changes were attributed to both chemical interactions in the rhizosphere and physiological responses of plants to salinity and pH stress. Moreover, the interaction between CLA and PGPR significantly influenced both plant growth traits and nutrient uptake, suggesting a complex relationship between soil amendments and microbial activity.

In conclusion, while chicken litter ash can serve as a potential nutrient source, its application should be carefully managed based on soil analysis, and preferably in combination with beneficial microbial inoculants. This integrated approach may enhance nutrient efficiency and support plant growth, particularly under challenging soil conditions. Further studies under field conditions are recommended to validate the long-term implications of CLA and PGPR co-application for sustainable crop production.

### **Ethical Statement**

Ethical approval is not required for this study.

### **Conflict of Interest**

The authors declare that there are no conflicts of interest.

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### **Author Contributions**

Conceptualization and methodology, F.S. and Y.A.; data collection, investigation and data analysis, preparation, H.U. and B.İ.; writing-review and editing, F.S. and Y.A. All authors have read and approved the published version of the article.

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