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Araştırma Makalesi / Research Article

Influence of Nickel on Urease Activity and Nitrogen Dynamics in Maize (*Zea mays*) Under Saline Conditions

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Abstract

Nickel (Ni) is a vital micronutrient that significantly contributes to essential plant functions, including normal growth, enzymatic activities, and nitrogen metabolism. Soil urease activity can markedly decrease under saline conditions, and both plants and soil organisms necessitate Ni for the effective assimilation of urea. This study investigates the effects of varying levels of Ni supplementation (0, 25, 50, and 100 mg kg^{-1}) under saline soil conditions on soil and plant urease activity, biomass yield, chlorophyll content, nitrogen (N) content, and nitrate assimilation. Thermal imaging was employed to assess plant stress by measuring leaf temperature. Results showed that the 25 mg kg^{-1} Ni treatment notably improved soil urease activity, leading to enhanced nitrogen uptake and biomass production. This Ni dose also resulted in the lowest leaf temperature in maize, indicating reduced stress. Low Ni levels increased nitrate reductase (NR) activity, nitrate content, and free amino acids in maize, while sharply reducing ammonium accumulation, suggesting that Ni promotes plant growth by regulating nitrogen assimilation. However, the 100 mg kg^{-1} dose caused toxicity in maize. All Ni concentrations decreased plant urease activity, indicating decreased urea uptake from the soil. Overall, these findings suggest that low doses of Ni support nitrogen metabolism and plant performance by regulating urease activity in both soil and plants, while higher doses have toxic effects.

Keywords: Nickel, urease, salinity, nitrogen uptake, nitrogen metabolism

1.Introduction

Soil salinity is a major global challenge, significantly limiting agricultural productivity (Singh, 2022). Salinity causes physiological stress in plants, including osmotic stress and ion imbalance, negatively impacting their growth, development, and nutrient uptake (Arif et al., 2020; Yasar et al., 2020; Balasubramaniam et al., 2023; Canavar et al., 2023; Gören et al., 2024). Salinity significantly affects the biological properties of soil, in addition to its direct effects on plants. The inhibition of microbial activity and enzymatic processes in saline environments restricts the availability of vital nutrients required for optimal plant growth (Zhou et al., 2017; Shahid et al., 2020; Ceritoğlu et al. 2020; Sun et al., 2021; Zhang et al., 2024). Urease activity, a crucial enzymatic process, is significantly influenced by salinity and is essential for nitrogen transformation in soils (Wang et al., 2021; Yao et al., 2021). Saline conditions can significantly reduce urease activity, thereby limiting nitrogen availability to plants and ultimately diminishing their growth and productivity (Wei et al., 2016; Zhu et al., 2023; Ramazanoglu et al., 2024). Urease is an essential enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide (D'Agostino and Carradori, 2024). The ammonia generated is then transformed into nitrate via nitrification, supplying plants with an essential nitrogen source for growth in agricultural soils (Grzyb et al., 2021; Robertson and Groffman, 2024). However, salinity diminishes urease activity in soils, thereby disrupting the nitrogen cycle (Li et al., 2021; Erdel, 2022; Ramazanoglu et al., 2024). Decreased urease activity may force plants to uptake urea, which is less efficient and potentially toxic for various plant species, including tomatoes (Tan et al., 2000), cucumbers (Tabatabaei, 2009), lettuce (Khoshgoftarmanesh et al., 2011), and soybeans (Kutman et al., 2014). Consequently, the regulation of urease activity is essential for enhancing plant nitrogen use efficiency (Dimkpa et al., 2020). Nickel (Ni) is a essential element necessary for the optimal activity of the urease enzyme, and a shortage of Ni in soils can significantly hinder urease activity (Kamboj et al., 2018). In saline soils, where urease activity is already impaired, low Ni levels might worsen this issue, further reducing enzyme functionality and forcing plants to directly uptake urea. Under these conditions, Ni fertilization can increase urease activity, improve nitrogen absorption and hence promote plant growth (Lavres et al., 2016). For instance, Khoshgoftarmanesh et al. (2011) showed that urease activity was dramatically boosted by Ni supplementation in hydroponically grown lettuce fed with urea, resulting in improved plant nitrogen content. Similar results were reported for lettuce (Oliveira et al., 2013), onions (Alibakhshi and Khoshgoftarmanesh, 2016), and soybeans (Barcelos et al., 2018). Most studies have focused on the effect of Ni on urease activity under conditions where urea is the sole nitrogen source. Salinity limits urease activity, but at the same time, vital nitrogen sources such as ammonium and nitrate remain available to plants. The relationship between Ni addition and soil urease activity in saline soils, particularly with respect to plant nitrogen uptake efficiency, has not been adequately investigated.

This research investigates the effects of varying Ni doses on growth and nitrogen metabolism of maize plants under saline soil conditions. This study aims to improve the understanding of the role of Ni in plant nitrogen nutrition by investigating its regulation of soil urease activity.

2.Materials and Methods

The research was carried out in 2024 within the semi-controlled greenhouses at the Faculty of Agriculture, Harran University. Saline soils obtained from the Sanlıurfa Harran Plain in Turkiye served as the experimental soil, with their characteristics detailed in Table 1. Five maize seeds were sown in plastic pots containing 2.5 kg of saline soil each, and following germination, one plant was retained in each pot. Following germination, various doses of $NiCl_2$ (0 (control), 25, 50, and 100 mg kg^{-1}) were added to the pots. Ten days post-Ni treatment, 50 mg kg^{-1} of nitrogen (urea) was administered to each pot. Furthermore 50 mg kg^{-1} phosphorus and 80 mg kg^{-1} potassium were applied during seed planting as basic fertilization. The study utilized 12 pots, comprising 4 treatments, each replicated 3 times.

Table 1. General properties of the research soil

pH	EC (dS m^{-1})	Total N (%)	Total C (%)	DTPA Extractable – Ni (mg kg^{-1})
7.2	35.8	0.13	0.68	0.428

2.1. Data collection and analysis

Leaf samples obtained from plants were preserved at -20ºC for subsequent analysis. The remaining plants were dried at 70ºC until a constant weight was achieved for the assessment of dry weight and nitrogen content. The dried plant material was subsequently ground, and the nitrogen content was analyzed employing the Kjeldahl method. SPAD values were obtained by averaging three measurements per plant using a Konica Minolta SPAD 502 instrument. Thermal images were acquired with a FLIR T540 thermal camera, and the images were analyzed utilizing FLIR Tools software to ascertain plant temperature. Leaf urease activity was evaluated using the method outlined by Hogan et al. (1983), while nitrate reductase (NR) activity was determined according to Jaworski's (1971) procedure. To analyze nitrogen compounds in plants, fresh leaf samples were boiled in distilled water for 1 h. After incubation, the samples were centrifuged at 5,000 rpm for 15 minutes to obtain a clear extract. The extracts were then used to measure total free amino acid, nitrate and ammonium content. Total amino acids were measured following the method of Lee and Takahashi (1966), nitrate concentration was assessed following the protocol of Cataldo et al. (1975), and ammonium content was evaluated according to the procedure outlined by Husted et al. (2000). Soil samples were collected on days 1, 7, and 15 post-urea application to assess urease activity. Soil urease activity was assessed utilizing the methodology outlined by Tabatabai and Bremner (1972).

2.2. Statistical analysis

The analysis of the collected data for statistical significance was conducted using Duncan's Multiple Range Test in SPSS version 21.0.

3. Findings and Discussion

Figure 1 illustrates the effects of nickel (Ni) nutrition on temperature changes in maize plants grown under saline soil conditions. Maize plants treated with 25 mg kg^{-1} Ni exhibited the lowest temperature values, whereas higher temperatures were recorded in other treatments. Additionally, the temperatures in plants treated with 0 mg kg^{-1} and 100 mg kg^{-1} Ni remained at similar levels. Low Ni reduced canopy temperatures, suggesting that Ni may support certain physiological processes.

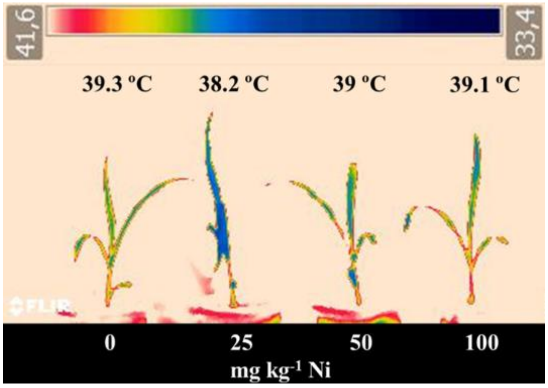

Figure 1. Thermal imaging of maize plants treated with 0, 25, 50 and 100 mg kg^{-1} Nickel (Ni) under saline soil conditions

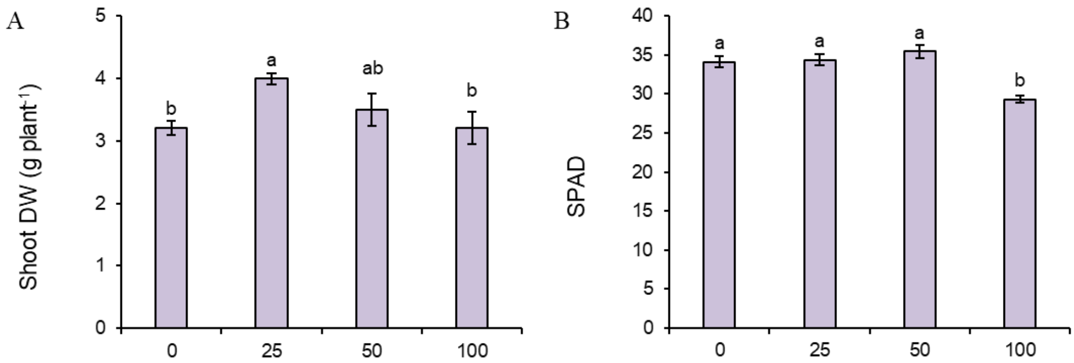

Figure 2. A) Shoot and root dry weight (DW) and B) SPAD value of maize seedlings treated with different doses of Nickel (0, 25, 50 and 100 mg kg^{-1} Ni) under saline soil conditions

Ni is a crucial micronutrient for plants, exhibiting both essential and harmful properties (Parvez et al., 2021). Ni is necessary at low quantities for vital functions such enzyme activity, nitrogen (N) metabolism, and normal growth (Kaur et al., 2023). Although the essential role of Ni in plants is well-documented, elevated amounts are detrimental to numerous plant species (Hassan et al., 2019; Turan, 2022). Sabir et al. (2011) declared that Ni increased microelement uptake and growth in maize. Gerendás and Sattelmacher (1997) demonstrated that Ni improved biomass yield and chlorophyll content in rye, wheat, soybean, rape, squash, and sunflower. In our study, 25 mg kg^{-1} Ni increased shoot dry weight by 24% compared to plants not treated with Ni, while higher concentrations (100 mg kg^{-1}) reduced biomass yield (Figure 2A). Regarding SPAD values, the 0, 25, and 50 mg kg^{-1} Ni treatments produced similar results, but the 100 mg kg^{-1} Ni treatment reduced SPAD values by 14% (Figure 2B). These findings suggest that low levels of Ni support plant growth under saline conditions, while higher concentrations are detrimental.

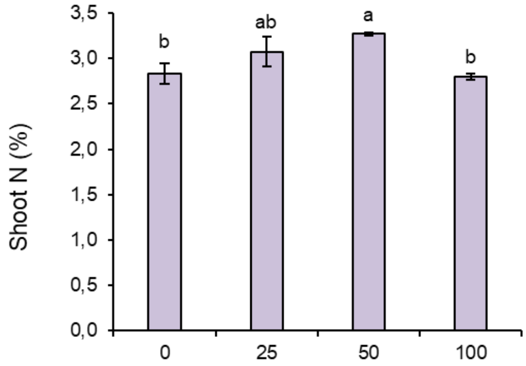

Figure 3. Shoot nitrogen (N) content in maize seedlings treated with different doses of Nickel (0, 25, 50 and 100 mg kg^{-1} Ni) under saline soil conditions

The positive effects of low Ni levels on plant growth may be due to improved nutrient uptake and assimilation. Singh et al. (2011) reported that low Ni levels increased N content and uptake in wheat, which led to improved dry matter yield, while higher Ni concentrations reduced dry matter yield compared to lower Ni levels but remained higher than the control. Similarly, in our study, the 25 and 50 mg kg^{-1} Ni treatments increased N content in maize by 8.6% and 15.5%, respectively (Figure 3). However, the 100 mg kg^{-1} Ni treatment did not significantly alter total N content. This improvement in plant N content may be related to the effect of Ni on N transformation processes in the soil, because Ni is an essential component of the urease enzyme and is necessary for the proper functioning of this enzyme (Mazzei et al., 2020). Urease catalyzes the hydrolysis of urea to ammonia, a key process for nitrogen assimilation (D'Agostino & Carradori, 2024). Saline conditions significantly reduce urease activity, which limits nitrogen uptake and, consequently, plant growth and productivity (Wei et al., 2016; Zhu et al., 2023; Ramazanoglu et al., 2024). Goswami et al. (2022) found that soil urease enzyme activity increased with rising Ni content in the soil. In our study, measurements taken on the first day after urea application showed that Ni increased soil urease activity by a large amount. The highest values were seen at the 50 mg kg^{-1} Ni treatment (Figure 4). Measurements taken on day 7 showed that the maximum urease activity occurred in the 25 mg kg^{-1} Ni treatment, while higher Ni levels suppressed urease enzyme activity. By day 15, we observed no significant differences in soil urease activity across Ni treatments.

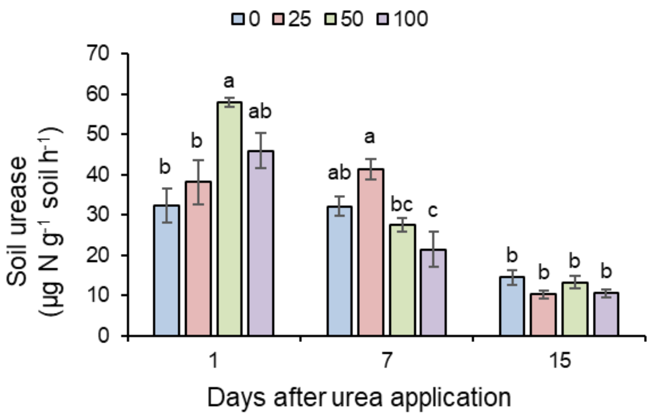

Figure 4. Soil urease enzyme activity in saline soil treated with different doses of Nickel (0, 25, 50, and 100 mg kg^{-1} Ni) at 1, 7, and 15 days after urea application

While urea serves as a crucial nitrogen source for field crops, excessive amounts of urea in plants, when it is the exclusive nitrogen source, might impede growth (Gerendás and Sattelmacher, 1997; Khoshgoftarmanesh et al., 2011). Ni is an essential component of urease, which is critical for urea assimilation (Sakamoto and Bryant, 2001; Tabatabaei, 2009; Witte, 2011; Mazzei et al., 2020). Ni has been shown to enhance urease activity in maize (Gheibi et al., 2009), lettuce (Khoshgoftarmanesh et al., 2011), and soybean (Barcelos et al., 2017), thereby decreasing urea contents. Contrary to previous studies, our findings show that Ni supplementation decreased urease activity in maize leaves (Figure 5). This may be related to decreased urea uptake by plants due to increased soil urease due to Ni supplementation. Khoshgoftarmanesh et al. (2011) indicated that urease enzyme activity was undetectable in plants supplied with nitrogen sources other than urea. The elevated urease activity in Ni-treated soils, relative to untreated soils, may have facilitated the uptake of alternative nitrogen forms. To validate this, we require additional research, including an examination of alterations in urea concentrations in both soil and plant.

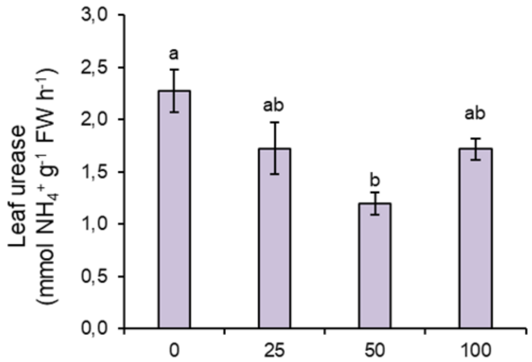

Figure 5. Leaf urease enzyme activity in maize seedlings treated with different doses of Nickel (0, 25, 50 and 100 mg kg^{-1} Ni) under saline soil conditions

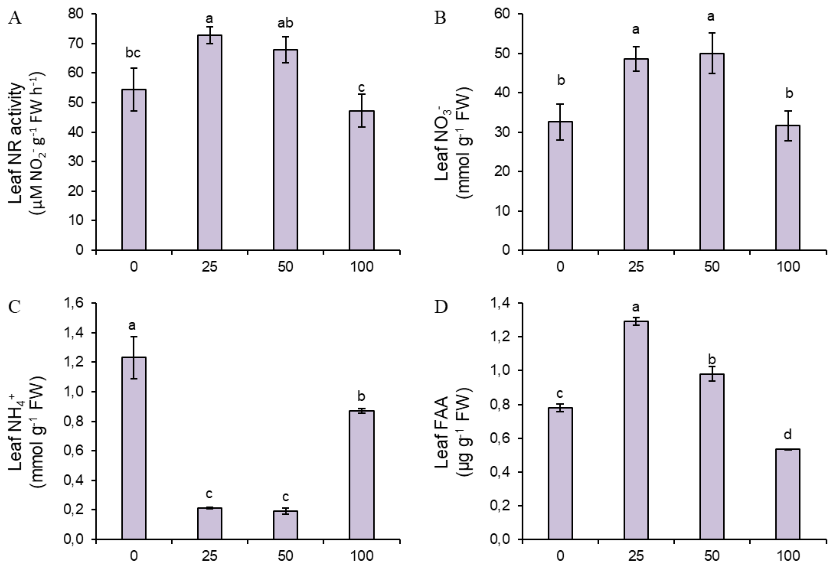

Figure 6. A) Leaf nitrate reductase (NR) activity, B) leaf nitrate (NO_3^-), C) leaf ammonium (NH_4^+) and D) leaf free amino acids (FAA) contents in maize seedlings treated with different doses of Nickel (0, 25, 50 and 100 mg kg^{-1} Ni) under saline soil conditions

Salinity disrupts nitrogen metabolism in plants by inhibiting the function of enzymes responsible for nitrogen assimilation (Liu et al., 2020; Hussain et al., 2021; Tian et al., 2022; 2023). Hessini et al. (2019) reported that salt stress reduced nitrate reductase (NR) activity and nitrate (NO_3^-) concentration in maize leaves while increasing ammonium (NH_4^+) accumulation. Furthermore, urea accumulation in urea-fed plants causes disruptions in N metabolism, leading to severe restrictions in ammonia supply for the synthesis of certain amino acids and diverse proteins (Gerendás et al., 1998). Besides its function in the urease enzyme, Ni also modulates the enzymes involved in the reduction of NO_3^-, therefore being essential for nitrogen metabolism in higher plants (Parvez et al., 2021). Zhang et al. (2022) demonstrated that Ni supplementation enhanced enzyme activities by up-regulating the gene expression of enzymes involved in carbon and nitrogen metabolism. In our study, 25 and 50 mg kg^{-1} Ni supplementation significantly increased NR activity and plant nitrate content compared to soils without Ni application (Figure 6A, B). However, 100 mg kg^{-1} Ni application suppressed NR activity and did not significantly change the nitrate content compared to untreated soils. Overall, Ni supplementation substantially reduced NH_4^+ concentrations (Figure 6C). Specifically, the 25, 50, and 100 mg kg^{-1} Ni treatments reduced NH_4^+ content in maize leaves by 83%, 84%, and 29%, respectively. Ni supplementation also increased free amino acid (FAA) accumulation in maize leaves (Figure 6D). Compared to the control (0 mg kg^{-1} Ni), the 25 and 50 mg kg^{-1} Ni treatments increased FAA content by 66% and 26%, respectively, while 100 mg kg^{-1} Ni application decreased the FAA content by 31%. These results indicate that Ni improves plant growth under saline conditions by regulating nitrate assimilation.

4.Conclusion

This study revealed the effects of Nickel (Ni) supplementation on soil urease activity, growth and nitrogen metabolism of maize plants under saline soil conditions. Ni supplementation at low levels (25 mg kg^{-1}) regulated soil urease activity and positively affected plant N uptake, plant growth and chlorophyll content. Furthermore, Ni supplementation supported plant growth by regulating nitrogen assimilation processes in plants. However, high Ni levels (100 mg kg^{-1}) negatively affected nitrogen assimilation by suppressing the nitrate reductase enzyme, leading to reduced growth. The results show that Ni may improve plant performance in saline soils at low doses, but may be toxic at high doses. Future studies on different plant species will provide additional evidence to support the findings. Long-term studies will contribute to the optimization of fertilization strategies by evaluating the persistence and effects of Ni added to the soil.

Declaration of Conflicts of Interest

The author has no conflict of interest to declare.

Ethical Committee Approval

No ethics committee approval required

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