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# Effects of nitrogen treatments and bacterial inoculation on macro- and micro-element contents of the *Halisbey* peanut variety

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## Highlights

- *The effect of nitrogen fertilization on nitrogen, iron, and copper elements was found to be positive in peanuts.*
- *Bacterial inoculation and different nitrogen dose applications did not affect the content of phosphorus, potassium, magnesium, sodium, manganese, and boron elements.*
- *Bacterial inoculation increased the nitrogen, calcium, and zinc contents of the peanut seed.*
- *Using nitrogen and bacteria application together (240N+B) increased the N content of the seed.*

## Abstract

This study was conducted to determine the effect of nitrogen (N) doses (0, 40, 80, 120, 160, 200, and 240 kg ha$^{-1}$) and bacterial inoculation on macro- (N, P, K, Ca, Mg, and Na) and micro- (Fe, Cu, Zn, Mn, and B) element contents of the *Halisbey* peanut variety. The nutrient requirements, specific nutrient management strategies, and adaptation of the variety to the regions where it was grown were also assessed. According to the average results of the N applications, different doses affected the levels of N (240N+B: 1.76%) alongside the content of other macro-elements, as well as sodium (200N: 0.09 mg kg$^{-1}$), iron (80N: 32.39 mg kg$^{-1}$), and copper (40N: 14.11 mg kg$^{-1}$) among the micro-elements. The bacterial application was not found to significantly increase N content (240N+B: 1.76%), calcium content (0.08%) and zinc content (49.68 mg kg$^{-1}$). At the same time, (240N+B) bacteria and N application increased the N ratio.

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## Introduction

Peanut is a plant that is grown in tropical and subtropical areas in almost all soil types and requires all macro- and micronutrients for growth. It is a crop with higher nutrient requirements than many others (Cox *et al*., 1970; Dwivedi, 1988; Hartzog and Adams, 1988; Singh, 1999). Peanuts contain an average of 40.10% fat, 25.30% protein, and 18% carbohydrates, along with vitamins A, B and E, as well as abundant minerals like K, Ca, Mg, P, and S (Woodroof, 1983; Maiti and Ebeling, 2002). The pulp remaining after the oil is extracted contains 45% crude protein, 24% organic compounds, and 5.5% mineral substances. Plant nutrient uptake in peanuts is most strongly needed at the maximum growth stage of the plant. Peanuts are susceptible to nutrient deficiency if the plant nutrients in the soil are inadequate. Fertilization has significant effects on yield and quality (Islam *et al*., 2012; Kaplan *et al*., 2016). It has been determined that the total need for different plant nutrients for the growth of the peanut plant is between 4.6-11.9% during the vegetative stage (0-25 days), 42.3-88.1% during the reproductive stage (25-75 days), and 6.4-53% in the pod development stage (75-105 days) (Polara *et al*., 1991). If the Ca mineral, which is essential for the plant, cannot be taken, the seeds inside the pod cannot develop normally, and the inside of the pods remains empty. The maximum absorption of Ca, Mg, and K is in the 25-75-day period, and the absorption of P, Mn, and Fe occurs during the capsule development stages (Polara *et al*., 1991; Singh and Chaudhari, 1995). Since nitrogen (N) affects the production of peanut plants, N fertilizer must be provided at a sufficient level for plant growth and yield

(Awadalla and Mohammed, 2017). If it cannot provide the total N content in the soil, inoculation of peanut plants with *Rhizobium* is considered a useful practice (Hadad *et al*., 1998). *Rhizobium* inoculation in legumes promotes growth and is an alternative to expensive inorganic N fertilizers (Ndakidemi and Semoka, 2006; Abbasi *et al*., 2010). It can offer a great opportunity to enhance legume growth and development. However, considering the cost of inoculation, producers are advised to carefully evaluate the decision to inoculate legume crops in all circumstances (Vessey, 2004).

Since peanut is a legume plant, it binds the free N in the air to the soil thanks to the *Rhizobium* sp. bacteria living symbiotically in its roots. In this way, the plant absorbs N from the soil and, in turn, releases N and organic matter for the benefit of the next plant (Arıoğlu, 2014). Soil analysis must be performed to determine whether there is sufficient N in the soil for the plant. To enable N fixation in peanuts, there must be a sufficient presence of *Rhizobium* bacteria in the soil, or the seeds need to be inoculated with these bacteria. As the population of *Rhizobium* bacteria increases, the plant's ability to fix N increases as well. N fixed by *Rhizobium* bacteria is stored in nodules located on the plant's roots. However, in conditions where the plant is not inoculated or there is a scarcity of *Rhizobium* bacteria in the soil, the amount of N biologically fixed by the plant is generally low (Gök and Onac, 1995). The peanut plant has the capacity to fix approximately 45-150 kg $ha^{-1}$ of N during its growing period (Woodroof, 1983). While a significant portion of the accumulated N is utilized by the plant itself, approximately 30-40% remains in the soil (Arıoğlu, 2014). However, in many peanut planting areas, there is often an insufficient population of *Rhizobium* bacteria in the soil to meet the N requirements of the plant. As a result, the plant's N needs are typically supplemented through fertilization. Tilak *et al*. (2006) reported that the inoculation of plants with *Rhizobium* promotes plant growth and enhances the availability of N in the soil. Differences in elemental contents in peanuts may be affected by factors such as geographic region, peanut varieties, harvest season, and agricultural practices (Zhao *et al*., 2020; Chen *et al*., 2022). This study was conducted to investigate and better understand the effects of various levels of N and bacteria fertilizers in isolation and combination on the macro- and micro-element content of the peanut seed.

## Materials and Methods

### Materials

This study was conducted in the research and experiment area of Çukurova University, Faculty of Agriculture, Department of Field Crops, Adana (latitude 37˚0'N and longitude 35˚19'E), Turkey, in 2015 and 2016. The Çukurova Region is under the influence of the Mediterranean climate, with hot and dry summers and warm and rainy winters (Republic of Turkey, 2016). The average air temperature, total precipitation, relative humidity, and long-term averages of the growing period in 2015-2016 are given in Table 1.

The typical Mediterranean climate prevails in Adana, where the trial was performed, with warm and rainy winters and dry and hot summers. During the development period of peanuts in 2015 and 2016, the average temperature values of each month were higher than those of many years. The monthly average temperature values for 2016 were found to be slightly higher compared to the air temperature values of 2015. Meanwhile, in September and October, corresponding to the harvest time of the plant in 2015 and 2016, the average temperature values were lower than the average for many years. The *Halisbey* peanut variety, which is commonly used in the region, was used alongside the most commonly used N fertilizer mixtures (at doses of 0, 40, 80, 120, 160, 200, and 240 kg $ha^{-1}$) such as ammonium sulfate and ammonium nitrate as well as *Rhizobium* bacteria as materials in the present study. *Rhizobium* bacteria contains at least $2\times10^9$ live *Bradyrhizobium* sp. bacteria (Arachis) bacteria per g. Inoculant prepared in 400 g packages is recommended for 80 kg of peanut seeds. *Rhizobium* bacteria were obtained from the Soil, Fertilizer and Water Resources Research Institute Directorate, Ankara (latitude 39˚57'N and longitude 32˚48'E), Turkey (Table 2) (Çağlar, 1949; Bouyoucos, 1951; Olsen *et al*., 1954; Richards, 1954; Jackson, 1958; Bremmer, 1965; Lindsay and Norwell, 1978).

### Methods

The experiment was conducted as a randomized complete block experimental design with 3 replications. The land on which the trial was established was deeply plowed in autumn and mixed with a cultivator in spring to prepare it for planting. According to the soil analysis conducted before planting, 100 kg $ha^{-1}$ pure $P_2O_5$ fertilizer was mixed with the goble disc, containing 42-44% $P_2O_5$, for each parcel. Besides, the trial field was sprayed with 1000 cc ha Spectrum EC against weeds, Dursban 25 WP against under-

**Table 1.** Monthly temperature, total precipitation, relative humidity in the 2015-2016 growing seasons, and long-term (1997-2016) averages in Adana Province, Turkey.

| Months | Average temperature (°C) | | | Monthly total precipitation (mm) | | | Relative humidity (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | 1997-2016 | 2015 | 2016 | 1994-2016 | 2015 | 2016 | 1994-2016 |
| April | 16.9 | 20.5 | 13.8 | 21.5 | 36.6 | 44.5 | 61.2 | 59.2 | 57.1 |
| May | 22.5 | 21.6 | 17.7 | 65.7 | 87.9 | 44.7 | 64.8 | 69.3 | 60.5 |
| June | 25.0 | 27.1 | 22.0 | 4.8 | 45.6 | 15.1 | 69.6 | 66.1 | 55.1 |
| July | 28.4 | 29.5 | 26.0 | 0.4 | 0.2 | 4.7 | 69.8 | 67.5 | 62.0 |
| August | 30.0 | 29.9 | 28.7 | 10.9 | 8.2 | 7.4 | 63.4 | 69.0 | 64.0 |
| September | 28.4 | 26.3 | 29.3 | 130.0 | 39.8 | 24.4 | 64.8 | 61.8 | 62.5 |
| October | 23.4 | 23.1 | 26.4 | 32.1 | - | 37.8 | 63.7 | 56.4 | 61.6 |

ground pests, and Captan against seed-borne diseases. The seeds were treated with 2 pesticides, Corconil 75W and Corban 25W, in a manner where 1.0 kg of pesticide was applied to 100 kg of seeds. In the trial, the parcel size was 2.8×5 m, and each parcel consisted of 4 rows. Before planting, the rows were determined by drawing marker at a distance of 70 cm between the rows, and the planting was made by hand on 12 April 2015 and 12 May 2016, with 15 cm above the row. 21% N was mixed directly into the soil twice during planting and 33% N after germination (maximum and in the last flowering period). Bacterial applications were made directly to the soil together with the seed during sowing. Following the planting, a sprinkler system was installed to provide adequate germination. The maturity status of the pods before harvest was determined using the "sell-out method", and the plants in the parcels were picked and harvested. During the harvest, 20 plants were randomly taken from the edge rows of each plot, their pods were milled, and their macro- and micro-element contents were quantified.

The seed samples of the plant were dried at 65˚C to a constant weight in a pneumatic dryer and prepared for analysis by the plant grinding mill. Seed samples were digested with a mixture of nitric acid + hydrogen peroxide (3 mL $HNO_3$+4 mL $H_2O_2$) in a Berghof MWS2 model microwave oven (Berghof Products + Instruments GmbH Eningen unter Achalm, Germany). Elements were analyzed in the extracts using ICP OES (Perkin Elmer OPTIMA 2100 DV, Waltham, MA, USA) (Kacar and İnal, 2010).

### Nitrogen content

Total N in seed samples was determined using a Buchi K-437/K-350 digestion/distillation unit according to the Kjeldahl method (Bremmer, 1965).

### Phosphorus content

In the solution obtained as a result of wet digesting, P was determined according to the Vanado molibdo phosphoric-yellow color method (Lott *et al*., 1956) by means of the PG T60 model spectrophotometer (Alma Park, Wibtoft, UK).

### Sodium, potassium, calcium and magnesium content

Na, K and Ca were determined in the solution obtained from seed samples digested with $HNO_3$ and $H_2O_2$ in a microwave oven using an Ependorf Elex 6361 Flame photometer (Horneck and Hanson, 1998). While Mg was determined *via* the Perkin Elmer OPTIMA 2100DV model ICP OES (Perkin Elmer, Waltham, MA, USA).

### Micro-elements (copper, iron, manganese, boron and zinc)

They were determined by Perkin Elmer OPTIMA 2100DV model ICP OES (Perkin Elmer, Waltham, MA, USA) in the solution obtained from seeds samples digested with $HNO_3$ and $H_2O_2$ in the microwave oven (Isaac and Johnson, 1998).

### Statistical analysis

The research data were analyzed through the use of the JMP 7.0 statistics package program according to the randomized complete block experimental design (JMP, 2007). The differences between the average values were compared to the significance level ($p<0.05$) using a least significant difference multiple comparison test.

## Results

The differences between the years 2015 and 2016 were not significant except for N, P and Zn (Tables 3 and 4). It was determined that different N doses and bacterial inoculation applications significantly affected the averages of N, P, Ca, Mg, Fe, Cu, Mn and Zn elements at the level of 5%, but did not affect the K, Na and B contents (Tables 3 and 4).

The years × treatments interaction was significant only in P, it was not significant in terms of N, K, Ca, Mg, Na, Fe, Cu, Mn, Zn and B (Tables 3 and 4).

### Nitrogen content

Considering the average N content values of peanut seeds over the 2 years, the highest N content value was obtained from the parcels where the application of bacteria (240N+B) was performed with 240 kg ha$^{-1}$ N application, while the lowest average N content value was obtained from the control (Figure 1). Increasing N doses and bacterial application also increased the N ratio in the peanut seeds. For all treatments, the average N content value over the 2 years was between 1.40-1.76% (Table 3). Treatment and years were determined to be significant. The N content was significant in both growing years of the plant.

**Table 2.** Some physical and chemical characteristics of the soils of the experimental sites.

| Properties | Year 2015 | Qualification classes | Year 2016 | Qualification classes | Methods |
|---|---|---|---|---|---|
| Texture | Loam | L | Loam | L | Bouyoucos, 1951 |
| pH | 7.76 | Slightly alkaline | 7.76 | Slightly alkaline | Richards, 1954 |
| EC, µS cm$^{-1}$ | 565.0 | Nonsaline | 624.3 | Nonsaline | Richards, 1954 |
| Lime, $CaCO_3$,% | 23.21 | High | 14.47 | Medium | Çağlar, 1949 |
| Organic matter % | 1.50 | Medium | 1.88 | Medium | Jackson, 1958 |
| Total N % | 0.100 | Low | 0.064 | Low | Bremmer, 1965 |
| Available $P_2O_5$ mg kg$^{-1}$ | 7.65 | Low | 10.05 | Low | Olsen *et al.* 1954 |
| Exc. K mg kg$^{-1}$ | 52.6 | Low | 86.9 | Low | Jackson, 1958 |
| DTPA-Fe mg kg$^{-1}$ | 2.03 | Low | 7.04 | High | Lindsay and Norwell, 1978 |
| DTPA- Cu mg kg$^{-1}$ | 0.71 | Sufficient | 2.09 | Sufficient | |
| DTPA-Mn mg kg$^{-1}$ | 5.06 | Low | 7.41 | Low | |
| DTPA-Zn mg kg$^{-1}$ | 0.56 | Low | 2.96 | High | |

EC, electrical conductivity; DTPA, diethylenetriaminepentaacetic acid.

## Phosphorus content

Taking the average P content of the 2 years into consideration, the highest P content value was obtained from the control (0.54%) parcels where no supplementary fertilizer or bacterial application treatment was made. On the other hand, the lowest P content value was observed in the parcels that received an N dose of 240 kg ha$^{-1}$ (0.43%). Apart from control, phosphorus content decreased with bacteria and increased N dose treatments (Table 3 and Figure 1).

## Potassium content

The average K rate values of the 2 years differed by 0.37% to 0.43%. The highest mean K value (0.43%) was obtained from the 40 kg ha$^{-1}$ N treatment (Table 3 and Figure 1). The relationship between the year and treatment interaction was found to be insignificant.

**Table 3.** Average macro-element contents of *Halisbey* peanuts in 2015 and 2016.

| Treatments | N (%) | | | P (%) | | | K (%) | | | Ca (%) | | | Mg (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | Means | 2015 | 2016 | Means | 2015 | 2016 | Means | 2015 | 2016 | Means | 2015 | 2016 | Means |
| Control | 1.33 g | 1.47 g | 1.40 ı | 0.54 a | 0.53 a | 0.54 a | 0.42 | 0.39 | 0.40 | 0.07 | 0.07 | 0.07 ab | 0.23 | 0.22 | 0.23 a |
| Bacteria (B) | 1.40 f | 1.50 fg | 1.45 hı | 0.50 b | 0.49 a-e | 0.49 bc | 0.41 | 0.40 | 0.40 | 0.08 | 0.09 | 0.08 a | 0.21 | 0.21 | 0.21 b |
| 40N | 1.40 f | 1.55 e-g | 1.48 gh | 0.51 ab | 0.51 ab | 0.51 ab | 0.43 | 0.43 | 0.43 | 0.07 | 0.07 | 0.07 b | 0.20 | 0.20 | 0.20 bc |
| 40N+B | 1.47 e | 1.58 d-g | 1.53 fg | 0.43 cd | 0.50 a-d | 0.47 c-e | 0.40 | 0.40 | 0.37 | 0.07 | 0.07 | 0.07 b | 0.20 | 0.20 | 0.20 b |
| 80N | 1.48 e | 1.61 c-f | 1.55 f | 0.47 bc | 0.51 a-c | 0.49 bc | 0.42 | 0.42 | 0.42 | 0.07 | 0.08 | 0.07 ab | 0.20 | 0.21 | 0.21 b |
| 80N+B | 1.55 d | 1.60 d-f | 1.58 ef | 0.43 cd | 0.50 a-d | 0.46 c-e | 0.41 | 0.41 | 0.38 | 0.07 | 0.07 | 0.07 bc | 0.18 | 0.20 | 0.19 bc |
| 120N | 1.55 d | 1.68 b-d | 1.61 de | 0.47 bc | 0.48 a-f | 0.48 cd | 0.41 | 0.38 | 0.39 | 0.07 | 0.07 | 0.07 b | 0.20 | 0.20 | 0.20 bc |
| 120N+B | 1.60 c | 1.65 b-e | 1.63 de | 0.48 b | 0.45 d-f | 0.46 c-e | 0.40 | 0.38 | 0.39 | 0.07 | 0.07 | 0.07 b | 0.21 | 0.19 | 0.20 bc |
| 160N | 1.60 c | 1.68 a-d | 1.64 cd | 0.48 b | 0.43 f | 0.46 d-f | 0.41 | 0.37 | 0.39 | 0.06 | 0.06 | 0.06 c | 0.20 | 0.19 | 0.19 bc |
| 160N+B | 1.64 b | 1.73 ab | 1.69 bc | 0.43 cd | 0.47 b-f | 0.45 d-f | 0.39 | 0.39 | 0.39 | 0.06 | 0.07 | 0.07 bc | 0.19 | 0.21 | 0.20 bc |
| 200N | 1.66 b | 1.72 a-c | 1.69 bc | 0.48 b | 0.48 a-f | 0.48 cd | 0.41 | 0.37 | 0.39 | 0.07 | 0.07 | 0.07 bc | 0.19 | 0.20 | 0.20 bc |
| 200N+B | 1.71 a | 1.75 ab | 1.73 ab | 0.43 cd | 0.46 c-f | 0.45 ef | 0.38 | 0.38 | 0.38 | 0.07 | 0.07 | 0.07 b | 0.18 | 0.21 | 0.20 bc |
| 240N | 1.72 a | 1.75 ab | 1.73 ab | 0.42 d | 0.44 ef | 0.43 f | 0.36 | 0.36 | 0.36 | 0.06 | 0.06 | 0.06 c | 0.18 | 0.19 | 0.19 c |
| 240N+B | 1.72 a | 1.79 a | 1.76 a | 0.49 b | 0.47 b-f | 0.48 cd | 0.41 | 0.37 | 0.39 | 0.06 | 0.07 | 0.07 bc | 0.21 | 0.20 | 0.20 b |
| Average | 1.56 B | 1.65 A | 1.61 | 0.47 B | 0.48 A | 0.48 | 0.40 A | 0.39 B | 0.39 | 0.07 | 0.07 | 0.07 | 0.20 | 0.20 | 0.20 |
| LSD (%5$_A$) | 0.03 | 0.11 | 0.06 | 0.04 | 0.05 | 0.03 | ns | ns | ns | ns | ns | 0.01 | ns | ns | 0.02 |
| LSD (%5$_B$) | | 0.02 | | | 0.01 | | | ns | | | ns | | | ns | |
| LSD(%5$_{AXB}$) | | ns | | | 0.04 | | | ns | | | ns | | | ns | |

LSD, least significant difference; LSD (%5$_A$), treatment; LSD (%5$_B$), years; LSD (%5$_{AXB}$), years × treatment inoculation; ns, not significant. Different letters in each row indicate that small letters for treatment and capital letter for average of years.

**Table 4.** Micro-element contents and averages of *Halisbey* peanuts in 2015 and 2016.

| Treatments | Na (mg kg$^{-1}$) | | | Fe (mg kg$^{-1}$) | | | Cu (mg kg$^{-1}$) | | | Mn (mg kg$^{-1}$) | | | Zn (mg kg$^{-1}$) | | | B (mg kg$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | Means | 2015 | 2016 | Means | 2015 | 2016 | Means | 2015 | 2016 | Means | 2015 | 2016 | Means | 2015 | 2016 | Means |
| Control | 0.09 | 0.08 | 0.08 b | 30.34 ab | 26.84 bc | 28.59 b | 12.15 | 12.39 | 12.27 a-c | 23.26 | 22.68 | 22.97 a | 42.94 ab | 49.59 a-d | 46.27 a-d | 22.77 | 22.30 | 22.54 |
| Bacteria (B) | 0.09 | 0.08 | 0.08 b | 29.12 a-c | 25.65 b-d | 27.39 bc | 12.32 | 12.47 | 12.39 ab | 21.08 | 21.14 | 21.11 b-d | 45.62 a | 53.75 a | 49.68 a | 22.39 | 20.39 | 21.39 |
| 40N | 0.09 | 0.08 | 0.08 b | 29.65 ab | 25.12 b-d | 27.38 bc | 14.20 | 14.02 | 14.11 a | 21.69 | 21.67 | 21.68 ab | 42.93 ab | 52.56 ab | 47.75 ab | 22.76 | 22.64 | 22.70 |
| 40N+B | 0.09 | 0.08 | 0.08 b | 26.40 b-e | 27.64 ab | 27.02 bc | 12.81 | 12.73 | 12.77 ab | 21.07 | 21.04 | 21.06 b-d | 38.52 b-e | 46.62 b-e | 42.57 c-f | 20.28 | 21.36 | 21.82 |
| 80N | 0.09 | 0.08 | 0.08 b | 31.63 a | 32.15 a | 32.39 a | 12.14 | 13.02 | 12.58 ab | 19.33 | 21.15 | 20.24 b-e | 42.52 ab | 50.75 a-d | 46.64 a-c | 21.66 | 21.22 | 21.44 |
| 80N+B | 0.09 | 0.08 | 0.08 b | 24.44 c-e | 24.59 b-d | 24.52 c-e | 8.89 | 9.34 | 9.12 d | 20.89 | 20.42 | 20.66 b-e | 35.57 c-e | 42.45 e | 39.01 ef | 22.80 | 20.60 | 20.87 |
| 120N | 0.09 | 0.08 | 0.08 b | 22.06 e | 22.00 d | 22.03 e | 11.26 | 12.04 | 11.65 bc | 18.83 | 20.78 | 19.81 c-e | 39.24 a-d | 47.61 a-e | 43.42 b-e | 23.46 | 22.84 | 23.15 |
| 120N+B | 0.09 | 0.08 | 0.08 b | 26.97 a-d | 26.97 bc | 26.97 bc | 11.84 | 11.76 | 11.80 bc | 21.95 | 21.59 | 21.77 ab | 40.43 a-d | 45.55 c-e | 42.99 c-f | 22.28 | 22.32 | 22.30 |
| 160N | 0.09 | 0.07 | 0.08 b | 24.63 c-e | 24.93 b-d | 24.78 c-e | 10.54 | 11.29 | 10.92 b-d | 18.97 | 20.01 | 19.49 de | 37.43 b-e | 46.28 b-e | 41.86 d-f | 23.24 | 19.99 | 21.61 |
| 160N+B | 0.08 | 0.08 | 0.08 b | 26.01 b-e | 25.77 b-d | 25.89 b-d | 9.28 | 11.26 | 10.27 cd | 19.71 | 20.13 | 19.92 c-e | 34.05 de | 45.14 de | 39.59 ef | 21.36 | 22.86 | 22.11 |
| 200N | 0.10 | 0.09 | 0.09 a | 26.85 b-d | 26.98 bc | 26.92 bc | 12.41 | 12.91 | 12.66 ab | 18.94 | 19.37 | 19.16 e | 37.90 b-e | 51.98 a-c | 44.94 b-d | 26.60 | 25.53 | 26.07 |
| 200N+B | 0.09 | 0.08 | 0.08 b | 25.92 b-e | 25.66 b-d | 25.79 b-d | 12.31 | 13.29 | 12.80 ab | 20.51 | 21.76 | 21.14 b-d | 34.28 c-e | 51.49 a-d | 42.89 c-f | 23.26 | 21.43 | 22.35 |
| 240N | 0.09 | 0.08 | 0.08 b | 23.08 de | 23.23 b-d | 23.15 de | 12.65 | 11.23 | 11.94 bc | 18.58 | 20.65 | 19.62 de | 32.14 e | 44.83 de | 38.49 f | 22.44 | 23.53 | 22.99 |
| 240N+B | 0.09 | 0.07 | 0.08 b | 23.36 de | 23.00 cd | 23.18 de | 12.08 | 12.50 | 12.29 ab | 21.19 | 21.63 | 21.41 a-c | 40.89 a-c | 46.45 b-e | 43.67 b-e | 22.34 | 23.51 | 22.93 |
| Average | 0.09 A | 0.08 B | 0.08 | 25.26 A | 24.59 B | 26.14 | 11.78 B | 12.16 A | 11.97 | 20.43 B | 21.00 A | 20.72 | 38.89 B | 48.22 A | 43.56 | 22.69 | 22.18 | 22.45 |
| LSD (%5$_A$) | ns | ns | ns | 4.69 | 4.62 | 3.27 | ns | ns | 2.01 | ns | ns | 1.72 | 6.76 | 6.74 | 4.72 | ns | ns | ns |
| LSD (%5$_B$) | | ns | | | ns | | | ns | | | ns | | | | 1.78 | | ns | |
| LSD (%5$_{AXB}$) | | ns | | | ns | | | ns | | | ns | | | | ns | | ns | |

LSD, least significant difference; LSD (%5$_A$), treatment; LSD (%5$_B$), years; LSD (%5$_{AXB}$), years × treatment inoculation; ns, not significant. Different letters in each row indicate that small letters for treatment and capital letter for average of years.

## Calcium content

The average Ca content in both years was 0.07%. Upon analyzing the average values from both years, it was found that peanut seeds grown following the application of bacteria (0.08%) had the highest Ca content. In contrast, the treatments involving the application of 160N kg $ha^{-1}$ and 240N kg $ha^{-1}$ with 0.06% had the lowest average Ca content (Table 3 and Figure 1). The interaction between the year and treatments was found to be insignificant. Furthermore, the Ca content in the seeds decreased with increasing N doses.

## Magnesium content

For all treatments, the average Mg content in both years was 0.20%. When considering the average values from both years, the magnesium content ranged from 0.19 to 0.23%. The highest Mg content was observed in the control group (0.23%), whereas the lowest value of 0.19% was found in the 240N kg $ha^{-1}$ application (Table 3 and Figure 1). The year and the interaction between the year and treatments were determined to be insignificant.

## Sodium content

The average Na content in peanuts to which bacteria and different N doses were applied was between 0.08% and 0.09% (Table 4 and Figure 1). While the highest average Na content in peanuts was observed in the 200 kg $ha^{-1}$ N treatment, the other treatments were in the same group.

## Iron content

The average Fe content in the peanuts with bacteria and different N doses ranged from 22.03 mg $kg^{-1}$ to 32.39 mg $kg^{-1}$ (Table 4).



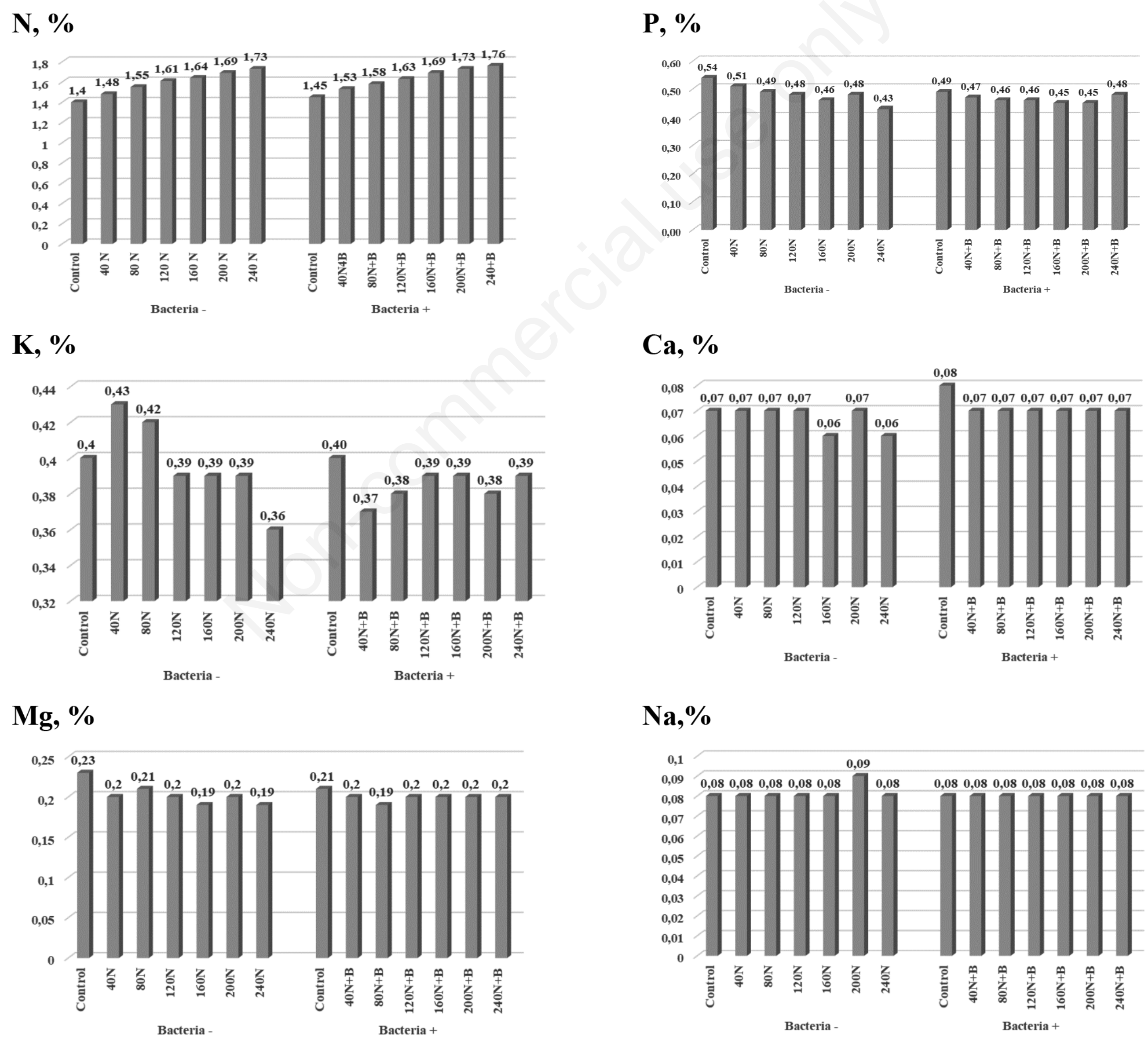


**Figure 1.** Macro-element content of *Halisbey* peanut seeds presented as the average for 2015 and 2016.

The highest average Fe content was obtained from the 80 kg ha$^{-1}$ N (32.39 mg kg$^{-1}$) application, while the lowest average Fe content was observed in the 120 kg ha$^{-1}$ N (22.03 mg kg$^{-1}$) treatment (Figure 2).

## Copper content

The average Cu content for the years 2015 and 2016 ranged from 9.12 mg kg$^{-1}$ to 14.11 mg kg$^{-1}$. The results showed that the highest average Cu content was obtained from the 40N kg ha$^{-1}$ (14.11 mg kg$^{-1}$) N application, while the lowest was obtained from the 80N+B kg ha$^{-1}$ (9.12 mg kg$^{-1}$) application. This indicates that the application of a low dose (40N kg ha$^{-1}$) of N affected the Cu content (Table 4 and Figure 2).

## Manganese content

Upon examining the average values of 2 years in terms of Mn content, the highest Mn content belonged to the control group (22.97 mg kg$^{-1}$) (Table 4). The Mn content demonstrated similar values (19.16 (200N), 20.66 (80N+B), 21.68 (40N), and 22.97 (control) mg kg$^{-1}$) in peanuts to which bacteria and increased N doses were applied (Figure 2).

## Zinc content

When the average values of the 2 years were analyzed in terms of Zn content, the bacterial treatment (49.68 mg kg$^{-1}$) was found to have the highest Zn content, while the lowest Zn value was obtained from the 240N kg ha$^{-1}$ treatment (38.49 mg kg$^{-1}$) (Table 4 and Figure 2).

## Boron content

The effect of bacteria and N applications on B in peanut seeds was found to be insignificant. The average values of the 2 years

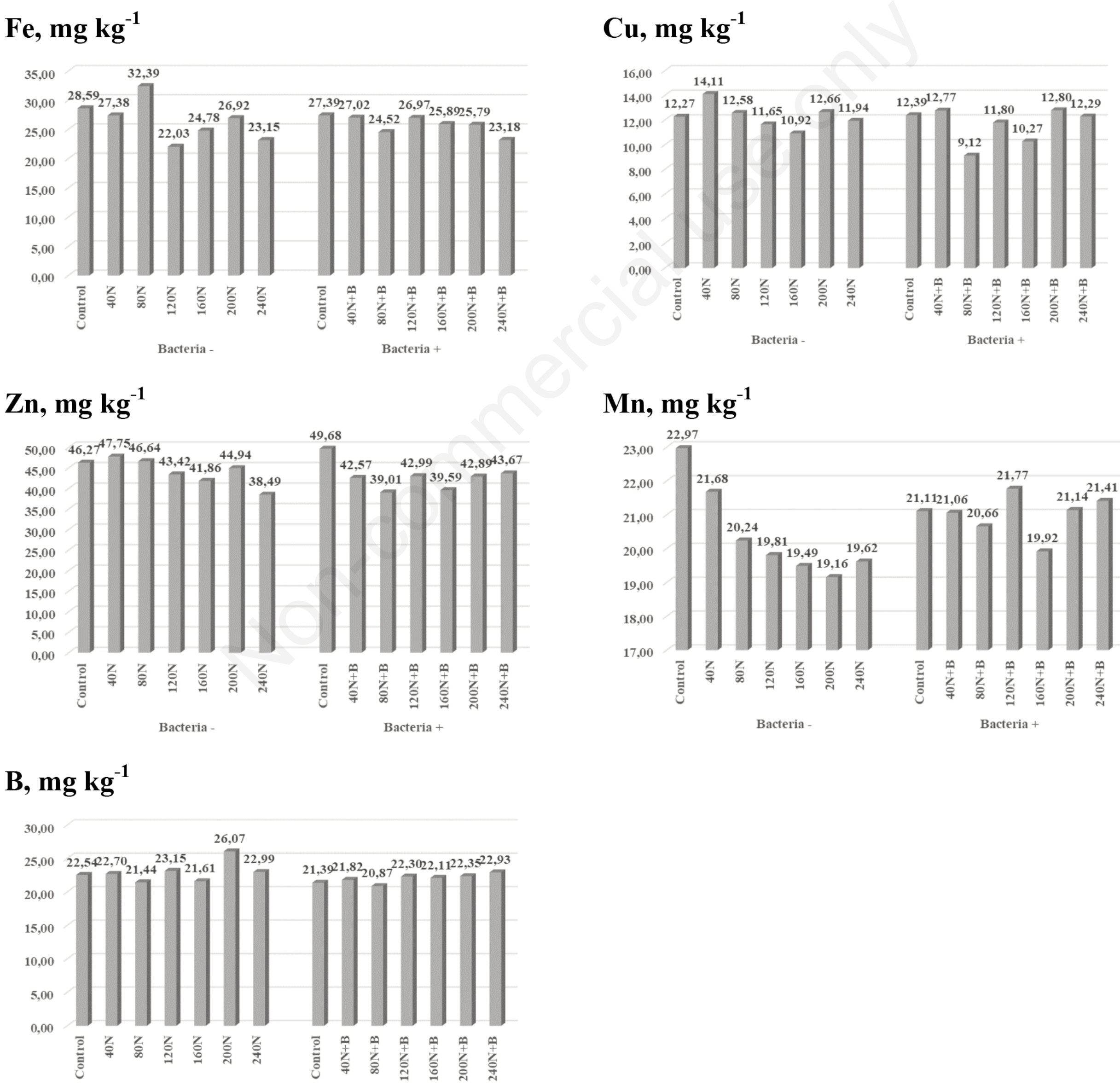


**Figure 2.** Micro-element content of *Halisbey* peanut seeds presented as the average of 2015 and 2016.

revealed that the highest value was obtained from the 200N kg ha$^{-1}$ application with 26.07 mg kg$^{-1}$, while the lowest value was obtained from the 80N+B treatment with 20.87 mg kg$^{-1}$ (Table 4 and Figure 2).

## Discussion

According to Figure 1, it is evident that the N content of the seeds is expected to increase with soil N levels. El-Kader *et al*. (2006), Solhi and Molahoseini (2013) and Kaplan *et al*. (2022) also emphasized that N fertilization has a positive effect on N accumulation in seeds. Purushotham and Hosman (1994) concluded that N had a beneficial effect on the plant's metabolic process and growth, thus having a positive effect on the chemical content of peanut seeds. In a study conducted with 0, 20, 40, and 60 kg ha$^{-1}$ different N doses, as well as *Rhizobium* and *Enterobacter* inoculation, Awadalla and Mohammed (2017) stated that N fertilizer doses and *Rhizobium* inoculation not only increased the yield of peanuts but also enhanced their nutritional value. Steer and Hocking (1984), in their study with different N doses (0, 30, 60, 90, 120 and 150 kg N ha$^{-1}$), stated that the N content of seeds was closely related to the N applied to the plant, as well as cultivar characteristics and environmental conditions. Our findings are consistent with this study. P content of the seed varied from 0.51%, 0.49%, 0.48%, 0.46%, 0.48% and 0.43% with 40N, 80N, 120N, 160N, 200N, and 240N kg ha$^{-1}$ N doses. In their study conducted with N, *Rhizobium*, and *Enterobacterium*, Awadalla and Mohammed (2017) identified that the %P content of peanuts was not significantly affected. In our findings, the highest P content was obtained from the control application (Table 3). Robinson *et al.* (1973), Mathers and Stewart (1982), and Lasztity (1983) reported that the Ca content in the seed decreased as N fertilization levels increased. Peanut plants remove the most N, K and Ca among macro- and micro-elements from the soil (Beasley, 1990; Shibata and Yano, 2003; Gök *et al*., 2004; Gök *et al*., 2005). Coarse grains (peanut seed) generally require twice as much Ca concentration as small grains in soils with varying Ca concentrations (Walker and Keisling, 1978; Gascho and Davis, 1994). Ca is used directly in the formation of seeds inside the pod. Therefore, the Ca mineral is highly significant for peanut plants (Arıoğlu, 2014). The soil analysis results suggested that peanuts grown in calcareous (Ca-rich) soils had high internal proportions of Ca. Our findings are in agreement with the study. In our experiment, the highest Ca content of peanut seeds was obtained in the bacterial inoculation treatment. Chen *et al*. (2022) found the Mg content, which is one of the macro-elements, in peanuts to be 0.161-0.176%. These results are consistent with our findings (0.185-0.228%) (Table 3). Different N doses and bacterial applications had no effect on Mg content. Na content of the plants was generally reported to vary between 0.01 and 10%, and legumes contained more Na than non-leguminous plants (Karaman, 2012). According to our study, the effect of treatments, years, and years × treatments interaction on Na content was found to be insignificant (Table 4 and Figure 1). Kaplan *et al*. (2022) reported that Na content decreased with increasing N doses in their research with different N doses on maize. Increasing N doses reduced the Fe content in the seed (Figure 2). The interactions between years and treatments were identified as insignificant (Table 4). In their study, Bozkurt and Karacal (2000) mentioned that increasing N doses reduced the Fe content of sunflowers. On the other hand, Lasztity (1983) and Salama and Buzas (1987) pointed out that N fertilizer had no effect on the Fe content of sunflowers. Fe is the fourth most abundant element in the earth's crust and soil. Fe deficiency in peanuts is the most common of all microelements in highly calcareous soils (Kadiroğlu, 2023). Peroxidase activity in leaves, stems, and roots of varieties with good Fe content is higher than 0.3, 0.6 and 0.15, respectively, and those showing lower activities are susceptible to iron chlorosis (Singh, 1999). Gowda *et al*. (1993) concluded that Fe absorption efficiency is dominant and infertility is hesitant in peanuts. The effect of different N doses on the Cu element content was positive. The application of a 40N dose resulted in the highest Cu content, measured at 14.11 mg kg$^{-1}$.

Kaplan *et al*. (2022) reported that the N doses applied to the corn grain increased the Cu content. The Mn content of the *Halisbey* peanut variety showed an irregular increase and decrease with bacteria and increasing N doses, and the highest value was obtained from the control application (Table 4). The Zn rate obtained from the seeds in the plots where both N and bacterial application were applied together was lower than that of the plots with only bacterial application (Figure 2). The effects of the applications on B were not found to be significantly different. Kaplan *et al*. (2022), in their study on both irrigation levels and N doses, found that N doses did not have a significant effect on the B content of maize grains. In their study conducted with N, *Rhizobium*, and *Entrobacterium* applications, Awadalla and Mohammed (2017) pointed out that N fixation increased the yield and yield components in *Rhizobium* inoculation to the seeds, and soil pH decreased in the rhizosphere region, which increased the amount of absorbable macro- and micronutrients.

## Conclusions

While different N doses significantly affected the N, Na, Fe, and Cu content of *Halisbey* peanuts, there was no change in P, K, Ca, Mg, Mn, Zn, and B contents. Bacterial inoculation significantly increased the Ca and Zn contents. The N content of the *Halisbey* variety increased significantly with the application of the highest N dose and bacterial inoculation (240N+B). This information will help determine the optimal nutrient requirements of the *Halisbey* peanut variety and its adaptation to the region where it is grown.

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