

Foliar biofortification with iron, zinc and copper in maize under rainfed climatic conditions and its nutritional quality

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ABSTRACT

Objective: To evaluate nutrient absorption and forage quality in corn microsilos (*Zea mays* L.) cultivated under rainfed conditions and foliar biofortified with iron (Fe), zinc (Zn), and copper (Cu).

Design/Methodology/Approach: The study was carried out at the Juan Rodríguez Clara Higher Technological Institute, located in the municipality of Juan Rodríguez Clara, Veracruz. Foliar applications of Zn, Cu, and Fe were administered throughout the crop's growth cycle. Bromatological parameters and mineral content were analyzed through correlation and interpretation in relation to climatic variables, specifically temperature and precipitation.

Results: Moderate temperatures (22-23 °C) promoted Fe absorption (202 ppm); however, they also led to increases in neutral detergent fiber (NDF) (59%) and acid detergent fiber (ADF) (33%), enhancing plant structural rigidity. Conversely, cumulative rainfall of 200 mm negatively impacted Zn (29 ppm) and Cu (19 ppm) uptake due to leaching and water stress, which subsequently reduced the organic matter content (%OM) in the microsilos to 88%. A positive correlation was identified between Fe concentration and structural fiber content, while a negative correlation was observed between protein levels and hemicellulose, indicating a potential metabolic imbalance.

Study Limitations/Implications: Rainfall interfered with Zn and Cu absorption, underscoring the importance of aligning foliar fertilization strategies with prevailing weather conditions.

Findings/conclusions: Fe biofortification influences plant structure; water stress limits the absorption of Zn and Cu. Foliar applications must be adjusted according to the climate to optimize the nutritional quality of the forage.

Keywords: Maize, biofortification, microminerals, fibers.

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INTRODUCTION

Crop biofortification has emerged as a sustainable strategy to enhance the nutritional quality of food and ensure global food security (Sandhu *et al.*, 2023). Essential

micronutrients such as zinc (Zn), copper (Cu), and iron (Fe) play pivotal roles in plant metabolic processes, directly influencing their development, productivity, and nutritional quality (Niu *et al.*, 2020). However, the deficiency of these elements in agricultural soils remains a persistent challenge, limiting crop yields and compromising the quality of forage used in animal feed (Shukla & Behera, 2024; Rahman *et al.*, 2020). Among fertilization strategies, foliar application of micronutrients has been identified as an effective technique to enhance nutrient uptake, particularly in soils with low availability of essential elements or under water stress conditions (Fageria *et al.*, 2012; Fernández & Brown, 2013). This method enables nutrients to be directly absorbed through the leaves, thereby avoiding losses due to soil immobilization (Niu *et al.*, 2020). Nonetheless, various environmental factors such as temperature, precipitation, and accumulated heat units (growing degree days, GDD) significantly influence the efficiency of biofortification by affecting the absorption and distribution of these micronutrients within plant tissues (Havlin *et al.*, 2014; Ullah *et al.*, 2020). Scientific research has demonstrated that adverse climatic conditions, including drought stress and thermal variability, directly impact enzymatic activity in plants, altering fundamental physiological processes such as transpiration, root uptake, and nutrient mobilization (Shukla & Behera, 2024; Rao & Takahashi, 2022). These disruptions can lead to metabolic imbalances in which the plant prioritizes the development of robust structural components such as neutral detergent fiber (NDF) and acid detergent fiber (ADF) at the expense of nitrogenous compound accumulation, ultimately reducing forage digestibility and nutritional value (White & Broadley, 2009). In this context, the aim of this study is to evaluate nutrient absorption and forage quality in corn (*Zea mays* L.) microsilos developed under rainfed conditions and foliar biofortified with Fe, Zn, and Cu, while analyzing the influence of temperature and precipitation on the absorption of these minerals. Understanding these interactions will help optimize biofortification strategies, contributing to the development of more efficient and sustainable agricultural systems.

MATERIALS AND METHODS

Study site

The experiment was conducted at the Juan Rodríguez Clara Higher Technological Institute, located in the municipality of Juan Rodríguez Clara, Veracruz, Mexico (18° 00' 6.1" N, 95° 24' 1.7" W), at an altitude of 133 meters above sea level. According to the modified Köppen climate classification (García, 2004), the region presents a warm sub-humid climate (AW₀), with an average annual temperature of 24.5 °C and annual precipitation of approximately 1100 mm. The predominant soil type is a distric Cambisol (FAO-UNESCO, 1977), characterized by a sandy loam texture and strongly acidic pH (NOM-021-RECNAT-2000, 2000) (Tosquy-Valle *et al.*, 2020).

Crop establishment

The field trial was established on a 960 m² plot using a planting configuration of 80×0.15 cm with commercial hybrid maize seed. A randomized complete block design (RCBD) with three replications was implemented. Foliar applications of zinc (Zn), iron

(Fe), and copper (Cu) were carried out at a concentration of 1000 ppm. Applications were performed at three key growth stages: when the crop had three fully developed leaves, at stage V5-V6, and at stage V10-V12, prior to flowering (Crosby-Galván *et al.*, 2024).

Evaluated variables

Nutritional and mineral content of the corn microsilos were assessed. Silos were constructed using 8 PVC tubes, filled with chopped maize plants (cut to <5 cm particle size) harvested at the milk-dough stage (45 days after sowing). Once filled, the containers were hermetically sealed and opened after 45 days for bromatological analysis. The following parameters were determined: dry matter (DM, %), ash (A, %), crude protein (CP, %), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), hemicellulose (H, %), and lignin (L, %) following standard methods (AOAC, 1975; Van Soest *et al.*, 1994). Mineral content Fe, Cu, and Zn (ppm) was determined via atomic absorption spectrophotometry (AAS). Data were also collected to estimate growing degree days (GDD), based on maximum and minimum daily temperatures (°C) and the maize base temperature (10 °C) (Angel *et al.*, 2017).

Statistical analysis

To evaluate the effect of foliar treatments with Zn, Cu, and Fe (ppm) on the nutritional content (%), an analysis of variance (ANOVA) was performed under a randomized complete block design with three replications. Tukey's test ($p < 0.05$) was applied to detect statistically significant differences among means. In the absence of significant differences, a Pearson correlation analysis was conducted between nutritional content variables (CP, NDF, ADF, H, and L) and mineral content (Fe, Cu, and Zn in ppm) to identify linear associations and interpret the physiological and structural effects of biofortification. Statistical analyses were performed using the SAS software package (SAS, 2009).

RESULTS AND DISCUSSION

The absence of statistically significant differences ($p > 0.05$) among treatments in the evaluated variables (Table 1) may be attributed to the natural variability of climatic conditions under rainfed systems (Figure 1), which likely elicited a moderate physiological response of the crop to the foliar biofortification treatments.

Table 1. Effect of foliar biofortification with Zn, Cu, and Fe on the nutritional and mineral composition of maize (*Zea mays* L.) microsilos under rainfed climatic conditions.

Treatment	pH	Temp (°C)	DM (%)	Ash (%)	OM (%)	CP (%)	NDF (%)	ADF (%)	H (%)	L (%)
Control	5	23	97	5	95	7	54	25	29	4
Zn	4	22	97	4	96	8	58	31	27	5
Cu	4	23	98	12	88	8	58	30	27	5
Fe	4	22	97	4	96	8	59	33	26	5

Temp=Temperature; DM=Dry Matter; Ash=Ash Content; OM=Organic Matter; CP=Crude Protein; NDF=Neutral Detergent Fiber; ADF=Acid Detergent Fiber; H=Hemicellulose; L=Lignin.

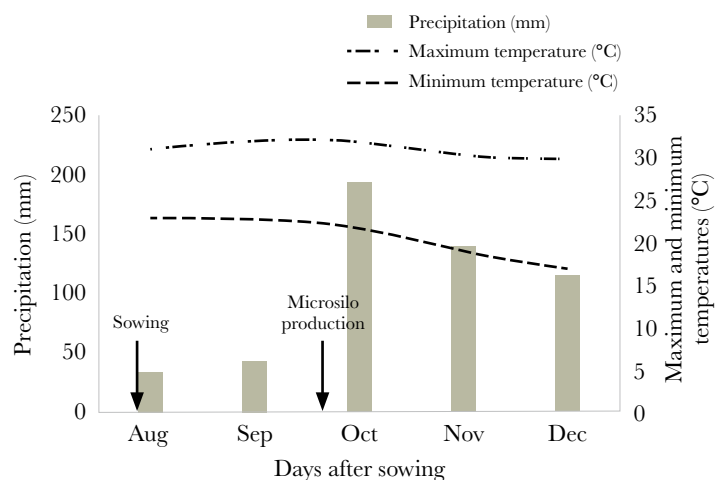


Figure 1. Precipitation and temperature behavior during the biofortification process of corn microsilsos (*Zea mays L.*).

On the other hand, the lack of statistical significance does not preclude the presence of physiologically relevant effects (Tables 1 and 2). Therefore, the analysis was complemented with a Pearson correlation matrix between bromatological and mineral variables (Table 3), which revealed association patterns that better explain the structural and nutritional responses of maize. During the crop cycle, temperatures ranged between 22 °C and 23 °C an optimal range for nutrient absorption according to maize physiological requirements (El-Sappah *et al.*, 2022; Waqas *et al.*, 2021). Conversely, under water stress conditions, foliar transpiration is reduced, limiting nutrient translocation from the leaves to growing organs. This leads to an increase in structural fiber content, specifically neutral detergent fiber (NDF) and acid detergent fiber (ADF). In the case of foliar-applied Fe, absorption occurred, but its mobilization to cellular structures was hindered. Consequently, lignification of foliar tissues was observed as a physiological defense response to stress, with NDF and ADF levels reaching 59% and 33%, respectively (Table 1).

The Fe concentrations obtained induced a structural response in the plant, characterized by an increase in structural fibers. Stein *et al.* (2019) reported that iron application can promote lignification in roots, particularly in cultivars tolerant to Fe excess, such as rice, where lignin concentration in roots increased under high Fe treatments. In the present study, Fe absorption reached 202 ppm under moderate temperature conditions although lower than that observed in the control treatment (254 ppm) (Table 2) suggesting effective micronutrient mobilization via the root pathway into plant tissues.

Table 2. Comparative concentration of mineral elements in ensiled maize forage. Juan Rodríguez Clara Higher Technological Institute, Spring-Summer 2023 growing season.

Micronutrient	Control (ppm)	Biofortified (ppm)	Critical Value Range (ppm) ¹
Copper (Cu)	16	19	5-20
Zinc (Zn)	26	29	20-70
Iron (Fe)	254	202	50-250

¹Critical values referenced from Mengel & Kirkby (1987) and Szerement *et al.* (2021).

This response can be attributed to the acidic nature of the soil (Tosquy-Valle *et al.*, 2017). Under acidic conditions, iron is predominantly present in its ferrous form (Fe^{2+}), which is more soluble and readily available for root uptake. In such environments, foliar absorption of Fe is generally less efficient than root uptake, due to structural barriers in the leaf and the dynamic behavior of the ion in solution. Additionally, physiological factors such as reduced foliar transpiration under water stress conditions limit the internal translocation of iron following foliar application. The effective absorption observed is likely due to moderate temperatures, under which Fe remains in its soluble (Fe^{2+}) form, facilitating its incorporation into plant tissues (Van Groeningen *et al.*, 2020). In contrast, elevated temperatures promote the oxidation of Fe into its insoluble ferric form (Fe^{3+}), reducing its bioavailability (Barker *et al.*, 2023). From a plant physiology perspective, thermal and water stress directly affect enzymatic activity, gene expression, and the function of ion channels involved in micronutrient uptake. When plants are exposed to excessive heat or water deficit, stomatal closure occurs as a survival mechanism, reducing foliar nutrient absorption and translocation. Although necessary for survival, this adaptive response compromises biofortification efficiency. Consequently, biofortified treatments with Cu and Zn, while showing slight increases, did not differ significantly from the control, suggesting inefficient biofortification (Table 2). This can be explained by the specific climatic and edaphic conditions during the study, characterized by acidic soil (pH 4.67) and low cation exchange capacity ($3.9 \text{ cmol}^+ \text{ kg}^{-1}$) (Tosquy-Valle *et al.*, 2017). These conditions enhance micronutrient solubility but increase susceptibility to leaching and reduce nutrient retention. Therefore, even when foliar absorption is targeted, application immediately before heavy rainfall (200 mm accumulated) reduces effective nutrient uptake due to surface wash-off. Although Zn levels reached 29 ppm slightly higher than the control (26 ppm) this cannot be considered a significant improvement. Cu concentrations also increased marginally (19 ppm *vs.* 16 ppm in the control), suggesting limited phloem mobility and potential interference from Fe, which showed high concentration in the control (254 ppm). These findings align with reports by Ullah *et al.* (2020), Szerement *et al.* (2021), and Luo *et al.* (2024), who emphasize that the efficiency of foliar micronutrient application largely depends on timing, nutrient mobility, and post-application environmental conditions. Moreover, Zn and Cu absorption is also temperature-sensitive (Barker *et al.*, 2023). This may be due to ionic competition among micronutrients and the plant's transpiration rate, which regulates foliar uptake. Despite strategic timing, cumulative rainfall of 200 mm after microsilos establishment did not significantly affect nutrient absorption (Figure 1). Heavy rains washed away foliar-applied nutrients before absorption (López-Salazar *et al.*, 2019) and reduced mobility (Luo *et al.*, 2024). Lower concentrations of Zn and Cu were likely due to leaching or moisture saturation, which slows nutrient uptake. Additionally, water-saturated soils limit root oxygenation, thereby reducing soil nutrient uptake (Zhou *et al.*, 2024; Drechsel *et al.*, 2023). While foliar applications can serve as mitigation strategies under rainfed conditions, careful timing is essential (Wasaya *et al.*, 2017). Environmental conditions also influenced the nutritional composition of ensiled forage (Figure 2, Table 1). Biofortified treatments showed an increase in crude protein from 7% (control) to 8%, suggesting that thermal conditions favor nitrogen compound synthesis (Waqas *et al.*, 2021).

In contrast, the Fe treatment increased NDF to 59% and ADF to 33%, indicating greater development of structural components (lignification) (Table 2). Iron contributes to cell wall lignification, a process driven by enzymatic activity stimulated by moderate temperatures (Shoormij *et al.*, 2022). Antonio-Medina *et al.* (2021) also reported that Cu application in green wheat forage under controlled conditions increased ADF and NDF fractions, likely due to structural reorganization of the cell wall. This aligns with foliar Cu biofortification in maize, which also resulted in elevated NDF (58%) and ADF (30%) compared to the control (54% and 25%, respectively), though not statistically significant (Table 1). This suggests that Cu may exert a structural effect across plant species, even under uncontrolled edaphoclimatic conditions. Moreover, at moderate temperatures (22-23 °C, Figure 2), the accumulation of growing degree days (GDD) favored Fe absorption (202 ppm), but also increased NDF and ADF, leading to more fibrous plant tissues via lignification (Wu *et al.*, 2024). During the cropping cycle, planting occurred on August 2, 2023, coinciding with a steady rise in GDD, which promoted vegetative development until reaching the milk-dough stage (Figure 2). This phase, characterized by high metabolic activity, is critical for the accumulation of essential nutrients.

However, the occurrence of cumulative precipitation totaling 200 mm created a conducive environment for water stress, which adversely affected physiological processes and reduced the efficiency of micronutrient absorption (Figures 1 and 2). Physiologically, water and heat stress disrupt key processes such as transpiration, root absorption, and nutrient mobilization. These effects are evident in Table 1. Additionally, Table 3 presents the structural and nutritional components of biofortified maize (*Zea mays* L.) microsilos with zinc (Zn), copper (Cu), and iron (Fe), which were directly influenced by plant physiology and the absorption dynamics of these micronutrients. A strong positive correlation was observed between neutral detergent fiber (NDF) and acid detergent fiber (ADF) ($r=0.88$, $p=0.0002$), indicating that increased Fe absorption promotes the synthesis of structural components such as cellulose and lignin, thereby enhancing the mechanical strength of plant cell walls.

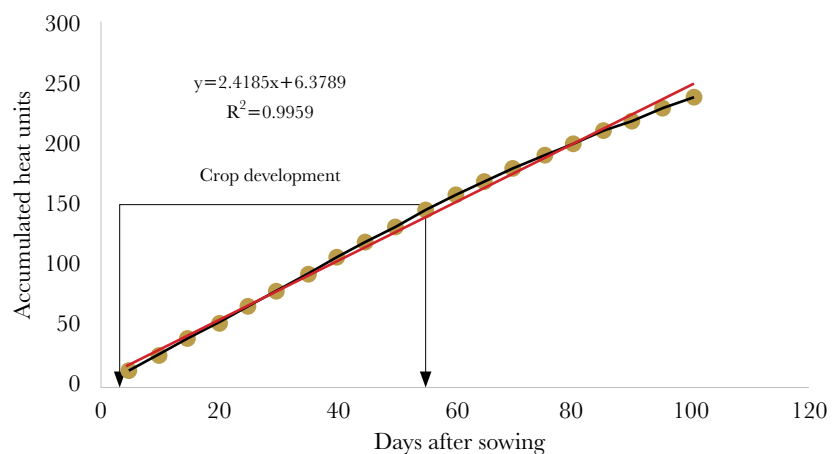


Figure 2. Relationship between days after sowing (DAS) and accumulated heat units with the maize (*Zea mays* L.) crop. Juan Rodriguez Clara Higher Technological Institute. 2023.

Table 3. Correlation between nutritional composition and mineral content in maize (*Zea mays* L.) microsilos biofortified with Fe, Cu, and Zn. Juan Rodríguez Clara Higher Technological Institute, Spring–Summer 2023 growing season.

Variables	NDF	ADF	H	L	Fe	Cu	Zn
CP	0.08 (p=0.798)	0.40 (p=0.204)	-0.69 (p=0.013)	0.49 (p=0.105)	0.03 (p=0.928)	0.03 (p=0.917)	-0.58 (p=0.048)
NDF		0.88 (p=0.0002)	-0.11 (p=0.741)	0.53 (p=0.074)	0.20 (p=0.526)	-0.31 (p=0.334)	0.10 (p=0.753)
ADF			-0.56 (p=0.056)	0.78 (p=0.003)	-0.03 (p=0.923)	-0.23 (p=0.463)	-0.04 (p=0.905)
H				-0.70 (p=0.011)	0.42 (p=0.176)	-0.04 (p=0.896)	0.27 (p=0.398)
L					-0.01 (p=0.978)	-0.10 (p=0.747)	-0.35 (p=0.268)
Fe						-0.19 (p=0.549)	-0.17 (p=0.597)
Cu							-0.24 (p=0.455)

PC=Proteína Cruda; FDN=Fibra Detergente Neutro; FDA=Fibra Detergente Ácida; H=Hemicelulosa; Li=Lignina Detergente Ácida; Fe=Hierro; Cu=Cobre; Zn=Zinc.

This physiological process occurs when the plant is subjected to stress conditions, prioritizing the formation of structural support tissues over other metabolic processes (Santiago & Malvar, 2010). A strong correlation was also observed between ADF and lignin (Li) ($r=0.77$, $p=0.003$), confirming that absorbed iron is closely linked to the lignification of plant tissues. This suggests that while the plant may tolerate adverse climatic conditions, it compromises forage digestibility by increasing cell wall rigidity, thereby negatively affecting forage quality once ensiled. Moreover, a significant negative correlation was found between crude protein content and hemicellulose (H) ($r=-0.69$, $p=0.0129$), indicating a metabolic shift in the plant toward the production of structural fibers at the expense of protein synthesis. This physiological response may result from climatic stress and biofortification with Fe and Cu elements known to stimulate the formation of protective tissues, but which can simultaneously reduce the nutritional value of the forage. These results demonstrate that the absorption of Fe, Zn, and Cu directly influences the structural composition of biofortified maize and that this effect does not improve upon silage conversion. The prioritization of structural tissue formation enhances plant resilience but compromises forage nutritional quality by decreasing protein availability and increasing fiber levels. Therefore, it is essential to achieve a balanced biofortification strategy that maximizes both micronutrient absorption and the nutritional value of the silage, thereby ensuring optimal nutrient supply for animal production systems.

Climatic conditions and micronutrient absorption

The climatic conditions recorded during the cropping cycle significantly influenced the absorption of zinc (Zn), copper (Cu), and iron (Fe) in biofortified maize (*Zea mays* L.), and no significant differences were observed once the crop was processed into silage.

Stable temperatures between 22 °C and 23 °C favored Fe absorption (202 ppm) in the biofortified treatment a result consistent with findings by Niu *et al.* (2020), who highlighted that moderate temperatures enhance the solubility and translocation of foliar-applied micronutrients. Under these thermal conditions, Fe remains in its soluble ferrous form (Fe^{2+}), facilitating incorporation into plant tissues and contributing to increased neutral detergent fiber (NDF) (59%) and acid detergent fiber (ADF) (33%) levels, which reinforce cellular structure through lignification (White & Broadley, 2009). However, the 200 mm of cumulative precipitation following foliar application negatively affected Zn (29 ppm) and Cu (19 ppm) absorption, likely due to leaching and water stress. According to Ullah *et al.* (2020), soil saturation reduces transpiration and limits foliar nutrient uptake. Rao and Takahashi (2022) noted that water stress alters enzymatic activity, stomatal conductance, and nutrient translocation. The behavior of Zn and Cu may also be explained by ionic competition, as the high Fe concentration could have interfered with their absorption. This interaction is especially relevant in moisture-saturated environments, where transpiration is limited (Szerement *et al.*, 2021). Water stress prior to rainfall, followed by excess moisture, hindered efficient nutrient uptake and limited the internal mobility of minerals such as Cu, which are critical to physiological processes related to plant growth (Antonio-Medina *et al.*, 2021).

Relationship between micronutrients and forage composition

The correlation matrix revealed key relationships between absorbed micronutrients and the structural composition of the forage. A strong positive correlation was observed between NDF and ADF ($r=0.88$, $p=0.0002$), indicating that Fe absorption promoted the synthesis of cellulose and lignin essential components for structural reinforcement in plant tissues (Liu, 2012). This finding is consistent with iron's role in cell wall lignification, enhancing mechanical resistance but potentially compromising forage digestibility (White & Broadley, 2009; Basnet & Khanal, 2022). Similarly, a positive correlation was found between ADF and lignin (Li) ($r=0.77$, $p=0.003$), supporting the notion that Fe absorption stimulates the formation of more rigid and lignified tissues, improving plant tolerance to environmental stress while potentially reducing the nutritional value of silage (Puren *et al.*, 2023). On the other hand, a significant negative correlation was identified between crude protein and hemicellulose (H) ($r=-0.69$, $p=0.0129$), suggesting a metabolic imbalance in which the plant shifts resources toward structural fiber production at the expense of nitrogen compound accumulation. This behavior may result from physiological responses to water stress and the presence of Fe and Cu, which stimulate the formation of defensive structures but compromise protein content (Szerement *et al.*, 2021; Rao & Takahashi, 2022). The findings highlight the need to synchronize foliar biofortification with climatic conditions to maximize micronutrient uptake and improve forage quality. Although pre-rainfall foliar applications helped prevent nutrient leaching, the proximity of heavy rainfall events affected the mobility and assimilation of Zn and Cu. This reinforces the importance of aligning agronomic management with climatic factors to optimize biofortification efficiency (Niu *et al.*, 2020; Xue *et al.*, 2023). The correlation between Fe absorption and increased NDF and ADF illustrates that while plant structural resistance improves, forage

digestibility may decline. Therefore, it is crucial to balance micronutrient application to enhance both absorption and silage nutritional value (Khulbe *et al.*, 2020).

CONCLUSIONS

Foliar application of iron, zinc, and copper in maize microsilos under rainfed conditions and acidic soils did not achieve effective biofortification. However, it did induce changes in structural and bromatological composition. Iron, in particular, influenced crude protein content but compromised nutritional quality by promoting forage lignification. Foliar biofortification is not solely dependent on dosage or nutrient type but also on timing, fertilizer formulation, and, most importantly, edaphoclimatic conditions. Therefore, it is recommended to adjust foliar application strategies according to soil characteristics and rainfall patterns to optimize crop response and forage quality. To ensure agronomic strategies such as biofortification are effective in animal production systems, a balanced approach must be implemented one that enhances nutrient uptake without compromising the nutritional value of silage.

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