

EFFECT OF *AZOTOBACTER CHROOCOCCUM* XH2018 STRAIN ON THE UPTAKE OF CERTAIN ELEMENTS IN WHEAT (*TRITICUM AESTIVUM* L.) AT THE TILLERING STAGE

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Abstract. *The effect of the biological preparation "Bioazot," developed on the basis of Azotobacter chroococcum XH2018 strain, on the uptake of macro- and microelements in wheat (Triticum aestivum L.) was investigated. The "Mezon" winter wheat cultivar was analyzed at the tillering stage, and elemental concentrations in root and shoot samples were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The experiment was conducted in 14 variants, with the Bioazot preparation applied in various combinations with mineral fertilizers. The results demonstrated that A. chroococcum XH2018 strain enhances the bioavailability of nutritional elements in plants, particularly promoting the accumulation of physiologically essential elements such as Fe, Zn, Mn, K, and Mg in the root system. Correlation analyses revealed strong synergistic and antagonistic relationships between certain elements. Specifically, strong positive correlations were recorded among Fe, Mn, Ba, and Cr, whereas potassium exhibited negative correlations with certain microelements. The findings, consistent with data from other studies, confirm the significant role of Azotobacter-based biofertilizers in improving wheat nutrition and mineral metabolism. The study demonstrates the potential of Bioazot as an ecologically safe and effective biological fertilization agent.*

Keywords: *bioazot, macro-and microelements, ICP-OES, correlation relationships, root, stem.*

INTRODUCTION

Microorganisms, particularly rhizobacteria, play an important role in enhancing plant growth, development, and productivity [8]. Rhizobacteria influence nutrient uptake, immune system formation, and element assimilation processes in plants [5,6,21]. In addition, rhizobacteria participate in the translocation of macro- and microelements within plants [14]. In particular, they exert a positive effect on the mobility of elements such as iron (Fe), zinc (Zn), and magnesium (Mg), which are of special significance in plant metabolism [12,14]. These elements play a crucial role in protein and chlorophyll synthesis as well as enzymatic activity in plants [9,15]. Deficiency of these elements may lead to suppression of vegetative growth, chlorosis, disruption of metabolic processes, and reduction in yield [13].

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide and holds strategic significance in ensuring global food security. Under conditions of increasing population growth, global climate change, declining soil fertility, and intensifying anthropogenic pressure on agroecosystems, sustainably improving wheat yield and enhancing mineral nutrition efficiency represent one of the most pressing directions of modern agrobiotechnology [3].

The tillering stage, one of the key vegetative phases of wheat ontogenesis, represents a physiologically critical period in the formation of yield structure. It is during this stage that lateral shoot formation, active root system development, expansion of leaf area, and intensive accumulation of vegetative biomass are observed. The formation and viability of tillers directly influence the number of productive stems, spike elements, and final grain yield [19]. Long-term agrobiological observations have emphasized that physiological processes and nutritional conditions during the tillering stage are among the primary factors determining the yield potential of wheat [18].

During the tillering stage, the plant's demand for nutritional elements increases sharply, as this period is characterized by intensified cell division, tissue differentiation, chloroplast formation, enzymatic reactions, and development of the photosynthetic apparatus [9].

The aim of this study was to determine the accumulation, quantitative distribution, and translocation characteristics of macro- and microelements in the roots and shoots of wheat (*Triticum aestivum* L.) at the tillering stage under the influence of Bioazot suspension prepared on the basis of *A. chroococcum* XH2018 strain.

MATERIALS AND METHODS

2.1. Research Material

The "Mezon" winter wheat cultivar (*Triticum aestivum* L.), propagated at the Institute of Genetics and Experimental Biology of Plants of the Academy of Sciences of the Republic of Uzbekistan, was selected as the research object. This cultivar is distinguished from other registered varieties by its high yield potential, early maturity, resistance to yellow rust, lodging tolerance, and good adaptability to diverse soil and climatic conditions. According to data from the institute's appraisal commission, the "Mezon" cultivar has an average stem height of 95 cm, a 1,000-grain weight of 44.8 g, a gluten content of 29.4%, and an average yield of 70–80 centners per hectare.

The study was conducted at the tillering stage of wheat development. Root and shoot samples were collected from plants for analysis.

2.2. Bacterial Strain and Biological Preparation

The free-living nitrogen-fixing *A. chroococcum* XH2018 strain was used throughout the study. This strain possesses the ability to convert atmospheric molecular nitrogen into forms assimilable by plants, synthesize phytohormones, and enhance the bioavailability of nutritional elements in the rhizosphere [1,10].

The strain was propagated in liquid nutrient medium under standard microbiological conditions, and the biological preparation "Bioazot" was produced from the active biomass. The prepared formulation was subsequently applied for the nutritional treatment of wheat plants in experimental trials.

2.3. Experimental Variants and Wheat Fertilization

The effect of the "Bioazot" biological preparation, developed on the basis of *A. chroococcum* XH2018 strain, on the accumulation and distribution of macro- and microelements in wheat plants was investigated throughout the study.

The experiment was organized in a total of 14 variants. The experimental variants are presented in Table 2.1.

Table 2.1 Experimental Variants and Applied Fertilization Scheme

Variant	Preparation	Bioazot (ml)	Urea (g)	Ammophos (g)	Potassium (g)
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V1	Control	–	–	–	–
V2	Bioazot	177.5	–	–	–
V3	Bioazot+NPK	177.5	177.5	106.5	35.5
V4	Bioazot+NPK	177.5	177.5	142.0	35.5
V5	Bioazot+NPK	177.5	177.5	177.5	3.55
V6	Bioazot+NPK	177.5	177.5	177.5	35.5
V7	Bioazot+NPK	177.5	213.0	106.5	35.5
V8	Bioazot+NPK	177.5	213.0	142.0	35.5
V9	Bioazot+NPK	177.5	213.0	177.5	35.5
V10	Bioazot+NPK	177.5	213.0	213.0	35.5
V11	Bioazot+NPK	177.5	213.0	106.5	53.25
V12	Bioazot+NPK	177.5	213.0	142.0	53.25
V13	Bioazot+NPK	177.5	213.0	177.5	53.25
V14	Bioazot+NPK	177.5	213.0	213.0	53.25

The volume of Bioazot preparation in each variant was 177.5 ml. Mineral fertilizers were applied in various combinations, including urea (177.5–213 g), ammophos (106.5–213 g), and potassium (35.5–53.25 g). The prepared suspensions were applied to wheat plants at the tillering stage.

2.4. Preparation of Plant Samples

Root and shoot samples were collected separately from wheat plants at the tillering stage. Under laboratory conditions, the samples were washed with distilled water and cleaned of external contaminants.

The cleaned samples were dried at 65–70°C until constant mass was achieved. The dried samples were ground in a laboratory mill and brought to a uniform granulometric fraction. The prepared samples were stored in hermetically sealed containers for subsequent chemical analyses.

2.5. Sample Mineralization

For elemental analysis, 0.1000 g of each dried sample was weighed on an analytical balance and placed into Teflon autoclaves.

To each sample, 3 ml of high-purity concentrated nitric acid (HNO₃) and 2 ml of hydrogen peroxide (H₂O₂) were added. The autoclaves were tightly sealed and placed into a microwave mineralization system - the Berghof Speedwave Xpert.

The wet digestion process was carried out at temperatures ranging from 50 to 230°C under a maximum pressure of 40 bar for 35–45 minutes. Following mineralization, the solutions were cooled to room temperature and quantitatively transferred into 50 ml volumetric flasks, diluted to the mark with bidistilled water, and thoroughly mixed.

2.6. Determination of Macro- and Microelements by ICP-OES

The quantitative composition of macro- and microelements in the mineralized solutions was determined using a Perkin Elmer Avio-200 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

During the analysis, macroelements including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), as well as microelements including iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), and others, were quantitatively evaluated by calibration against standard multi-element solutions. Analyses were conducted using argon gas of 99.995% purity. The obtained results were automatically recalculated by the instrument's software,

accounting for sample mass and dilution coefficients, and relative standard deviation (RSD) values were determined [2,4].

2.7. Statistical Analysis

Statistical processing of the obtained data was performed using Microsoft Excel and PAST software. Spearman's rank correlation analysis was conducted to evaluate the interrelationships among macro- and microelements across the experimental variants.

RESULTS

3.1. Accumulation Characteristics of Elements in the Wheat Root System

3.1.1. Quantitative Characterization of Elements in Root Tissues

A descriptive statistical analysis was performed to evaluate the quantitative composition of macro- and microelements in the root portion at the tillering stage. According to the analysis results, considerable variation was observed in the accumulation levels of elements in the root tissues of wheat (*Triticum aestivum* L.). Among the elements studied, the highest mean concentration was recorded for potassium (K), with a value of $13,459.07 \pm 2,518.65$ mg/kg. In addition, iron (Fe) ($6,037.74 \pm 1,661.67$ mg/kg), aluminum (Al) (438.03 ± 97.60 mg/kg), and manganese (Mn) (275.78 ± 110.39 mg/kg) were also found to accumulate at relatively high levels in root tissues. Among microelements, the mean concentrations of zinc (Zn) and barium (Ba) were 65.51 ± 16.47 and 60.66 ± 14.72 mg/kg, respectively. In contrast, mercury (Hg) (0.026 ± 0.003 mg/kg), lead (Pb) (0.95 ± 0.64 mg/kg), and lithium (Li) (0.22 ± 0.38 mg/kg) were recorded at the lowest concentrations. According to the coefficient of variation analysis, the highest variability was observed for lithium (Li; 172.72%), lead (Pb; 66.97%), and vanadium (V; 45.68%), indicating that the accumulation of these elements in response to fertilization variants is highly variable. The mean concentrations and standard deviation values of macro- and microelements in root tissues are presented in Figure 3.1.

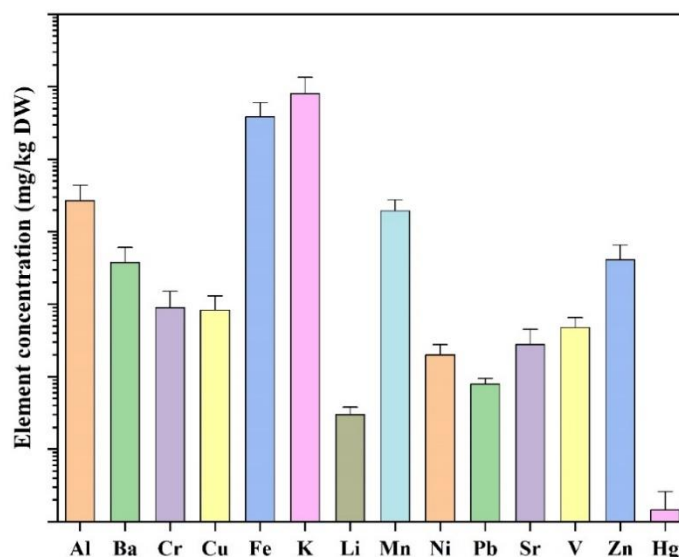


Figure 3.1. Mean concentrations of macro- and microelements accumulated in root tissues of *Triticum aestivum* L. at the tillering stage (mean $\pm$ SD, n = 14).

3.1.2. Correlation Relationships Among Elements in Root Tissues

Spearman's rank correlation analysis was performed to assess the interrelationships among macro- and microelements determined in the root portion. The correlation analysis confirmed the existence of statistically significant positive and negative associations among certain elements. The correlation coefficients between elements are presented in Table 3.1.

Table 3.1 Spearman Correlation Relationships Among Elements Determined in Wheat Root Tissue

Element Pair	r_s	p	Correlation Level
Al – Fe	0.91	<0.001	Very strong positive
Al – Mn	0.84	<0.001	Strong positive
Al – V	0.84	<0.001	Strong positive
Ba – Mn	0.97	<0.001	Very strong positive
Ba – V	0.92	<0.001	Very strong positive
Cr – Fe	0.96	<0.001	Very strong positive
Cr – Mn	0.84	<0.001	Strong positive
Cu – Mn	0.79	<0.001	Strong positive
Fe – Mn	0.81	<0.001	Strong positive
Fe – V	0.85	<0.001	Strong positive
K – V	-0.88	<0.001	Strong negative
K – Mn	-0.85	<0.001	Strong negative
K – Ba	-0.81	<0.001	Strong negative
Zn – Ni	0.70	0.006	Strong positive
Hg – Ni	0.71	0.005	Strong positive

Note: r_s — Spearman's rank correlation coefficient; p — significance level; $p < 0.05$ was considered statistically significant.

The interrelationships among macro- and microelements determined in the root portion of wheat (*Triticum aestivum* L.) were assessed using Spearman's rank correlation analysis. The results revealed statistically significant positive and negative associations among certain elements.

Aluminum (Al) showed strong positive correlations with barium (Ba) ($r_s = 0.88$; $p < 0.001$), chromium (Cr) ($r_s = 0.88$; $p < 0.001$), iron (Fe) ($r_s = 0.91$; $p < 0.001$), manganese (Mn) ($r_s = 0.84$; $p < 0.001$), and vanadium (V) ($r_s = 0.84$; $p < 0.001$). This suggests that these elements may share interdependent transport and accumulation mechanisms in the root system.

Barium (Ba) demonstrated very strong positive correlations with manganese (Mn) ($r_s = 0.97$; $p < 0.001$) and vanadium (V) ($r_s = 0.92$; $p < 0.001$). Similarly, chromium (Cr) exhibited a strong association with iron (Fe) ($r_s = 0.96$; $p < 0.001$).

Potassium (K) showed negative correlations with several elements, including aluminum (Al) ($r_s = -0.71$; $p < 0.01$), barium (Ba) ($r_s = -0.81$; $p < 0.001$), iron (Fe) ($r_s = -0.78$; $p < 0.01$), manganese (Mn) ($r_s = -0.85$; $p < 0.001$), and vanadium (V) ($r_s = -0.88$; $p < 0.001$). This indicates that an increase in potassium concentration may exert an antagonistic effect on the accumulation of certain microelements in the roots.

A moderate positive association was recorded between nickel (Ni) and zinc (Zn) ($r_s = 0.70$; $p < 0.01$). Mercury (Hg) showed a positive correlation with nickel (Ni) ($r_s = 0.71$; $p < 0.01$) and a negative correlation with lead (Pb) ($r_s = -0.61$; $p < 0.05$).

3.2. Accumulation Characteristics of Elements in the Wheat Shoot System

3.2.1. Quantitative Characterization of Elements in Shoot Tissues

A descriptive statistical analysis was performed to evaluate the quantitative composition of macro- and microelements in the shoot portion at the tillering stage. The analysis revealed considerable variation in the accumulation levels of elements in the shoot tissues of wheat (*Triticum aestivum* L.). Among the elements studied, the highest mean concentration was recorded for potassium (K) at 818.38 ± 192.00 mg/kg. Iron (Fe) (249.78 ± 104.44 mg/kg) and barium (Ba) (27.51 ± 13.73 mg/kg) were also detected at relatively high levels. The mean concentrations of lithium (Li), chromium (Cr), and zinc (Zn) were 10.52 ± 0.50 , 6.57 ± 1.90 , and 5.94 ± 2.60 mg/kg, respectively. In contrast, cadmium (Cd) (0.008 ± 0.002 mg/kg), lead (Pb) (0.47 ± 0.29 mg/kg), and osmium (Os) (0.59 ± 0.32 mg/kg) were recorded at the lowest concentrations. According to the coefficient of variation analysis, the highest variability was observed for lead (Pb; 61.86%), osmium (Os; 54.71%), and barium (Ba; 49.93%). The mean concentrations and standard deviation values of macro- and microelements in shoot tissues are presented in Figure 3.2.

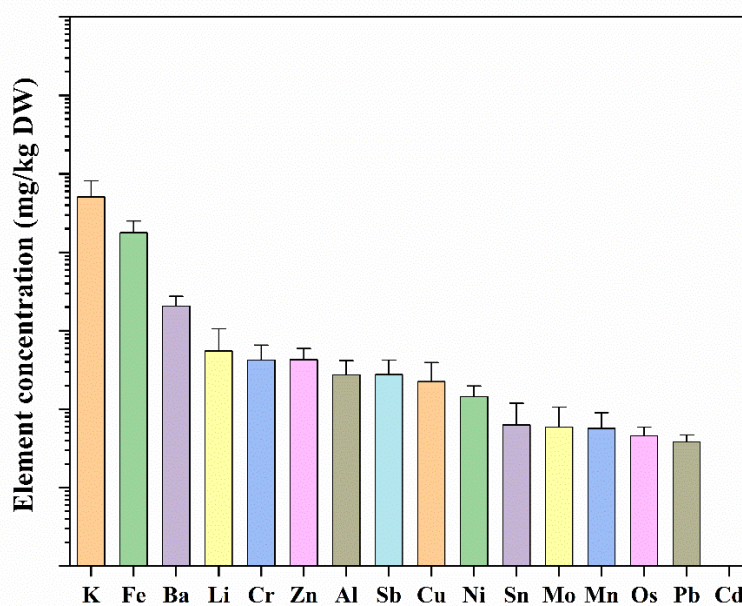


Figure 3.2. Mean concentrations of macro- and microelements accumulated in shoot tissues of *Triticum aestivum* L. at the tillering stage (mean $\pm$ SD, n = 14).

Among the elements studied in wheat shoot tissues, the highest concentration was recorded for potassium (K), with a mean value of 818.38 ± 192.00 mg/kg. Iron (Fe) (249.78 ± 104.44 mg/kg) and barium (Ba) (27.51 ± 13.73 mg/kg) were also detected at relatively high levels. The highest variability based on the coefficient of variation was observed for lead (Pb; 61.86%), osmium (Os; 54.71%), and barium (Ba; 49.93%), indicating that fertilization variants had a considerable influence on the accumulation of these elements in shoot tissues. In contrast, lithium (Li; 4.72%) and tin (Sn; 5.78%) showed low variation, suggesting their relatively stable distribution along the shoot.

3.2.2. Correlation Relationships Among Elements in Shoot Tissues

The interrelationships among macro- and microelements determined in the shoot portion of wheat (*Triticum aestivum* L.) were assessed using Spearman's rank correlation analysis.

Statistically significant positive and negative associations were observed among a number of elements. The correlation coefficients between elements are presented in Table 3.2.

Table 3.2 Spearman Correlation Relationships Among Elements Determined in Wheat (*Triticum aestivum* L.) Shoot Tissue

Element Pair	r_s	p	Correlation Level
Al – Os	-0.61	0.020	Strong negative
Al – Fe	0.56	0.014	Moderate positive
Al – Sb	0.66	0.010	Strong positive
Cd – Zn	-0.69	0.012	Strong negative
Cd – Os	0.86	<0.001	Very strong positive
Cd – Pb	0.81	<0.001	Very strong positive
Ni – Os	0.84	<0.001	Very strong positive
Ni – Ba	0.92	<0.001	Very strong positive
Ni – Cr	0.93	<0.001	Very strong positive
Pb – Os	0.84	<0.001	Very strong positive
Zn – Cu	-0.93	<0.001	Very strong negative
Zn – Li	-0.89	<0.001	Very strong negative
Ba – Cr	0.98	<0.001	Very strong positive
Ba – Sb	0.93	<0.001	Very strong positive
Cr – Sb	0.95	<0.001	Very strong positive
Cu – Fe	-0.81	<0.001	Very strong negative
Fe – Mn	-0.73	0.003	Strong negative
K – Mn	-0.66	0.009	Strong negative
K – Mo	0.57	0.031	Moderate positive

Note: r_s — Spearman's rank correlation coefficient; p — significance level; $p < 0.05$ was considered statistically significant.

Among the elements determined in shoot tissues, barium (Ba) exhibited very strong positive correlations with nickel (Ni) ($r_s = 0.92$; $p < 0.001$), chromium (Cr) ($r_s = 0.98$; $p < 0.001$), and antimony (Sb) ($r_s = 0.93$; $p < 0.001$). Furthermore, a very strong positive association was also recorded between chromium (Cr) and antimony (Sb) ($r_s = 0.95$; $p < 0.001$), suggesting that these elements may share similar transport and accumulation mechanisms in shoot tissues.

Cadmium (Cd) showed positive associations with osmium (Os) ($r_s = 0.86$; $p < 0.001$), lead (Pb) ($r_s = 0.81$; $p < 0.001$), and nickel (Ni) ($r_s = 0.63$; $p < 0.05$), indicating a tendency for co-accumulation of certain heavy metals in wheat shoot tissues.

Iron (Fe) showed moderate positive correlations with molybdenum (Mo) ($r_s = 0.65$; $p < 0.05$) and potassium (K) ($r_s = 0.60$; $p < 0.05$), which may reflect functional interactions of these elements in metabolic processes and physiological transport systems.

Among the negative correlations, zinc (Zn) exhibited very strong negative associations with copper (Cu) ($r_s = -0.93$; $p < 0.001$) and lithium (Li) ($r_s = -0.89$; $p < 0.001$). A strong negative correlation was also recorded between iron (Fe) and copper (Cu) ($r_s = -0.81$; $p < 0.001$). These

findings indicate the presence of antagonistic transport or competitive uptake mechanisms between certain elements.

3.3. Translocation of Elements from Root to Shoot

The translocation factor (TF) was calculated to evaluate the intensity of element transfer from roots to aboveground vegetative organs. TF values were examined based on the mean concentrations of elements in root and shoot tissues. The translocation coefficients of elements from root to shoot are presented in Table 3.3.

Table 3.3 Translocation Factor of Elements from Root to Shoot Tissues in Wheat (*Triticum aestivum* L.)

Element	Root	Shoot	TF
Al	438.03	4.16	0.009
Ba	60.66	27.51	0.45
Cr	15.04	6.57	0.44
Cu	13.05	3.93	0.30
Fe	6037.74	249.78	0.04
K	13459.07	818.38	0.06
Li	0.22	10.52	47.64
Mn	275.78	0.90	0.003
Ni	2.77	1.98	0.71
Pb	0.95	0.47	0.49
Zn	65.51	5.94	0.09

Note: TF — Translocation Factor; $TF = C_{shoot} / C_{root}$. TF > 1 indicates active translocation; TF < 1 indicates that the element is predominantly retained in root tissues.*

For the majority of the elements studied, TF values were below 1, indicating their predominant retention in the root system. Particularly low translocation was observed for aluminum (Al; TF = 0.009), manganese (Mn; TF = 0.003), iron (Fe; TF = 0.04), and potassium (K; TF = 0.06). In contrast, lithium (Li) exhibited a TF of 47.64, indicating active transport of this element from roots to shoot tissues. Nickel (Ni; TF = 0.71) and lead (Pb; TF = 0.49) also showed relatively higher translocation.

DISCUSSION

Data obtained at the tillering stage of wheat demonstrated a non-uniform distribution of macro- and microelements across plant organs. The results showed that Fe, K, Mn, Al, and Zn accumulated predominantly in the root system. For instance, the mean concentrations of Fe and K in roots were 6,037.74 mg/kg and 13,459.07 mg/kg, respectively, while these values were considerably lower in shoot tissues. This difference reflects the important physiological role of roots in absorbing mineral elements and retaining them prior to subsequent transport. Similar findings have been reported by other authors during the vegetative development period of wheat [7,16].

The degree of elemental variability also revealed certain noteworthy patterns. In root tissues, Li and Pb, and in shoot tissues, Pb and Os, exhibited high variability. This implies that elements were not uniformly distributed across all variants, and that various combinations of Bioazot and mineral fertilization may have had a considerable effect on the uptake of certain elements. Numerous studies have demonstrated that bacteria belonging to the *Azotobacter*

chroococcum species can enhance the solubility of nutritional elements in the rhizosphere through the production of organic acids, siderophores, and phytohormones [1,10].

Correlation relationships in root tissues indicated that elements do not act independently of one another. The strong positive associations identified between Al and Fe, Ba and Mn, and Cr and Fe suggest that the entry of these elements into the plant or their accumulation in tissues may be regulated through shared physiological pathways. In contrast, the negative correlations between K and Ba, Mn, and V indicate the presence of competitive interactions among certain ions. Selective mineral transport and inter-ionic antagonism in wheat have been reported previously [11].

Results obtained from shoot tissues also indicated that certain patterns are maintained during upward element movement. The very strong associations of Ba with Cr, Ni, and Sb allow for the hypothesis that common mechanisms are involved in the xylem transport of these elements. On the other hand, the negative correlations of Zn with Cu and Li suggest that competition arises among certain microelements during transport or redistribution. This further confirms that the plant's nutrient management system is considerably complex.

The translocation factor results constituted one of the most significant aspects of the study. For nearly all elements examined, TF values were below 1, confirming their predominant retention in the root system. This was particularly evident for Mn, Al, Fe, and K. Thus, the root system restricts the transfer of these elements to aboveground organs to a certain degree. In contrast, Li exhibited a notably high TF value, indicating its relatively unrestricted movement into shoot tissues. The retention of certain microelements in wheat roots and their limited transfer to aboveground organs have been confirmed in other studies as well [17,20].

In particular, the data obtained demonstrated that combinations of the Bioazot preparation based on *A. chroococcum* XH2018 strain and mineral fertilization exerted a considerable influence on the processes of element uptake, accumulation, and translocation from root to shoot in wheat. The enhancement of microbiological activity in the rhizosphere in the presence of Bioazot, the conversion of nutritional elements into plant-available forms, and the activation of ion exchange processes may be among the primary reasons underlying these results.

CONCLUSION

The distribution characteristics of macro- and microelements in root and shoot tissues of *Triticum aestivum* L. at the tillering stage were investigated under the influence of the Bioazot preparation based on *A. chroococcum* XH2018 strain and various mineral fertilization variants. The results demonstrated that the majority of elements accumulated predominantly in the root system, with Fe, K, Mn, Al, and Zn in particular accumulating at considerably higher levels in root tissues compared to shoot tissues.

Correlation analysis revealed strong positive and negative associations among elements, confirming that the uptake and internal transport of mineral elements in wheat are governed by complex synergistic and antagonistic relationships. Translocation factor results indicated $TF < 1$ for the majority of elements, signifying their predominant retention in the root system. The high TF value recorded for lithium (Li) suggests that certain elements undergo more active translocation toward vegetative organs.

Furthermore, it was established that combinations of the Bioazot preparation based on *A. chroococcum* XH2018 strain and mineral fertilization may exert a considerable influence on the bioavailability of elements, their redistribution across organs, and their migration within the root–shoot system. The findings indicate that the use of biological fertilizers holds significant practical

value for enhancing the efficiency of nutrient element utilization and optimizing mineral nutrition processes in wheat.

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