

Microbial Bioinoculation as an Effective Strategy to Enhance Plant Potassium Uptake for Sustainable Agriculture

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Abstract

The present investigation was conducted to isolate and characterize potassium-solubilizing bacteria and to determine their potassium mobilization potential using in vitro assays and pot experiments. Among the soil-derived isolates, strain AOB28 showed a high K-solubilizing potential, reaching 50.09 mg L⁻¹ K relative to the uninoculated treatment. The K-solubilization capacity of the strain was further evaluated under varying environmental conditions, revealing maximum K release at 40°C, pH 6, and a mica concentration of 9 g L⁻¹. The enzymatic profile of strain AOB28 revealed notable amylase and protease activities, along with detectable CMCase activity, and the strain displayed considerable tolerance to both salt and heavy metals, supporting its suitability for biotechnological applications. Pot experiments demonstrated that bacterial inoculation significantly enhanced plant growth relative to the uninoculated control, as evidenced by a 62% increase in dry matter accumulation. In parallel with enhanced biomass production, bacterial treatment substantially modified mineral nutrient uptake. Notably, AOB28 *B. subtilis* enhanced K uptake in arugula plant grown under pot conditions (+ 63.5%), markedly decreased Ca uptake (-29%), and moderately increased Mg and P uptake (+ 19% and + 27%, respectively). For micronutrient accumulation, Mn and Zn increased significantly by 37.9% and 42.6%, respectively, in AOB28-treated plants, whereas Fe and Cu uptake were not statistically significant. Among the evaluated nutrients, potassium exhibited the most pronounced response, indicating that improved K acquisition was a key contributor to the observed growth stimulation and corroborating the results obtained from the in vitro solubilization assays. These findings suggest that such bacterial strains could be used in balanced fertilization strategies for arugula, but their application should be carefully managed, and further research is needed to understand their mechanisms and broader potential in agriculture.

Introduction

Potassium (K) is a vital cationic macronutrient involved in key plant functions, including osmoregulation, membrane potential regulation, carbon assimilation, nutrient use efficiency, and stress tolerance (Sanyal et al. 2020; Sardans and Peñuelas 2021; Dahuja et al. 2021). The majority of soil K exists predominantly in mineral and fixed forms, rendering it largely inaccessible to plants without specific mobilization mechanisms (Soumare, 2023). Approximately 2% of the total K present in the soil is directly available for plant uptake, while the fixation and release of K in soils are influenced by various soil physical and chemical properties, plant–soil interactions, and soil microbial activities (Zörb et al. 2014).

Using eco-friendly microbial fertilizers can be an effective approach to achieving sustainable agriculture and minimizing chemical fertilizer use (Bakhshandeh et al. 2015; Kumar et al. 2022; Rai et al. 2023). While conventional fertilizers provide short-term increases in K availability, mica-supported K-solubilizing bacteria (KSB) represent a long-term alternative for improving soil K availability (Akkaya et al. 2024). In this context, certain bacteria such as *Pseudomonas*, *Bacillus*, *Klebsiella*, and *Pantoea* have been shown to solubilize K from insoluble minerals like mica and illite through the secretion of organic acids and various other mechanisms (Meena et al. 2015). K-solubilizing bacteria release K from mineral structures by secreting organic acids such as citric, oxalic, tartaric, succinic, and α-ketoglutaric acids. Additionally,

they enhance the dissolution of K compounds by proton extrusion and complexation of metal ions such as Fe^{2+} , Al^{3+} , and Ca^{2+} (Meena et al. 2016).

Evidence indicates that plants inoculated with *Bacillus* spp. exhibit significant increases in root length and total biomass, underscoring their potential as bio-stimulants, particularly under stress conditions such as saline soils that restrict plant growth (Neta et al. 2024). Among these bacteria, *Bacillus* spp. has attracted increasing attention due to its ability to promote plant growth and improve soil quality through the modulation of diverse biochemical and physiological processes (Saha et al. 2016; Nawaz et al. 2020). Its growth-promoting effects are associated with beneficial interactions within the rhizosphere, enhancing plant tolerance to multiple stress factors and facilitating adaptation across diverse ecosystems, as supported by metagenomic studies (Dinu et al. 2019; Umiralieva et al. 2021). Extracellular enzymes such as cellulase, amylase, and protease secreted by microorganisms facilitate the decomposition of organic waste (Saha and Santra 2014; Priyadarshini et al. 2019; Su et al. 2021). Moreover, *B. subtilis* has been reported to synthesize indole-3-acetic acid (IAA), thereby stimulating root development and increasing nutrient uptake (Anguiano Cabello et al. 2019). In this study, *B. subtilis* was isolated and characterized, and its potassium-solubilizing capacity was evaluated under varying pH, temperature, and mica concentrations. The strain's enzymatic activities and tolerance to stress conditions were also determined, and its effects on plant growth and nutrient uptake were assessed through controlled pot experiments using arugula (*Eruca vesicaria*).

Material and Method

Bacterial Isolation from Soil Environment

Isolates were obtained from an agricultural surface soil sample (0–20 cm depth) collected from a walnut orchard located in the Kahramanmaraş–Afşin region (WGS 84: 39.4347° N, 37.6371° E; elevation 1434 m). The sample were initially sterilized at 80 °C for 10 min to eliminate heat-sensitive microorganisms and to select for spore-forming bacteria, and were subsequently subjected to serial dilution up to 10^{-5} . For bacterial isolation, 0.1 mL of each dilution was spread onto Nutrient Agar plates (pH 7.0). The resulting colonies were subcultured to obtain pure cultures. The isolate was subsequently subjected to phenotypic characterization, including gram staining, spore staining, catalase activity, and motility tests. Finally, the purified cultures were stored on Nutrient Agar slants at 4 °C for further analyses.

Assessment of K Solubilization Potential of Isolates

The K solubilization potential of the isolates was assessed using Aleksandrov medium (Glucose 5 g L⁻¹, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.005 g L⁻¹, FeCl_3 0.1 g L⁻¹, CaCO_3 2 g L⁻¹, $\text{Ca}_3(\text{PO}_4)_2$ 2 g L⁻¹, mica 1 g L⁻¹, and agar 20 g L⁻¹). Overnight cultures were spot-inoculated and incubated at 35 ± 2 °C for up to 8 days. Strains forming clear zones were identified as K solubilizers, with AOB28, AOB89, AOB90, AOB92, AOB93, and AOB94 showing the strongest activity.

Quantitative Analysis of K Solubilization

For the quantitative assessment of K solubilization by KSB, 10 mL of Aleksandrov broth medium (Glucose 5 g L⁻¹, MgSO₄·7H₂O 0.005 g L⁻¹, FeCl₃ 0.1 g L⁻¹, CaCO₃ 2 g L⁻¹, Ca₃(PO₄)₂ 2 g L⁻¹, and mica 1 g L⁻¹) was employed (Prajapati et al. 2013). To conduct the assay, 0.1 mL of cultures corresponding to 0.5 McFarland standard turbidity (1×10⁸ cfu mL⁻¹) suspended in sterile physiological saline were inoculated into Aleksandrov broth media.

Following inoculation, the media were incubated in a shaker incubator (New Brunswick Scientific, Edison, NJ, USA) at 150 rpm and 30 ± 2 °C for 72 h. Bacterial growth was determined by measuring optical density at 600 nm using a spectrophotometer (Model EZ-150, PerkinElmer, Waltham, MA, USA). Cultures were then centrifuged at 3350 × *g* for 10 min using a Hettich Mikro 22R centrifuge (Hettich, Tuttlingen, Germany), and the concentration of dissolved K in the supernatant was quantified by Atomic Absorption Spectrophotometry (AAS, Agilent 240AA; Agilent Technologies, Santa Clara, CA, USA) following the method of Parmar and Sindhu (2013).

pH Effect on K Solubilization by Bacterial Isolates

Potassium solubilization by *Bacillus* spp. isolates was evaluated across a pH range of 5.0 to 9.0. Aleksandrov broth (10 mL) adjusted to 0.5 McFarland turbidity was inoculated with 0.1 mL of bacterial culture and incubated at 30±2°C for 72 hours. To maintain stable pH during incubation, media were prepared using buffer solutions instead of distilled water: Phosphate buffer for pH 5.0–8.0 and boric acid–NaOH buffer for pH 9.0. K concentrations in the supernatants were quantified via AAS.

Temperature Effect on K-Solubilization by Bacteria

Bacillus spp. isolates were evaluated for their K solubilization efficiency at temperatures ranging from 20°C to 45°C. Pre-activated bacterial cultures, adjusted to a turbidity equivalent to 0.5 McFarland standard, were inoculated into 10 mL of Aleksandrov broth medium at a rate of 0.1 mL and incubated for 72 hours. K concentrations in the resulting supernatants were quantified using AAS.

Impact of Mica Dosage on K Solubilization of Isolates

The effect of mica concentration on K solubilization was evaluated by supplementing Aleksandrov broth medium with different mica concentrations (1.0, 3.0, 5.0, 7.0, and 9.0 g L⁻¹). Each prepared medium was inoculated with 0.1 mL of bacterial culture standardized to 0.5 McFarland turbidity and incubated at 35±2°C for 72 hours. Following incubation, the optical density of the cultures was measured at 600 nm using a spectrophotometer to determine bacterial growth. Subsequently, cultures were centrifuged at 3350 *g* for 10 minutes, and the concentration of soluble K in the supernatant was quantified using AAS.

Identification and biochemical characterization of Isolates

The selected AOB28 strain was cultured in Nutrient Broth (NB) and incubated overnight at 37°C, followed by preliminary identification procedures including Gram staining, endospore staining, and motility tests (Barrow and Feltham 1993; Aygan and Arkan 2007).

16S rRNA Gene Sequence analyze

Genomic DNA of strain AOB28 was extracted using a commercial kit (GF-1 Bacterial DNA Extraction Kit, Invitrogen). DNA quality and concentration were assessed using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and samples were standardized to 50 ng/μL for 16S rDNA amplification. PCR products were verified by 2% agarose gel electrophoresis, enzymatically purified, and sequenced bidirectionally using the BigDye Terminator v3.1 Cycle Sequencing Kit on a 3130 xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence similarity searches were performed using BLAST against the NCBI GenBank database, and sequences showing ≥99% similarity were used for taxonomic identification and subsequent analyses (Orak et al. 2022).

Assessment of Extracellular Enzymatic Activities of the Isolates

The extracellular enzyme production potential of AOB28 was qualitatively assessed for amylase, protease, xylanase, CMCase, and avicelase. Amylase activity was tested on starch-containing agar medium, the isolates were inoculated and incubated at 35 °C for 24–48 h. Plates were then stained with iodine solution, and clear zones indicated positive amylase activity. Protease production was evaluated on gelatin-containing medium, with clear zones around colonies considered indication of protease activity. Xylanase, CMCase, and avicelase activities were tested on media containing xylan, CMC, or Avicel, respectively, and stained with 0.1% (w/v) Congo red. Clear zones on a red background were interpreted as positive enzyme activity (Gupta et al. 2012; Ghani et al. 2013).

Determination of salinity and heavy metal resistance

The salt tolerance of *B. subtilis* AOB28 was evaluated using Nutrient Agar (NA) supplemented with 0.5, 1, 2, 4, 8, 16, 20, and 25% (w/v) NaCl. The media were prepared taking into account the total salt concentration. The isolate was inoculated onto the media using the streak method, and the plates were incubated at 35 ± 2 °C for 24 h. Bacterial growth was visually assessed and recorded as either positive (+) or negative (–). The tolerance to heavy metals was determined on Mueller Hinton Agar (MHA) supplemented with CoCl₂·6H₂O, NiCl₂·6H₂O, CuSO₄·5H₂O, FeSO₄·7H₂O, ZnCl₂, AgNO₃, and Pb(NO₃)₂ at concentrations of 100, 200, 400, 800, and 1600 mg L⁻¹. Bacterial suspensions adjusted to a 0.5 McFarland standard were streaked onto the metal-containing media under aseptic conditions, followed by incubation at 35 ± 2 °C for 24 h. Growth was then visually evaluated to determine the isolate's tolerance to each metal. (Rosas-Ramirez et al. 2023).

Pot Experiment: Establishment and Harvest

Pot experiments were conducted using 2 kg of soil sieved through a 2-mm mesh and placed into seedling bags. The pot experiment consisted of a control group and a bacterial inoculation treatment. The study was designed according to a completely randomized design (CRD) with three replications. Bacterial cultures grown in Nutrient broth were centrifuged (3350 × *g*, 10 min), and the resulting pellets were suspended in distilled water to a turbidity of 1.0 McFarland (1.8 × 10⁷ cfu mL⁻¹). Arugula (*E.*

vesicaria) seeds were inoculated with the bacterial suspension and sown into the soil, while uninoculated seeds served as controls. All treatments received 50 mg kg⁻¹ nitrogen fertilizer and were irrigated to maintain field capacity. After a 10-week growth period, aboveground plant biomass was harvested.

Soil and plant analyses

Soil samples were air-dried under laboratory conditions following the protocols established by Jackson (2005). Subsequently, they were mechanically crushed using a wooden mallet and passed through a 2 mm sieve for analysis. The pH levels and soluble salt concentrations were measured using a pH meter and an electrical conductivity meter, respectively, employing the saturated paste method as described by Thomas (1996) and Rhoades (1996). The total lime content of the soil was determined using a Scheibler calcimeter, following the procedure outlined by Loeppert and Suarez (1996). Soil organic matter content was measured using the Walkley-Black method, adhering to the procedural guidelines defined by Nelson and Sommers (1996).

Leaf samples were digested following a block digestion procedure using nitric acid (HNO₃) and perchloric acid (HClO₄), as detailed by Jones and Case (1990). The resulting digests were then diluted to a final volume of 25 mL. Phosphorus concentrations were determined spectrophotometrically via the vanadomolybdophosphoric acid method, according to Kuo (1996). Concentrations of K, Ca, Mg, and micronutrients in the leaf samples were quantified using atomic absorption spectrometry (AAS).

Statistical Analyses

Graphical representations were generated using GraphPad Prism 10.4.2 software (GraphPad Software, San Diego, CA, USA). Statistical differences between the control and bacterial treatments were evaluated using an unpaired t-test, whereas comparisons among multiple groups were performed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test, using JMP Pro 13 software (SAS Institute Inc., Cary, NC, USA). Different lowercase letters indicate statistically significant differences among treatments, and non-significant comparisons are denoted as "ns".

RESULTS

Bacterial identification and biochemical Properties

A total of 175 bacterial isolates were evaluated for their biochemical characteristics. The AOB28 strain was found to be aerobic, Gram-positive, rod-shaped, motile, catalase-positive, and capable of forming spores. Based on 16S rDNA sequence analysis, the strain showed 99% sequence identity with *Bacillus subtilis* 16S rDNA sequences deposited in GenBank (accession number: PX944923).

Extracellular Enzyme Activities of the Isolates

The isolates were qualitatively evaluated for their ability to produce extracellular enzymes, including amylase, xylanase, protease, cellulase, and avicelase. The results indicated positive activities for amylase, CMCase, and protease, whereas no xylanase or avicelase activity was observed (Table 1).

Table 1. Colony and zone diameters for enzyme activities of *B. subtilis* AOB28 strain

Isolate AOB 28	Colony Diameter	Zone Diameter (mm)
Amylase	4	15
Xylanase	0	0
CMCase	4	5
Avicelase	0	0
Protease	4	20

The enzyme activities of AOB28 were examined. Amylase activity showed a colony diameter of 4 mm and an inhibition zone diameter of 15 mm, while protease activity exhibited a colony diameter of 4 mm and an inhibition zone diameter of 20 mm. No measurable activity was observed for xylanase and avicelase enzymes (Figure 1).

Salt and metal tolerance

AOB28 was observed to grow at NaCl concentrations up to 4%, while no growth was detected at 8% NaCl and above, and *B. subtilis* exhibited consistent tolerance to all tested heavy metals across concentrations ranging from 100 to 1600 mg L⁻¹.

Screening and Quantitative Assessment of Potassium Solubilization Potential in Bacterial Isolates

Among 175 bacterial strains screened on Aleksandrov agar, six isolates forming clear zones were selected as potential potassium solubilizers and subsequently evaluated in broth culture. After 8 days of incubation, potassium release was quantified in both mica-free and 1% mica-supplemented media. Among these isolates, strain AOB28 exhibited the highest potassium-solubilization capacity, showing a statistically significant increase in K release in the presence of mica ($p < 0.01$), with K concentrations rising from 7.6 mg L⁻¹ to 50.9 mg L⁻¹. (Figure 2).

Effects of pH, Temperature, and Mica Concentration on Bacterial Growth and K Solubilization

To determine the optimum conditions for the K solubilization activity of the AOB28 strain, the effects of temperature, pH, and mica concentration were evaluated. All tested factors significantly influenced cell concentration and K solubilization ($p < 0.01$). The optimum temperature was 40°C, at which the highest cell concentration was observed and K solubilization reached 60.2 mg L⁻¹. Maximum K solubility (58.7 mg L⁻¹) was recorded at pH 6.0, while the highest K solubilization (62.0 mg L⁻¹) occurred at 9.0 g L⁻¹ mica (Figure 3).

Selected Properties of Experimental and Isolation Soil

The soil used for bacterial isolation was characterized by a slightly alkaline pH (7.60), relatively low organic matter content (2.44%), and low electrical conductivity (330 $\mu\text{S cm}^{-1}$), indicating non-saline conditions, as well as a notably high lime content (30.22%), which may influence nutrient availability and soil structure (Akkaya et al. 2024). The pH values of the soil used in the pot experiment indicated a slightly alkaline reaction, while it was characterized by low salinity and high lime content. Organic matter levels of the soil observed to range from low to medium (Ulgen and Yurtsever 1995). The phosphorus and K concentrations in the experimental soil were determined to be 4.55 mg kg^{-1} and 221 mg kg^{-1} , respectively (Table 2).

Table 2. Some characteristics of soils used for pot experiment and the bacterial isolation

	pH	OM	EC	Lime	P	K
		%	$\mu\text{S cm}^{-1}$	%	mg kg^{-1}	
Exp. Soil	7.86	0.86	350	16.55	4.50	227
Isolation soil	7.60	2.44	330	30.22	-	-

OM: Organic matter, EC: Electrical Conductivity

Plant Analyses of the *B. subtilis* AOB28 Strain

Pot experiments with arugula (*E. vesicaria*) demonstrated that *B. subtilis* AOB28 significantly promoted plant growth compared to the control. Significant increases were detected in total plant height ($p < 0.05$) and root length ($p < 0.01$). Specifically, plants treated with *B. subtilis* AOB28 exhibited a 53.75% increase in root length and a 24.44% increase in total plant height relative to the control (Figure 4).

The dry matter weights of arugula (*E. vesicaria*) plants indicated that the control group had root, stem, leaf, and total dry weights of 0.10 g pot^{-1} , 0.06 g pot^{-1} , 1.30 g pot^{-1} , and 1.41 g pot^{-1} , respectively. Compared with the control, plants treated with *B. subtilis* AOB28 showed significantly higher dry matter accumulation, with values of 0.21 g pot^{-1} for roots ($p < 0.05$), 0.11 g pot^{-1} for stems ($p < 0.01$), 2.01 g pot^{-1} for leaves ($p < 0.01$), and a total dry weight of 2.33 g pot^{-1} ($p < 0.01$) (Figure 5).

Considering tissue nutrient concentrations together with nutrient uptake amounts, potassium concentration did not differ significantly between treatments, remaining at 2.80% in the control group and 2.76% in plants treated with *B. subtilis* AOB28; however, K uptake (mg pot^{-1}) was significantly higher in the AOB28-treated plants ($p < 0.05$), increasing from 3925 to 6417 mg pot^{-1} . In contrast, tissue Ca and Mg concentrations were significantly reduced in the treated plants, with Ca decreasing significantly ($p < 0.05$) and Mg showing a more pronounced reduction ($p < 0.01$), whereas the decrease in P concentration was not statistically significant. Regarding nutrient removal, the control plants removed 31.85 mg Ca, 7.20 mg Mg, and 247 mg P pot^{-1} , while plants treated with *B. subtilis* AOB28 removed a significantly lower amount of Ca (22.55 mg pot^{-1} ; $p < 0.05$). Although Mg (8.62 mg pot^{-1}) and P (3.12 mg pot^{-1})

uptake values were numerically higher in the treated plants, these differences were not significant (Figure 6A–B).

Tissue micronutrient analysis revealed significant changes in Fe and Cu concentrations after *B. subtilis* AOB28 treatment. Tissue Fe concentration increased significantly from 24.5 mg kg⁻¹ in the control to 29.7 mg kg⁻¹ in the treated plants ($p < 0.05$), whereas Cu concentration decreased significantly from 1.15 to 0.74 mg kg⁻¹ ($p < 0.01$). In contrast, although Zn and Mn concentrations were numerically higher in the treated plants, these differences were not statistically significant (Figure 7A). With respect to micronutrient uptake (mg pot⁻¹), Mn and Zn removal increased significantly in the AOB28-treated plants, with Mn uptake rising from 5.24 to 7.23 mg pot⁻¹ ($p < 0.05$) and Zn uptake increasing from 6.44 to 9.18 mg pot⁻¹ ($p < 0.05$). Although Fe uptake increased numerically (24.5 to 29.07 mg pot⁻¹) and Cu uptake decreased (1.15 to 0.74 mg pot⁻¹), these changes were not statistically significant (Figure 7B).

DISCUSSION

This study aimed to investigate whether *B. subtilis* AOB28 can enhance K bioavailability by mobilizing mineral-bound K and whether this function leads to improved plant nutrient acquisition under soil conditions. By integrating mineral solubilization assays, physiological characterization, and pot experiments, the study establishes a functional link between microbial activity, nutrient mobilization, and plant performance. Notably, the identification of *B. subtilis* AOB28 as exhibiting a substantially higher K-solubilization capacity under in vitro conditions (50.9 mg L⁻¹) compared with the other tested strains is particularly important for understanding its role in mineral K solubilization (Figure 2).

The optimal K solubilization exhibited by *B. subtilis* AOB28 at elevated temperature reflects enhanced microbial activity and improved K mobilization efficiency. Similarly, the strain displayed its highest growth performance under mildly acidic conditions, accompanied by a concomitant enhancement in K mobilization (Figure 3A–B). Together, these responses indicate that temperature and pH play critical roles in regulating both bacterial activity and mineral K availability (Figure 3 C–D). Optimal activity under moderately acidic conditions and elevated temperature aligns with established principles of microbe-mineral interactions, whereby proton availability and metabolic rates jointly influence nutrient mobilization. Acidification enhances ion exchange and weakens mineral lattices, while increased temperature accelerates enzymatic and metabolic processes, collectively amplifying solubilization efficiency (Özdemir and Demirkıran 2019).

The highest K release (62.0 mg L⁻¹) was observed to be associated with cultures supplemented with mica, coinciding with enhanced bacterial growth under these conditions (Figure 3 E–F). These data reveal the potential of *B. subtilis* AOB28 for applications in agriculture and biotechnology. The incorporation of mica into the culture medium is considered an effective strategy to enhance the growth of various microorganisms due to its mineral composition. As a silicate mineral, mica not only provides structural support but also facilitates the release of K⁺ ions, which are vital for various plant physiological functions (Pendse et al. 2022). The stimulatory impact of elevated mica concentrations on the growth of

B. subtilis AOB28 aligns with prior research indicating that the addition of mineral substrates can boost microbial activity and biomass yield. This trait is ecologically relevant, as many soil microorganisms persist in environments where nutrient availability is largely governed by mineral dissolution rather than organic inputs. Accordingly, the capacity to maintain growth under such conditions suggests that *B. subtilis* AOB28 is a functionally resilient member of the soil microbiome.

The enzymatic profile of *B. subtilis* AOB28 marked revealed amylase activity, indicating that this enzyme represents the dominant functional component among the strain's enzymatic activities (Table 1). The effectiveness and prominence of amylase activity relative to other functional enzymes have been suggested in previous studies (Mutlu and Poyraz 2017; Yurt 2020). Although xylanase and avicelase activities were not detected, the presence of CMCase activity indicates a measurable capacity for cellulose degradation (Table 1). Given the critical role of CMCase in lignocellulosic breakdown, this moderate level of activity underscores the potential functional relevance of *B. subtilis* AOB28 in lignocellulose-associated biotechnological and industrial applications (Berikten and Kivanç 2021; Binek and Hameş 2024). Additionally, the substantial protease activity observed suggests that protein hydrolysis constitutes another key functional trait of this strain. Since proteases are integral to numerous industrial processes, *B. subtilis* AOB28 may be regarded as a promising biological resource for diverse biotechnological applications (Table 1). The enzymatic activities of strain were reflected by the relatively hydrolysis zones observed in relation to colony growth (Figure 1).

B. subtilis AOB28 has demonstrated a strong stress response to increasing salt concentrations in the present study, indicating a moderate level of halotolerance, as the strain was able to sustain growth at salinity levels of up to 8% NaCl. Salt tolerance is an indicator of the ability of *Bacillus* spp. to survive under stress conditions and is critical in the fields of environmental microbiology and agricultural biotechnology (Li et al. 2024). *Bacillus* spp. possess the ability to produce various proteins, particularly molecules such as ectoine, to facilitate osmoregulation (Liang et al. 2015). This feature plays a crucial role in protecting plants from salt stress, which can negatively impact their growth and productivity in regions with saline irrigation water. The application of *Bacillus* spp. to pepper has been demonstrated to mitigate the detrimental effects of salt stress and significantly improve key plant growth parameters (Kazerooni et al. 2021; Öztürk and Dursun 2023). In addition to salt tolerance, the heavy metal tolerance exhibited by *B. subtilis* AOB28, as demonstrated by its ability to tolerate concentrations of up to 1600 ppm in the present study, suggests a high resistance capacity. This resistance may be associated with the development of genetic and biochemical resistance mechanisms commonly reported in bacteria (Rosas-Ramirez et al. 2023; Eroğlu and Yasa 2025). The heavy metal binding capabilities of *Bacillus* spp. further demonstrate the significant role these microorganisms play in mitigating environmental pollution (Inggraini, 2017).

The inoculation of *B. subtilis* AOB28 has been shown to exert a significant impact on the growth performance and nutrient acquisition of *E. vesicaria*. Notably, arugula plants grown in soils inoculated with *B. subtilis* AOB28 exhibited significant increases in dry matter accumulation across root, stem, and leaf, which collectively contributed to a significant enhancement in total plant biomass. Consequently,

total dry biomass increased from 1.40 to 2.27 g pot⁻¹ following bacterial inoculation (Figure 5). Additionally, total plant height increased by 24.44%, suggesting concurrent increases in biomass accumulation and morphological development (Figure 4). This finding supports the potential of *B.subtilis* AOB28 to promote plant growth. Numerous studies have reported the positive impact of *Bacillus* spp. on enhancing plant growth, improving nutrient acquisition, and increasing agricultural yield (Calvo et al. 2019; Yağmur et al. 2020; Koyun et al. 2022; Tsotetsi et al. 2022).

The evaluation of *B.subtilis* AOB28 mediated plant responses require an integrated assessment of nutrient dynamics, as microbial inoculation can simultaneously influence the uptake of multiple macro- and micronutrients under soil-based growth conditions. In this context, the effects of *B.subtilis* AOB28 on arugula were assessed in a pot experiment, not only in terms of the significant increase in K uptake, but also with respect to the changes observed in the uptake of other macronutrients. *B.subtilis* AOB28 notably enhanced K uptake in arugula plant grown under pot conditions (+63.5%), while it substantially decreased Ca uptake (-29%) and led to moderate increases in Mg and P uptake (+19% and +27%, respectively) (Figure 6A-B). The reduction in Ca uptake under pot conditions may be attributed to competitive interactions between K and Ca at the root uptake sites, a phenomenon reported under elevated K availability (Fageria, 2001). Under the same experimental conditions, *Bacillus* inoculation enhanced the uptake of micronutrients such as Fe, Zn, and Mn, whereas a decrease in Cu uptake was observed (Figure 7A–B). These findings suggest that such bacterial strains could be integrated into balanced fertilization strategies for arugula plants; however, their use should be regulated carefully (Yalçın and Çimrin 2019; Yağmur et al. 2020; Çelik 2023). *B.subtilis* AOB28 contributes to improved nutrient uptake by creating a favorable chemical and physical environment in the rhizosphere (Magotra et al. 2021). In addition, certain *Bacillus* spp. have been reported to produce lipopeptide metabolites that promote nutrient acquisition in plants (Chen et al. 2019; Verma et al. 2017). The ability of *Bacillus* spp. to enhance root morphology and nutrient uptake has been well-documented in numerous studies (He and Dijkstra 2014; Calvo et al. 2019; Tsotetsi et al. 2022; Ahmad et al., 2023).

Alongside the positive effects of *B.subtilis* AOB28 on arugula, the observed decreases in tissue concentrations of certain nutrients (Ca and Cu) indicate that such applications should be carefully evaluated. Therefore, developing a more balanced and integrated fertilization strategy is essential for improving overall plant health and nutrient quality. The interactions of bacteria such as *B.subtilis* AOB28 within the rhizosphere and their effects on plant growth are of great importance for enhancing agricultural productivity. In sustainable agricultural practices, the use of such bacteria can contribute not only to improving soil fertility but also to the conservation of natural resources. The cultivation of *B.subtilis* AOB28 in the presence of mica, along with the determination of optimal growth conditions and plant tests using arugula, supports its potential use as a natural biofertilizer. Therefore, the K-solubilizing capabilities of such bacteria and their integration into modern agricultural practices should be aligned with sustainable farming strategies.

Conclusions and future recommendations

This study demonstrates that *B. subtilis* AOB28 is a highly effective potassium-solubilizing bacterium with strong enzymatic activities, including amylase, protease, and CMCase, as well as notable tolerance to salt and heavy metal stress. The strain exhibited optimal K solubilization under moderately acidic conditions, elevated temperature, and in the presence of mica, demonstrating its resilience and functional versatility. Pot experiments with arugula (*E. vesicaria*) revealed that *B. subtilis* AOB28 inoculation significantly enhanced plant growth, biomass accumulation, and nutrient uptake, particularly potassium, while also influencing the uptake of other macro- and micronutrients; notably, calcium uptake was reduced, possibly due to antagonistic interactions with the elevated potassium levels. These findings underscore the potential of *B. subtilis* AOB28 as a biofertilizer for sustainable agriculture, emphasizing its dual role in improving soil nutrient availability and promoting plant productivity. These results emphasize its applicability in sustainable agriculture by promoting soil fertility and supporting plant growth. Further research is recommended to reveal the fundamental mechanisms by which this bacterium acts and to explore its broader potential applications in sustainable agriculture. Such studies will help to maximize its benefits for crop productivity and soil health.

Declarations

Authors' contribution

The idea of conducting this study belongs to Nazan Çömlekçioğlu. Bacterial isolations from soil samples were carried out Ashabil Aygan and Osman Bedrettin Akkaya. K concentrations of supernatants were measured by Ömer Faruk Demir and Dilek Özkılıç. The article was written by Osman Bedrettin Akkaya, Ömer Faruk Demir, and Ashabil Aygan.

Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statements

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

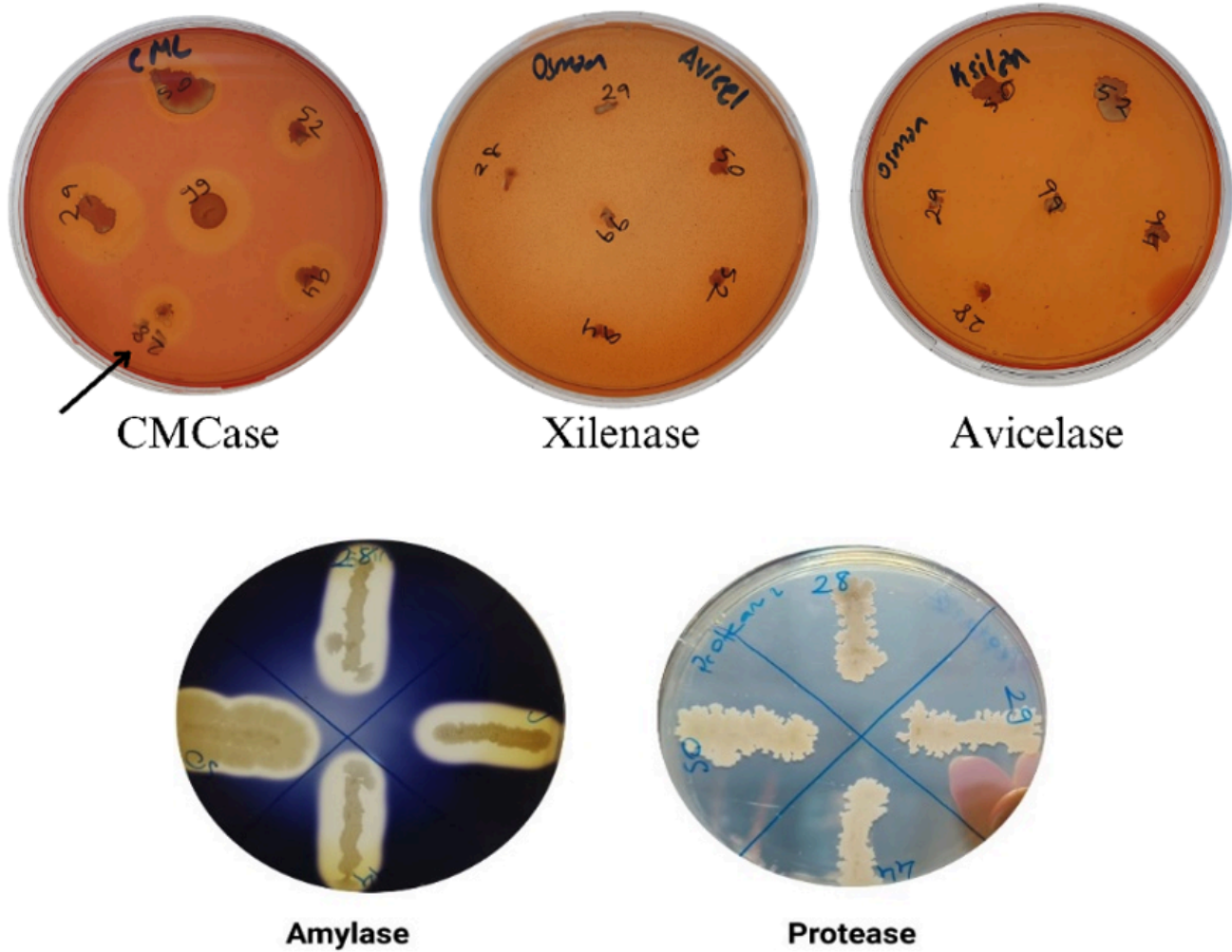


Figure 1

Qualitative images of Protease, CMCase Avicelase and Xylanase enzyme activities of AOB28 isolate

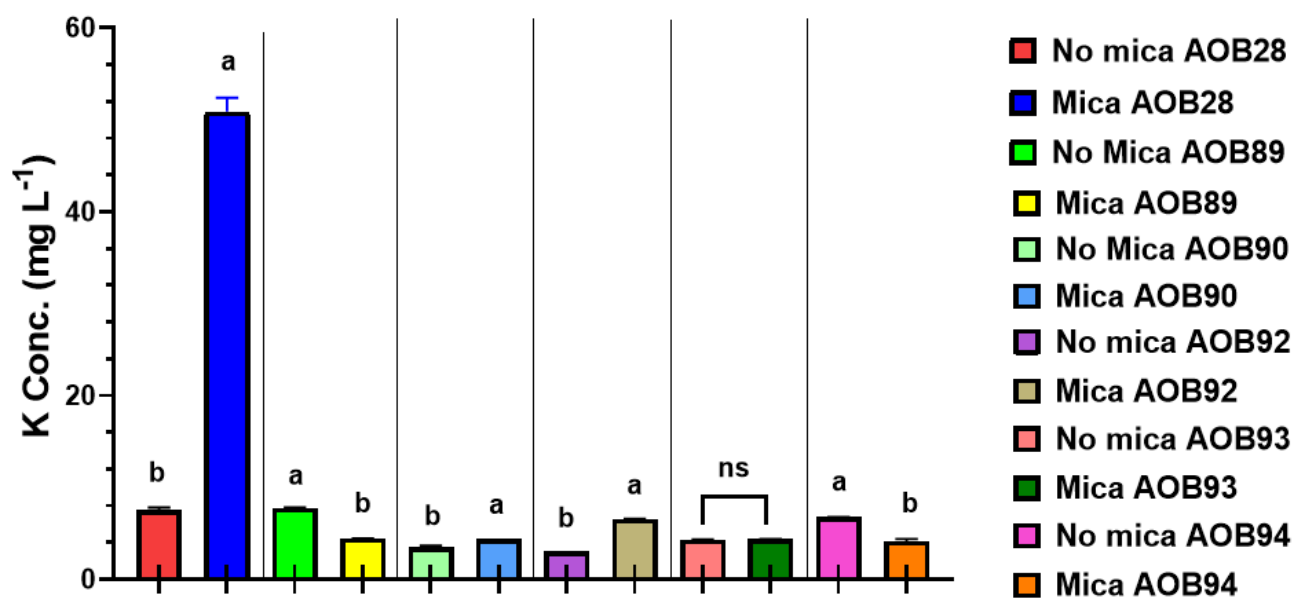


Figure 2

K solubilization amount of *B. subtilis* AOB28 in Aleksandrov broth medium

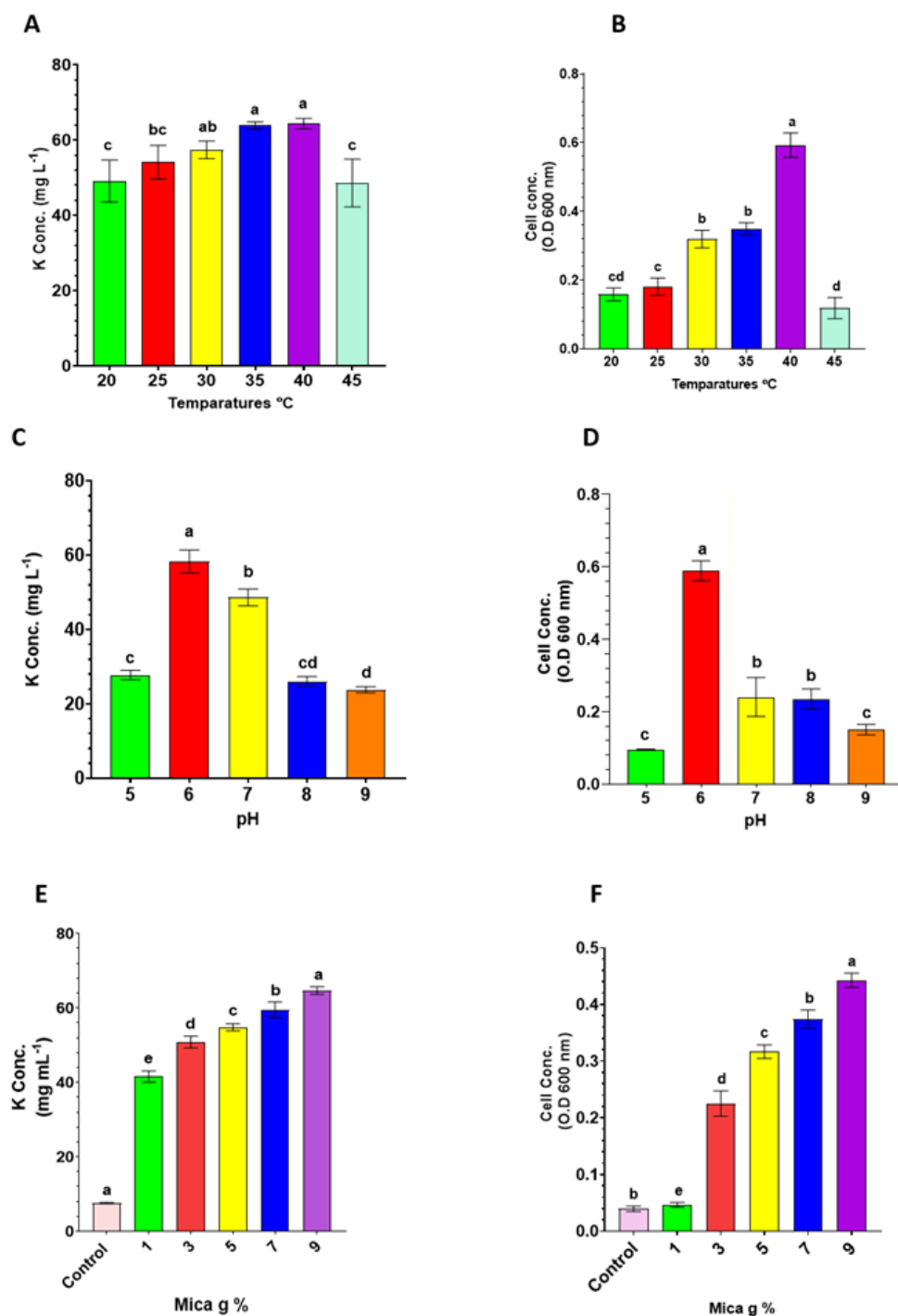
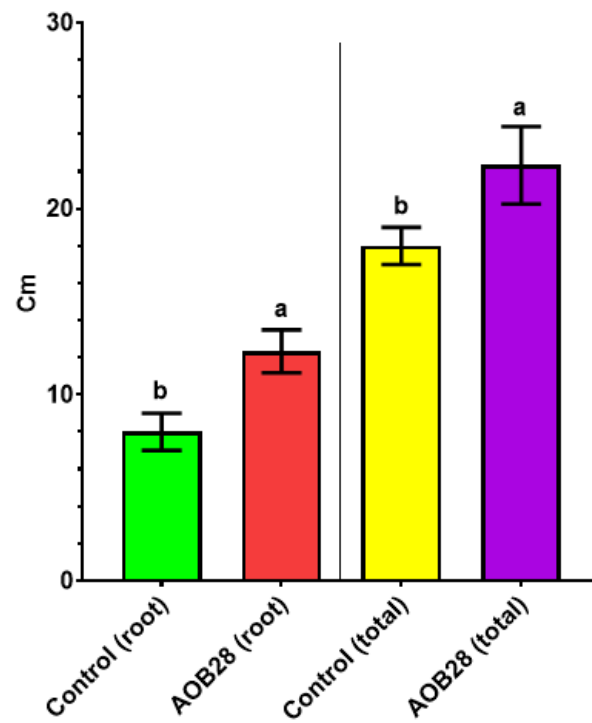


Figure 3

K solubilization ability and cell concentration of *B. subtilis* AOB28 strain incubated in Aleksandrov broth under different temperatures (A, B), pH levels (C, D), and mica concentrations (E, F).



Control



AOB28



Control



AOB28

Figure 4

Root and total lengths of arugula (*E. vesicaria*).

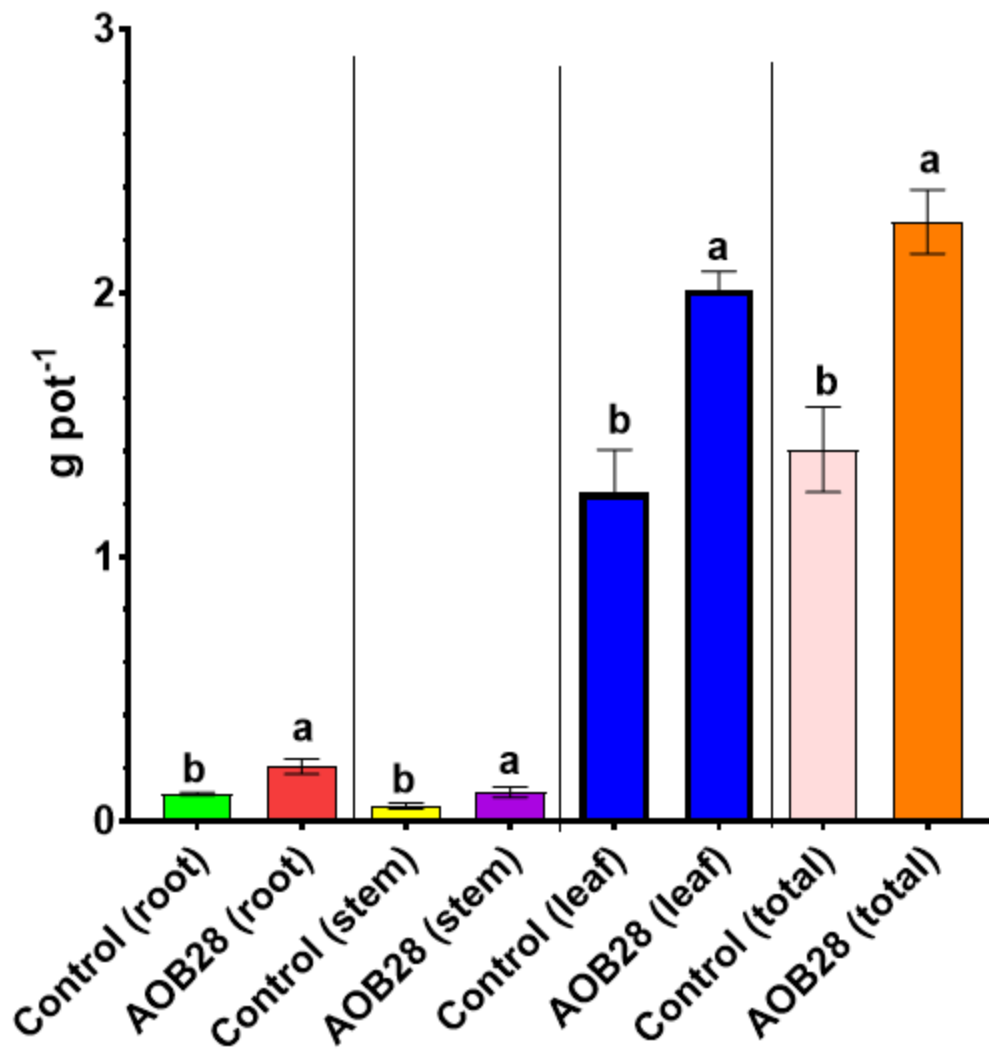
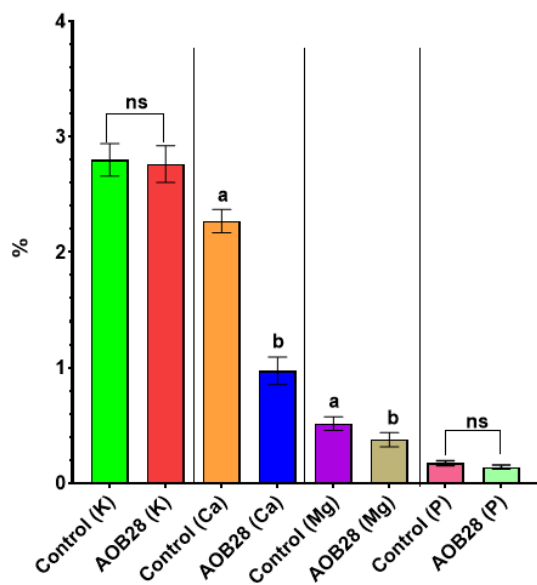


Figure 5

Dry Matter Accumulation of Arugula (*E. vesicaria*) plant.

A



B

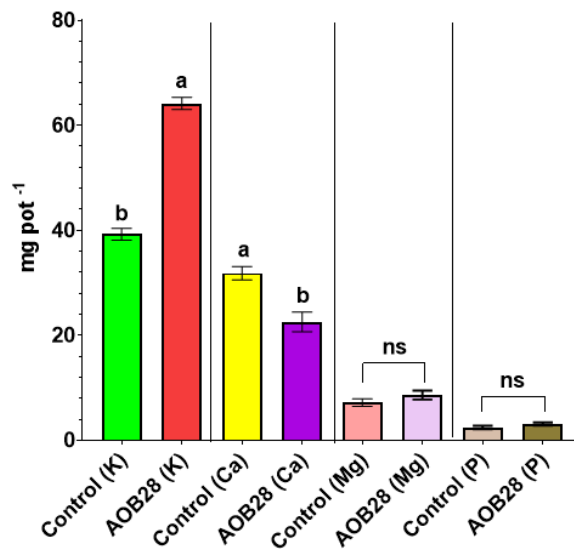
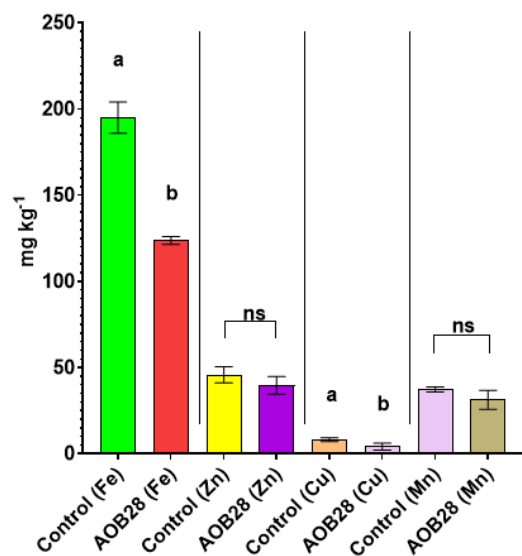


Figure 6

Concentrations and Uptake of macronutrients in arugula (*E. vesicaria*). Bars with different letters differ significantly according to Tukey's HSD test.

A



B

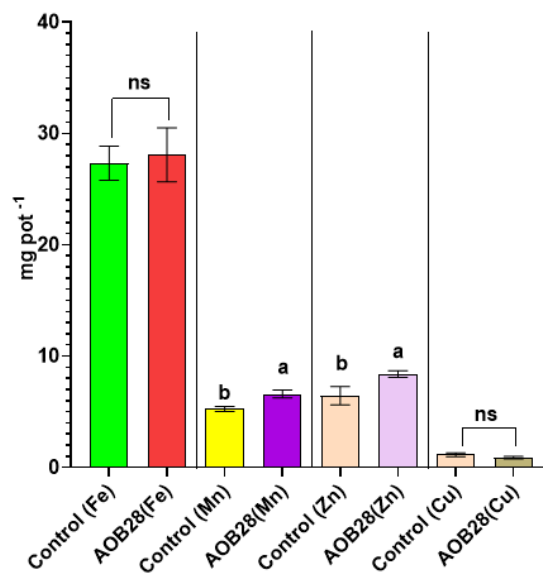


Figure 7

Concentrations and Uptake of micronutrients in arugula (*E. vesicaria*).