Toxicity and bioaccumulation of Cu and Ni in plants during the vegetative stage of hybrid maize grown in dystroferric red latosol

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Abstract

Heavy metals such as Cu^{2+} and Ni^{2+} can promote bioaccumulation and induce phytotoxic effects in various plant groups, including agriculturally important species such as maize (*Zea mays* L.). In this study, we assessed the effects of different concentrations of these metals, which act as micronutrients at low levels but become toxic at elevated doses. Increasing concentrations of Cu and Ni (mg L^{-1}) were applied via nutrient solution to dystroferric Red Latosol cultivated with the hybrid maize Pioneer P3601 PWU. The experiment was conducted in a greenhouse and evaluated at the V5 growth stage. Our results showed that concentrations above 5 mg L^{-1} impaired both shoot and root development, with severely toxic effects observed at the highest concentrations (350 and 600 mg L^{-1}). We conclude that Cu and Ni levels exceeding 5 mg L^{-1} negatively affect the vegetative growth of hybrid maize and exhibit significant translocation between roots and shoots, ultimately compromising plant development.

Keywords: toxic effects of heavy metals, micronutrients, *Zea mays* L., roots, soil contamination.

Toxicidade e bioacumulação de Cu e Ni em plantas no período vegetativo em milho híbrido cultivado em solo vermelho distroférrico

Resumo

Metais pesados como Cu^{2+} e Ni^{2+} podem promover bioacumulação e causar efeitos fitotóxicos em diferentes grupos vegetais, incluindo espécies de alto interesse agrícola, como o milho (*Zea mays* L.). Neste estudo, avaliamos os efeitos de diferentes concentrações desses metais, que em baixas doses atuam como micronutrientes, mas em níveis elevados tornam-se tóxicos às plantas. Foram aplicadas concentrações crescentes de Cu e Ni (mg L^{-1}) via solução nutritiva em solo vermelho distroférrico cultivado com milho híbrido Pioneer P3601 PWU. O experimento foi conduzido em casa de vegetação e avaliado no estádio V5. Os resultados mostraram que doses acima de 5 mg L^{-1} comprometeram o desenvolvimento da parte aérea e das raízes, com efeitos severamente tóxicos observados nas maiores concentrações testadas (350 e 600 mg L^{-1}). Conclui-se que concentrações superiores a 5 mg L^{-1} de Cu ou Ni prejudicam o crescimento vegetativo de milho híbrido, além de apresentarem elevada translocação entre raízes e parte aérea, impactando negativamente o desenvolvimento da cultura.

Palavras-chave: efeito tóxico por metais, micronutrientes, *Zea mays* L., raízes, solo contaminado

1. Introduction

Heavy metals (HMs) represent an important source of environmental contamination resulting from anthropogenic activities such as mining, smelting, application of chemical fertilizers, the use of biofertilizers such as sewage sludge, and pesticide application (Andresen; Küpper, 2013). Among the most extensively studied

HMs are Cd, Pb, Cr, As, Hg, Ni, Cu, and Zn, which pose serious threats to agriculture due to their propensity to accumulate in soils and bioaccumulate in plants and animals (Wang et al., 2023). If not absorbed by plants or removed through leaching, these elements exhibit high environmental persistence and may remain in the soil for hundreds of years (Ghori et al., 2019; Tóth et al., 2016).

Several metals, such as Cd, Cr, and Hg, do not play essential physiological roles in plants, even at low concentrations. In contrast, micronutrients such as Cu and Ni are required in small amounts for normal physiological processes, although concentrations above adequate levels result in phytotoxicity (Tubotu et al., 2024). Metals such as Cu^{2+} and Ni^{2+} have been widely investigated for their bioaccumulation capacity and their impacts on economically important crops, including soybean, maize, and beans.

Copper is recognized as an essential micronutrient, playing a key role in plant growth and development as well as in maintaining morphophysiological processes. Nickel, in turn, although not universally classified as a micronutrient, is considered a common heavy metal in Brazilian soils. Tipu et al. (2020) reported that elevated Ni concentrations reduce plant growth, decrease photosynthetic pigments, and limit P and Na uptake in *Zea mays*. Brown et al. (1987a), however, proposed its essentiality, given that Ni participates in the structure and function of the urease enzyme (Brown et al., 1987b), influences the hydrogenase enzyme complex by increasing the efficiency of biological nitrogen fixation in legumes (Klucas et al., 1983), and contributes to phytoalexin synthesis, thereby enhancing disease resistance. At high levels, however, Ni causes significant physiological disorders (Walker et al., 1985; Paiva et al., 2002).

It is estimated that, globally, between 106,000 and 544,000 tons of Ni and approximately 10 g ha^{-1} of Cu are added to soils annually, mainly through metallurgical activities, fossil fuel combustion, metal-processing industries, leaching, geobiogeochemical processes, and sewage sludge application (Berton et al., 2006; Wiggenhauser et al., 2024; Rasheed et al., 2024). According to Uren (1992) and Kicińska et al. (2022), soil pH is the primary factor regulating the distribution of Cu and Ni between the solid and soluble phases, with their availability being inversely related to this parameter. At low pH, the higher concentration of H^+ competes for adsorption sites, reducing the binding of Cu and Ni to soil particles and increasing their availability and toxicity. Under high pH conditions, adsorption and/or precipitation as hydroxides is enhanced, thereby decreasing metal availability (Tipu et al., 2020; Vasilachi-Mitoseru et al., 2023).

The phytotoxicity of Cu and Ni is mainly associated with their interference in the photosystem, resulting in disturbances in the Calvin cycle and inhibition of ATP and NADPH transport due to impaired dark-phase reactions (Krupa et al., 1993). Although the toxic effects of Cu are widely documented, little is known about Ni toxicity symptoms in plants during the vegetative stage. At moderate to high levels, Ni induces chlorosis similar to Fe^{2+} deficiency symptoms. Normal Ni concentrations in plant dry matter range from 0.1 to 5 mg kg^{-1} but may exceed 50 mg kg^{-1} under toxic conditions, except in accumulator and hyperaccumulator species (Adriano, 1986; Paiva et al., 2003).

Given this context, evaluating the doses of Cu and Ni capable of promoting bioaccumulation and inducing deleterious effects during the vegetative growth period of maize is essential. Therefore, this study aimed to assess different doses of Cu(II) and Ni(II), applied simultaneously in dystroferric Red Latosol, on the cultivation of the hybrid maize Pioneer P3601 PWU.

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 August to October 2025, at coordinates 17°48'16.6" S and 50°56'04.7" W, with an average altitude of 748 m.

2.2 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.

2.3 Experimental design

The experimental design was completely randomized (CRD), with four replications. The treatments consisted of eight doses of $CuSO_4 \cdot 5H_2O$ + $NiSO_4 \cdot 6H_2O$ P. A – ACS (0, 5, 15, 35, 85, 100, 125, and 600 mg L^{-1} of Cu + Ni), applied in the planting furrow at the time of maize sowing, totaling 32 sample plots. The Cu + Ni doses were applied simultaneously, using 33 mL of each solution per plot, according to the concentration in mg L^{-1}.

2.4 Soil amendment, sowing, and maize hybrid description

After soil analysis, correction was carried out, considering the area to have high fertility, with more than 20 years of continuous cultivation (soybeans, maize, and sorghum). Liming was performed to raise base saturation to 70%. A total of 200 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 the interpretations of Ribeiro et al. (1999), base fertilization was considered unnecessary.

2.4.1 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.

$Cmol_c\ dm^{-3}$				mg dm^{-3}				
Ca	Mg	Ca+Mg	Al	K	K	S	P	pH $CaCl_2$
7.22	2.65	9.87	0.05	1.34	525	6	63.5	6.5
Micronutrients mg dm^{-3}						g dm^{-3}	$Cmol_c\ dm^{-3}$	
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 = $Ca(H_2PO_4)_2$ in HOAc (acetic acid); Organic Matter (O.M.) = Colorimetric method; Total P = Sulfuric acid digestion, and B = $BaCl_2$. Source: Authors, 2025.

After preparation, the soil was transferred to experimental units (transparent plastic cups, 1 L), and three maize seeds were sown per unit. After germination, thinning was performed at the V2 growth stage, leaving one plant 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 reaches an average plant height of 2.70 m, an ear height of 1.46 m, a flowering GDU of 1593, and has a semi-hard yellow–orange kernel type. It presents high yield potential and stability, good foliar disease resistance, and nematode tolerance: high reduction of the reproduction factor (RF) for *Pratylenchus brachyurus* and *Meloidogyne incognita*, and moderate RF reduction for *Meloidogyne javanica*. Additionally, it shows high responsiveness to crop management practices.

2.5 Cultural practices

At the V3 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).

2.6 Sampling and variable analysis

Sampling was carried out on maize plants at the V5 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 Copper and Zinc contents in the roots and shoots were also analyzed.

2.7 Analysis of Cu and Zn

According to EMBRAPA (2011), the determination of Cu and Ni was carried out by atomic absorption spectrometry (AAS) after acid digestion (wet method), using standard solutions of Cu and Ni with concentrations ranging from 0.1 to 10.0 mg L^{-1}, prepared from $CuSO_4$ and $NiSO_4$ standard salts. The wavelengths (λ) employed were Copper (Cu) 324.7 nm and Nickel (Ni) 232.0 nm, using flame mode (F-AAS) with acetylene gas. The digestion of root and shoot samples was performed using a nitric–perchloric acid mixture.

2.8 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 were conducted using the SISVAR statistical software (Ferreira, 2019).

3. Results

3.1 Agronomic and bioaccumulative parameters

According to the F-test, the agronomic and bioaccumulative parameters of Cu + Ni — including shoot traits, root length, shoot fresh mass, root fresh mass, shoot dry mass, and root dry mass — showed significant effects and fitted a linear model, as presented in (Table 2) and (Figures 1, 2, and 3).

Table 2. Agronomic and bioaccumulative parameters of Cu + Ni in hybrid maize grown in dystroferric Red Latosol.

	PH (cm)	RL (cm)	SFM (g)	RFM (g)	SDM (g)	RDM (g)
Doses	*	*	*	*	*	*
Equation	Linear	Linear	Linear	Linear	Linear	Linear
CV (%)	15.61	35.54	26.92	58.90	33.17	98.96

Note: *Significant. Plant height (PH), Root length (RL), Shoot fresh mass (SFM), Root fresh mass (RFM), Shoot dry mass (SDM), and Root dry mass (RDM). Source: Authors, 2025.

Figure 1 presents the results for plant height (PH) and root length (RL) in hybrid maize grown in dystroferric Red Latosol under different combined doses of Cu + Ni. High concentrations of Cu and Ni exhibit a bioaccumulative effect in both shoot and root tissues. Doses above 5 mg L^{-1} lead to a reduction in plant height (Figure 1a). A similar pattern was observed for root length, with significant decreases occurring at concentrations higher than 5 mg L^{-1} (Figure 1b). Doses ranging from 350 to 600 mg L^{-1} of Cu + Ni are considered highly detrimental due to their severe toxicity, resulting in plant intolerance and marked physiological impairment.

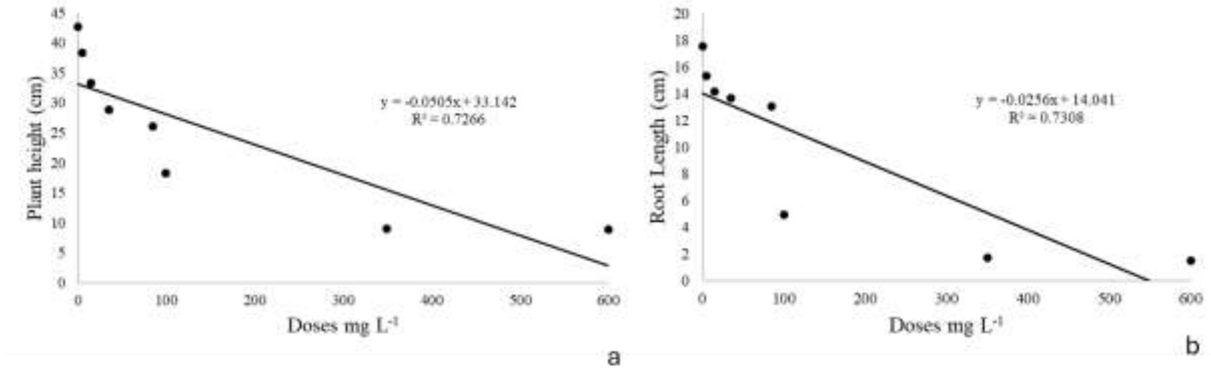

Figure 1. (**a**) Plant height and (**b**) root length under different Cu and Ni doses in hybrid maize grown in dystroferric Red Latosol. Source: Authors, 2025.

Shoot and root fresh mass were negatively affected by the bioaccumulation of Cu + Ni (Figure 2a,b). Concentrations up to 35 mg L^{-1} resulted in only a slight decrease in shoot fresh mass. However, for root fresh mass, concentrations above 5 mg L^{-1} caused significant reductions. At the two highest concentrations, 350 and 600 mg L^{-1}, the plants exhibited marked intolerance and pronounced metal bioaccumulation, indicating severe toxicity.

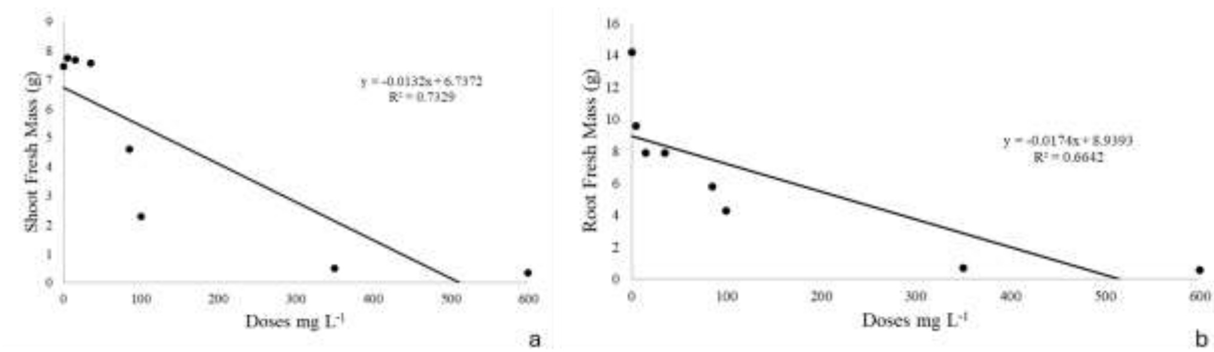

Figure 2. (**a**) Shoot fresh mass and (**b**) root fresh mass under different Cu and Ni concentrations in hybrid maize grown in dystroferric Red Latosol. Source: Authors, 2025.

The shoot litter mass (SDM) showed a noticeable reduction at concentrations above 85 mg L^{-1}. Root litter (RDM), however, exhibited negative effects at concentrations starting from 5 mg L^{-1}, indicating greater sensitivity of this compartment to chemical stress (Figure 3a,b).

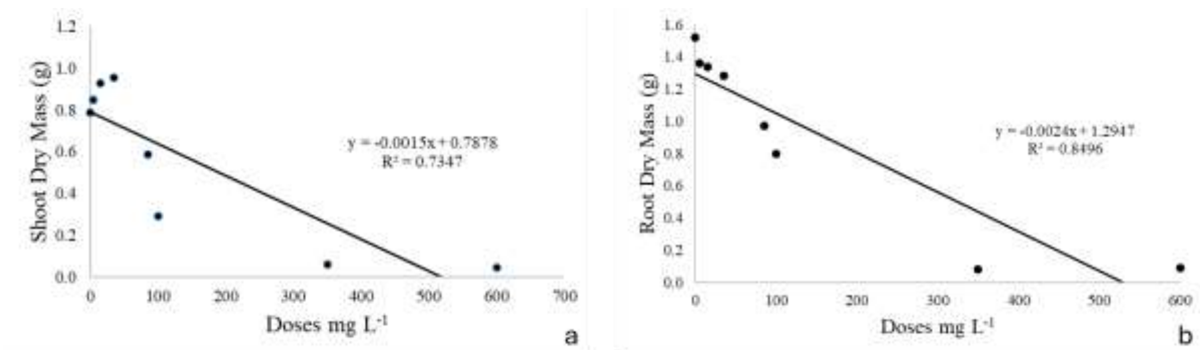

Figure 3. (**a**) Shoot dry mass and (**b**) root dry mass of a maize hybrid grown in dystroferric Red Latosol under different Cu and Ni concentrations. Source: Authors, 2025.

4. Discussion

Copper (Cu) and nickel (Ni) play essential roles in biochemical and structural processes in plants, acting as micronutrients at low concentrations and contributing to the development of economically important crops such as maize. However, our results show that doses above 5 $mg \cdot L^{-1}$ induced toxicity (Figure 1a,b), significantly reducing shoot and root length. These findings are consistent with Kuinchtner et al. (2023), who reported toxicity in *Handroanthus heptaphyllus* even at lower Cu concentrations (4, 6, and 8 $mg \cdot L^{-1}$). Similarly, studies in maize demonstrate strong Ni toxicity at 600 $mg \cdot L^{-1}$ (Silva Filho et al., 2025) and even at considerably lower ranges, such as 20–40 $mg \cdot L^{-1}$ (Amjad et al., 2020).

The literature consistently indicates a dose-dependent relationship between these metals and their physiological effects, ranging from beneficial responses to severe developmental impairment. At trace levels, both Cu and Ni can promote growth. Nazarova (2022) found that seed dressing with Fe+Ni metallic nanopowders increased plant height at the V5–V6 stage by 7.1% and expanded leaf area during flowering by 18.4%. Productivity components also improved, with increases of 12.6% in green mass with cobs, 14.3% in husked cobs, and 10.9% in the weight of 10 cobs. These results reinforce the micronutrient role of Ni when supplied at appropriate levels.

In contrast, excess Cu and Ni disrupt water and nutrient uptake due to their high mobility and competition for binding sites, interfering particularly with nutrients such as N, P, and K. These effects are even more pronounced in the root system. Xu et al. (2024) reported significant morphometric changes in roots under metal-induced stress, supporting our observations.

Boros-Lajszner et al. (2025) further demonstrated that soils individually contaminated with Zn, Cu, or Ni—or with combinations such as ZnCu, ZnNi, CuNi, and ZnCuNi—drastically reduced maize productivity, with Ni being the most detrimental, followed by Cu. Metal combinations intensified yield losses, corroborating our findings. Moreover, Cu alone or in combination exerted strong inhibitory effects on soil enzymatic activity, whereas sunflower-husk biochar successfully mitigated metal toxicity, highlighting its potential as an immobilizing agent.

In our highest Ni doses, the metal exhibited pronounced toxicity to the root system, reducing length and both fresh and dry root biomass (Figures 1–3). Similar outcomes were reported by Cheraghvareh et al. (2024), who found substantial accumulation of Ni, nitrate, and nitrite in maize roots exposed to 75 and 150 µM $NiCl_2$, along with increased nitrate reductase activity and altered amino acid profiles, suggesting the activation of stress-adaptation mechanisms.

The reduction in shoot and root biomass observed in our study aligns with findings by Amanullah & Khan (2023), who showed that Ni and Cu impose strong stress on maize, although their impacts can be alleviated through the application of Trichoderma asperellum combined with biochar. Biomass losses during vegetative development compromise straw production—essential for soil protection—thereby increasing susceptibility to nutrient losses and environmental stressors. According to Kumar et al. (2022), heavy-metal toxicity directly affects root elongation and branching rates, reducing biomass accumulation.

Although toxic at high levels, Ni can also confer beneficial effects related to induced resistance. Oliveira et al. (2022) demonstrated that foliar Ni applications enhanced maize resistance to northern corn leaf blight (NCLB), caused by *Exserohilum turcicum*. This response involved improvements in the photosynthetic apparatus, increased ethylene and reactive oxygen species production, and activation of antioxidant and defense enzymes. Copper (Cu), although widely recognized for its toxic potential at elevated concentrations, has been shown to induce resistance responses in plants when supplied at adequate levels, acting as a signaling element that activates defense pathways. At non-toxic concentrations, Cu participates in the regulation of reactive oxygen species (ROS) production, which function as secondary messengers in triggering antioxidant and structural defense mechanisms. Recent studies have demonstrated that Cu can enhance the activity of antioxidant enzymes—such as superoxide dismutase (SOD), peroxidases (POD), and polyphenol oxidases (PPO)—thereby promoting greater tolerance to biotic stress (Chen et al., 2022; Panda et al., 2024; Bouzayani et al., 2024).

Supporting these findings, Zheng et al. (2023) reported that moderate Cu applications significantly increased SOD and CAT activities, strengthening the antioxidant system and reducing cellular damage under fungal stress. Similarly, Li et al. (2022) and Rabeh et al. (2025) showed that Cu, when supplied at appropriate levels, enhanced lignification and phytoalexin production, increasing resistance against necrotrophic pathogens. In addition, Park et al. (2022) and Akhtar et al. (2026) demonstrated that Cu induces the expression of genes associated with the phenylpropanoid pathway—crucial for the formation of structural barriers and antimicrobial compounds.

Thus, despite their potential phytotoxicity at high concentrations, both Cu and Ni can act as signaling micronutrients, activating physiological and biochemical pathways that contribute to plant resistance. However, it is important to emphasize that the proposal to classify Ni as an essential micronutrient still requires substantial additional research. Overall, our results confirm that Cu and Ni concentrations above 5 mg·L^{-1} promote severe toxicity, impairing root morphology and shoot development in hybrid maize, whereas adequate concentrations contribute to physiological performance and may even induce biotic resistance.

5. Conclusions

Cu and Ni concentrations above 5 mg L^{-1} exhibit high toxicity to the maize hybrid, impairing both shoot and root development. Both elements show substantial translocation and bioaccumulation within plant tissues, leading to adverse physiological effects that reduce plant growth and overall tolerance. Future studies may investigate the effects of Cu and Ni during the reproductive stage of hybrid maize, focusing on parameters such as ear size, grain number, and overall yield. Evaluating these traits in soils contaminated with high concentrations of Cu and Ni will provide a deeper understanding of how these metals influence crop productivity.

6. Authors' Contributions

João Vitor Ramos da Silva Dantas: project writing; sample preparation; planting; experimental maintenance; harvesting; data analyses; and manuscript writing. *Roberto Castro Pereira Filho*: project writing; sample preparation; planting; experimental maintenance; harvesting; data analyses; and manuscript writing. *Matheus Vinícius Abadia Ventura*: data analysis and statistical evaluation. *Antonio Carlos Pereira de Menezes Filho*: supervisor; project oversight; data analysis; manuscript writing; and publication procedures.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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