

## Influence of NiSO<sub>4</sub> doses on agronomic and bioaccumulative parameters in maize (*Zea mays*) plants during the vegetative stage

Elano Carmo Silva Filho<sup>1</sup>, Gilberto Moura da Silva Neto<sup>1</sup>, Carlos Frederico de Souza Castro<sup>2</sup>, Elizabete Nunes da Rocha<sup>1</sup>, Matheus Vinícius Abadia Ventura<sup>1,2</sup> & Antonio Carlos Pereira de Menezes Filho<sup>1</sup>

<sup>1</sup> UniBRAS University Center of Rio Verde, Rio Verde, Goiás, Brazil

<sup>2</sup> Goiano Federal Institute, Rio Verde Campus, Rio Verde, Goiás, Brazil

Correspondence: Antonio Carlos Pereira de Menezes Filho, Soil and Foliar Laboratory, UniBRAS University Center of Rio Verde, Rio Verde, Goiás, Brazil. E-mail: antonio.menezes@braseducacional.com.br

Received: May 26, 2025

DOI: 10.14295/bjs.v4i7.759

Accepted: June 16, 2025

URL: <https://doi.org/10.14295/bjs.v4i7.759>

### Abstract

According to the list of essential micronutrients for agriculturally important plants, nickel (Ni) plays a key role in various physiological processes. This study aimed to evaluate the agronomic and bioaccumulative parameters of Ni in shoot and root tissues of transgenic maize plants during the vegetative phase under different Ni concentrations. Concentrations ranging from 0 to 600 mg L<sup>-1</sup> of Ni, using NiSO<sub>4</sub>·5H<sub>2</sub>O as the source, were prepared and applied directly into the planting furrow at sowing of the transgenic maize hybrid Pioneer P3601 PWU. Standard cultural practices were carried out throughout the experimental period. Maize plants at the V7 stage were collected and analyzed for root and shoot length, fresh and dry biomass of roots and shoots, and Ni bioaccumulation in both tissues. The data showed that Ni doses affected shoot fresh mass, with a maximum of 0.82 g at 600 mg L<sup>-1</sup>, and bioaccumulative content in shoots and especially in roots, reaching 1.92 and 29.31 mg kg<sup>-1</sup>, respectively, at the same concentration. Ni doses influenced only shoot dry mass among the agronomic parameters, and the greatest bioaccumulative effect was observed in the roots of the transgenic maize hybrid Pioneer P3601 PWU.

**Keywords:** nickel, plant fresh mass, root dry mass, Ni bioaccumulation.

## Influência de doses de NiSO<sub>4</sub> sobre os parâmetros agronômicos e bioacumulativo em plantas de milho (*Zea mays*) fase vegetativa

### Resumo

Conforme lista de micronutrientes essenciais para os vegetais de interesse agrícola, o Níquel (Ni) faz parte estando envolvido em diversos processos fisiológicos. Este estudo teve por objetivo verificar os parâmetros agronômicos e bioacumulativos do Ni para parte aérea e raízes em plantas de milho híbrido transgênico fase vegetativa em diferentes concentrações. Concentrações 0 a 600 mg L<sup>-1</sup> de Ni como fonte o NiSO<sub>4</sub>·5H<sub>2</sub>O foram preparadas e aplicadas via suco durante a semeadura de milho híbrido transgênico Pioneer P3601 PWU. Tratos culturais foram aplicados ao longo do período de análises. Plantas de milho em V7 foram coletadas e analisadas quanto ao comprimento de raízes e parte aérea, massa fresca e seca raízes e parte aérea e teor bioacumulativo nas raízes e parte aérea. Os dados obtidos verificaram que as doses influenciaram na massa fresca aérea 0,82 g concentração 600 mg L<sup>-1</sup> e nas doses bioacumulativas da parte aérea e principalmente das raízes com 1,92 e 29,31 mg kg<sup>-1</sup> dose 600 mg L<sup>-1</sup>. Doses de Ni influenciaram apenas na massa seca aérea e maior efeito bioacumulativo nas raízes de milho híbrido transgênico Pioneer P3601 PWU.

**Palavras-chave:** níquel, massa fresca planta, massa seca raízes, bioacumulação de Ni.

### 1. Introduction

Maize (*Zea mays* L.) is one of the most widely cultivated cereals worldwide, playing a fundamental role in human and animal nutrition, as well as serving as a raw material for various industries (Lana et al., 2012;

Ferreira et al., 2014; Torres et al., 2016). Brazil ranks among the top five global maize producers, noted for its high yield potential and nutritional requirements. Maize cultivation demands considerable amounts of macro- and micronutrients, among which nickel (Ni) stands out as an essential element, although its physiological and toxicological behavior in crop plants remains underexplored (Patra et al., 2019).

Nickel is a vital micronutrient for plant development, primarily involved in the activation of urease, the enzyme responsible for converting urea into ammonia within the plant. It also participates in the synthesis of phytoalexins, compounds linked to plant resistance against pathogens (Hänsch; Mendel, 2009; Reis et al., 2014). When present in appropriate concentrations, Ni plays crucial roles in physiological processes from seed germination to grain production, making it indispensable for the completion of the plant life cycle. Consequently, Ni is currently recognized as an essential micronutrient for agriculturally important plants (Rizwan et al., 2024).

Despite its essentiality, the translocation, bioaccumulation, and physiological effects of Ni in different plant tissues are still not well understood. Studies have used soluble forms of Ni—such as sulfate, nitrate, or chloride—to investigate its uptake and effects on various plant parts. It is well established that excessive concentrations of Ni can trigger phytotoxic effects by disrupting metabolic pathways, altering water and nutrient balances, inhibiting enzymatic activity, impairing stomatal function, disrupting photosynthetic electron transport, degrading chlorophyll a/b pigments, and ultimately reducing photosynthetic rates and overall plant growth (Yusuf et al., 2011; Bhalerao et al., 2015).

Acceptable levels of Ni in plant dry matter typically range from 0.05 to 10 mg kg<sup>-1</sup>, depending on the species (Yusuf et al., 2011). Nevertheless, despite being essential, Ni toxicity is a significant concern. According to Berton et al. (2006), it is estimated that between 106 and 544 tons of Ni are introduced annually into various soil types worldwide—including agricultural soils—through metallurgical activities, fossil fuel combustion, and the application of sewage sludge as a nutrient source, raising environmental and agronomic risks.

In high concentrations, Ni becomes a serious pollutant, particularly due to its impact on soil microbial communities and its high absorption capacity through both foliar and root pathways, facilitating its entry into the food chain (Silva et al., 2007; Sreekanth et al., 2013; Rizwan et al., 2022). Moreover, Ni<sup>2+</sup> competes with other essential divalent cations such as Mg<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup>, negatively affecting nutrient uptake. Manganese (Mn), in particular, is among the elements most restricted by Ni competition (Palacios et al., 1998; Begum et al., 2022).

Given this context, it is essential to investigate the effects of different Ni concentrations on maize plants, especially regarding agronomic performance and bioaccumulation in root and shoot tissues during the early vegetative stage. Understanding these aspects will contribute to better micronutrient management and help mitigate potential Ni toxicity in agriculture.

Therefore, the objective of this study was to evaluate the effects of varying Ni concentrations on agronomic parameters and Ni bioaccumulation during the initial vegetative stage of transgenic maize plants (Pioneer P3601 PWU), thereby contributing to the physiological and chemical understanding of this micronutrient's behavior in genetically modified crops.

## 2. Materials and Methods

### 2.1 Experimental location

The experiment was conducted in the experimental area of the UniBRAS University Center in Rio Verde, Goiás, Brazil, from February to May 2025, at coordinates 17°48'16.6" S and 50°56'04.7" W, with an average altitude of 748 m.

### 2.2 Soil type

The soil used in the experiment was classified as a Dystroferric Red Latosol (LVdf), with a clayey texture, and the following chemical characteristics (Table 1).

Table 1. Chemical and physicochemical parameters of the planting soil.

Cmol <sub>c</sub> dm <sup>-3</sup>			mg dm <sup>-3</sup>					
Ca	Mg	Ca+Mg	Al	K	K	S	P	pH
								CaCl <sub>2</sub>
7.22	2.65	9.87	0.05	1.34	525	6	63.5	6.5
Micronutrients mg dm <sup>-3</sup>					g dm <sup>-3</sup>		Cmol <sub>c</sub> dm <sup>-3</sup>	
Na	Fe	Mn	Cu	Zn	B	O.M	CTC	SB
8.0	31.5	162.8	3.2	6.9	6.9	44.4	88.34	11.25
Texture			Relationships base			% bases CEC		
Clay	Silt	Sand	Ca/Mg	Ca/K	Mg/K	Ca/CEC	Mg/CEC	K/CEC
42.5	15.1	42.4	2.73	5.38	1.97	56.71	20.81	10.54

Note: CEC = Cation Exchange Capacity. Extractants P (Mel), K, Na, Cu, Fe, Mn, and Zn = Mehlich 1; Ca, Mg, and Al = 1N KCl; S = Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> in HOAc (acetic acid); Organic Matter (O.M.) = Colorimetric method; Total P = Sulfuric acid digestion, and B = BaCl<sub>2</sub>. Source: Authors, 2025.

### 2.3 Climate

The region's climate is classified as Aw (Tropical Savanna) according to the Köppen-Geiger system. The average temperature ranges from 20 to 25 °C. The rainy season occurs from October to April, while the dry season extends from May to September. The average annual precipitation ranges from 1,567 to 1,611 mm.

### 2.4 Experimental design

The experimental design was completely randomized (CRD), with four replications. The treatments consisted of eight doses of NiSO<sub>4</sub>·5H<sub>2</sub>O P.A - ACS (Vatten Soluções Ambientais, Brazil) (0, 5, 15, 35, 85, 100, 125, and 600 mg/L<sup>-1</sup> of Ni), applied in the planting furrow at the time of soybean sowing, totaling 32 sample plots. The Ni doses were randomly applied, ranging from low to high levels for soybean cultivation.

### 2.5 Soil amendment, sowing, and maize hybrid description

After soil analysis, correction was performed considering the area as having high fertility, with over 20 years of continuous cultivation (soybean, maize, and sorghum). Liming was carried out to raise base saturation to 70%. A total of 600 kg of soil was collected at two depths (0–20 cm and 20–40 cm) and transported to the experimental area. Based on the soil analysis results and interpretations of Ribeiro et al. (1999), base fertilization was unnecessary. After soil analysis, the soil was transferred to 15 L experimental units (pots), and five maize seeds were sown per unit. After germination, thinning was performed at the V2 growth stage, leaving three plants per experimental unit. The maize hybrid used was the transgenic Pioneer P3601 PWU. This hybrid has an early growth cycle and is intended for grain production. It has a plant height of 2.70 m, an ear height of 1.46 m, a flowering GDU of 1593, and a semi-hard yellow-orange kernel type. It presents high yield potential and stability, good foliar disease resistance, and nematode tolerance: high resistance factor (RF) reduction for *Pratylenchus brachyurus* and *Meloidogyne incognita*, and moderate RF reduction for *Meloidogyne javanica*. It also exhibits high responsiveness to crop management practices.

### 2.6 Cultural practices

At the V4 stage, the commercial product FICAM® (Bendiocarb, Brazil) was used for pest control, as *Diabrotica speciosa* was observed. Irrigation was performed every six days or when low soil moisture was detected with an electronic moisture probe (BOM, model 6810, China).

### 2.7 Sampling and variable analysis

Sampling was carried out on maize plants at the V7 phenological stage. The roots were washed with running

water to remove soil particles. The plants were then transferred to the Soil and Foliar Laboratory at UniBRAS Rio Verde, where vegetative parameters were analyzed, including Plant Height (PH), Root Length (RL), both expressed in centimeters (cm), Shoot Fresh Mass (SFM), Root Fresh Mass (RFM), Shoot Dry Mass (SDM), and Root Dry Mass (RDM), all expressed in grams (g). The nickel content in the root (Ni-root) and in the shoot (Ni-shoot) was also analyzed.

### 2.8 Nickel analysis

According to EMBRAPA (2011), Nickel (Ni) analysis was performed by atomic absorption spectrometry (AAS) following acid digestion (wet method) using a Ni standard solution with concentrations ranging from 0.1 to 5.0 mg L<sup>-1</sup>, based on a NiSO<sub>4</sub> standard salt. The wavelength ( $\lambda$ ) used was 232.0 nm or 231.6 nm (the main absorption line for Ni), with flame mode (F-AAS) and acetylene gas. The method used to digest the root and aerial part samples was the Nitric-perchloric mixture.

### 2.9 Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA), using the adopted experimental design. When significance was detected by the *F*-test ( $p < 0.05$ ), regression analysis was performed, selecting the model with the highest significance and best fit ( $R^2$ ). All analyses used the SISVAR statistical software (Ferreira, 2019).

## 3. Results

### 3.1 Agronomic and bioaccumulative parameters

According to the *F*-test for agronomic and bioaccumulative parameters of Ni in roots and shoots, the observed models were linear for SFM and quadratic for Ni-root and Ni-shoot content. The remaining parameters showed no significant differences and, therefore, did not fit any distinct statistical model (Table 2).

Table 2. Agronomic and bioaccumulative parameters of Ni in Pioneer P3601 PWU hybrid maize plants under different concentrations.

F-value	F-critical value	Model	CV (%)
Root length (cm)	1.04 ns	-	32.48
Plant height (cm)	3.67 ns	-	13.17
Root fresh mass (g)	3.56 ns	-	59.08
Root dry mass (g)	3.27 ns	-	31.64
Shoot fresh mass (g)	4.95 *	Linear	40.50
Shoot dry mass (g)	3.74 ns	-	28.78
Root Ni content (mg kg <sup>-1</sup> )	1052233.01 *	Quadratic	0.80
Shoot Ni content (mg kg <sup>-1</sup> )	6783.55 *	Quadratic	1.69

Note: \* = Significant. ns = not significant. CV% = Coefficient of variation. Source: Authors, 2025.

### 3.1 Agronomic parameters

Figure 1 presents the results of SFM in Pioneer P3601 PWU hybrid maize plants subjected to different Ni concentrations. According to the *F*-test ( $p < 0.05$ ), Ni treatments resulted in two distinct data clusters: one comprising the control and doses up to 125 mg L<sup>-1</sup> of Ni, with a maximum value of 0.61 g, and another at 600 mg L<sup>-1</sup> of Ni, showing a maximum value of 0.82 g. The data followed a linear statistical model.

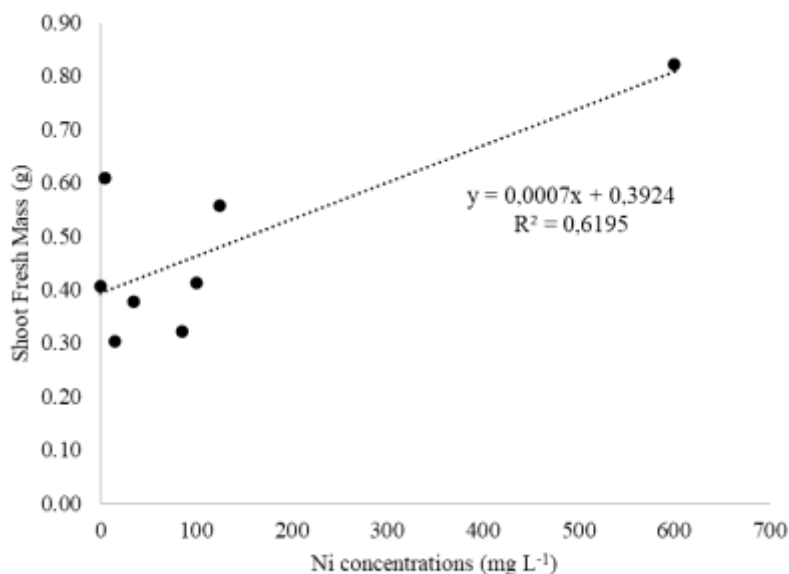


Figure 1. Agronomic parameter of shoot fresh mass in Pioneer P3601 PWU hybrid maize subjected to different Ni doses (mg L<sup>-1</sup>). Source: Author, 2025.

Figure 2 (A and B) shows the results of Ni bioaccumulation in the shoot and roots of the transgenic maize hybrid Pioneer P3601 PWU. According to the *F*-test, the best-fitting statistical model was quadratic. For both shoot and root tissues, two data groupings were observed: from the control up to 125 mg L<sup>-1</sup>, with maximum values of 1.25 mg kg<sup>-1</sup> and 27.48 mg kg<sup>-1</sup>, respectively. At the highest concentration of 600 mg L<sup>-1</sup>, a lower Ni uptake was observed in the shoot (1.92 mg kg<sup>-1</sup>) (Figure 2, A), whereas the roots showed higher bioaccumulation at 29.31 mg kg<sup>-1</sup> (Figure 2, B).

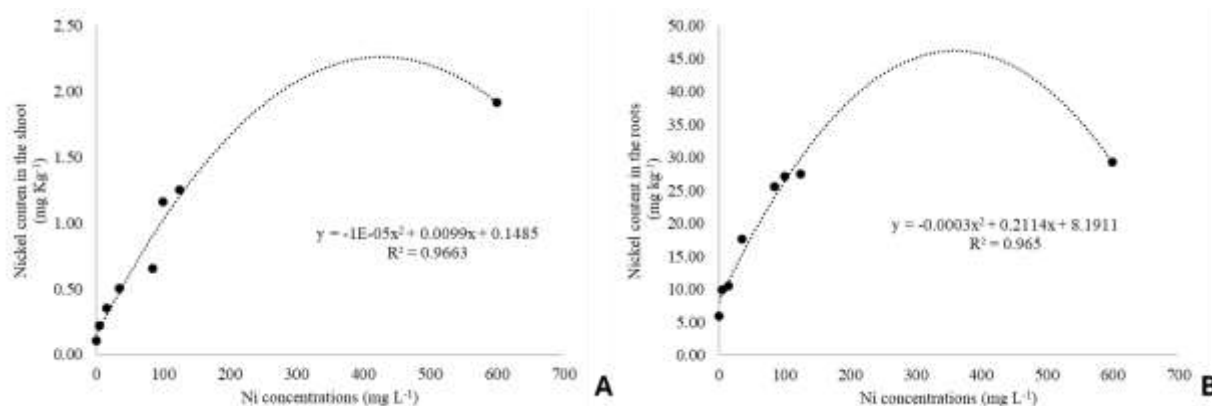


Figure 2. Bioaccumulative parameters of Ni (mg kg<sup>-1</sup>) in roots and shoots of Pioneer P3601 PWU hybrid maize plants under different doses (mg L<sup>-1</sup>). Source: Authors, 2025.

#### 4. Discussion

It is well established that both macro- and micronutrients can become toxic when applied at doses exceeding the recommended levels for each plant species, affecting not only ornamental plants but also small- and large-scale crops. In our study, we observed that the different doses of the nickel (Ni) source used had a positive effect only on the agronomic parameter of shoot fresh mass.

Santiago et al. (2015), evaluating the effect of NiCl<sub>2</sub>·6H<sub>2</sub>O on the AI Bandeirante maize hybrid, reported a significant effect on shoot dry mass at doses ranging from 0.016 to 10 mg kg<sup>-1</sup> in different soil types. However,

the authors noted a decrease in dry mass production with increasing Ni doses, suggesting a tolerance threshold for the crop.

Similarly, Campanharo et al. (2013) observed that cowpea plants were susceptible to Ni-induced phytotoxicity at concentrations ranging from 0 to 100 mg L<sup>-1</sup>, with primary symptoms including reddish spots on the first true leaves. In another study on common bean, Berton et al. (2006) found that a Ni dose of 210 mg kg<sup>-1</sup> significantly reduced dry mass production, indicating that excess Ni negatively affects several essential physiological processes.

Regarding bioaccumulation, our results demonstrated a higher concentration of Ni in the roots compared to the shoots during the vegetative stage, indicating low translocation and a preferential accumulation of the metal in below-ground organs with lower affinity for aerial tissues. This finding differs from the study by Cheng et al. (2024), who reported that soluble Ni is readily absorbed by the roots and presents high mobility, being easily translocated to the upper parts of the plant.

Although the effects of Ni on foliar tissues are not yet fully understood, it is known that this metal can inhibit photosynthesis and respiration, cause necrotic lesions, and retard plant growth. Our results indicate a pronounced affinity of Ni for root tissues rather than foliar tissues, even during the vegetative phase. Similar results were reported for sorghum plants treated with Ni-containing sludge, where the researchers found greater Ni bioaccumulation in the roots compared to the aerial organs (leaves, stems, and grains) (Revoredo & Melo, 2006). Divergent findings have also been reported; for example, Campanharo et al. (2013) showed that foliar application of Ni (0 to 100 mg L<sup>-1</sup>) to cowpea plants resulted in preferential accumulation in mature and young leaves as well as in pods, confirming a tendency for Ni to concentrate in aerial tissues. However, Ni concentrations in the roots were significantly different from both the control and the aerial tissues.

Nickel also interferes with the absorption and metabolism of other essential micronutrients, such as zinc (Zn) and manganese (Mn), with which it has antagonistic interactions, and may exert either antagonistic or synergistic effects with copper (Cu) and iron (Fe). Torres et al. (2016) found that the application of different Ni doses in maize plants led to reduced shoot development, chlorosis, abscission of basal leaves, root necrosis, and decreased dry biomass production, especially at higher concentrations (7.5 and 10 mg dm<sup>-3</sup>).

Given these findings, future studies should focus on evaluating the cumulative translocation of Ni throughout the entire maize growth cycle, including the potential transfer of the metal from the plant to the grains. According to Krupa et al. (1993), Ni phytotoxicity is primarily due to its disruptive action on the photosystem, causing disturbances in the Calvin cycle and inhibiting electron transport as a result of excessive accumulation of ATP and NADPH due to inefficiency in dark reactions.

## 5. Conclusions

The application of different nickel (Ni) doses significantly affected only the shoot fresh mass parameter. Regarding the bioaccumulation effect, greater translocation of Ni to the roots and lower affinity for the shoot were observed during the vegetative stage of the Pioneer P3601 PWU hybrid maize plants, corroborating the results of the agronomic parameters.

## 6. Authors' Contributions

*Elano Carmo Silva Filho*: project design, experimental procedures, and manuscript writing. *Gilberto Moura da Silva Neto*: project design, experimental procedures, and manuscript writing. *Carlos Frederico de Souza Castro*: analysis of Ni concentrations, preparation of solutions, and application. *Elizabete Nunes da Rocha*: manuscript writing and post-review revisions. *Matheus Vinícius Abadia Ventura*: data analysis and statistics. *Antonio Carlos Pereira de Menezes Filho*: supervisor, project development, experimental oversight, data collection, manuscript writing, translation, and publication.

## 7. Conflicts of Interest

No conflicts of interest.

## 8. Ethics Approval

Not applicable.

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#### **Funding**

Not applicable.

#### **Institutional Review Board Statement**

Not applicable.

#### **Informed Consent Statement**

Not applicable.

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