ISSN 2175-6813

Revista Engenharia na Agricultura

Viçosa, MG, DEA/UFV - DOI: 10.13083/reveng.v32i1.16812 v.32, p.16-26, 2024

NITRIC OXIDE AS AN ATTENUATOR OF COPPER TOXICITY IN THE CONCENTRATION OF NUTRIENTS IN MAIZE SEEDLINGS

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Keywords:
Bioaccumulation
Heavy metal
Micronutrient
Zea mays L.

ABSTRACT

The maize crop is highlighted in the worldwide and Brazilian agribusiness, presenting itself as a raw material for both human and animal nutrition. Nitric oxide (NO) stands out as a signalling molecule playing a crucial role in plant responses to abiotic stresses as caused by heavy metals. Therefore, the objective of this work was to evaluate the effect of nitric oxide on the levels of macro and micronutrients, such as cationic magnesium, calcium, iron, copper, zinc and manganese. The copper bioaccumulation and translocation factor, in the initial growth of maize seedlings were subjected to copper toxicity. The seeds were soaked for 48 hours in Germitest paper using a solution containing sodium nitroprusside $Na_2[Fe(CN)_5NO]2H_2O$ as a donor of nitric oxide, sodium ferrocyanide $Na_4Fe(CN)_6$ as compensator and deionized water (control). The experiment was carried out in a 4 x 3 completely randomized factorial design with 12 treatments and 8 repetitions, totaling 96 trays containing 25 seeds per repetition. The results showed that the doses of nitric oxide were not sufficient to attenuate the copper toxicity, highlighting the metal accumulation in the roots. The doses of sodium nitroprusside and sodium ferrocyanide provided toxicity, changing the mineral balance in the mobilization of macro and cationic micronutrients and their translocation to the aerial part of K9606VIP3 maize seedlings in the initial growth.

Palavras-chave:
Bioacumulação
Metal pesado
Micronutriente
Zea mays L.

ÓXIDO NÍTRICO COMO ATENUADOR DA TOXICIDADE DO COBRE NA CONCENTRAÇÃO DE NUTRIENTES EM MUDAS DE MILHO

RESUMO

A cultura do milho tem destaque no agronegócio mundial e brasileiro, apresentando-se como matéria-prima tanto para a nutrição humana quanto animal. O óxido nítrico (NO) destaca-se como uma molécula sinalizadora que desempenha um papel crucial nas respostas das plantas aos estresses abióticos causados por metais pesados. Portanto, o objetivo deste trabalho foi avaliar o efeito do óxido nítrico nos teores de macro e micronutrientes, como magnésio catiônico, cálcio, ferro, cobre, zinco e manganês. O fator de bioacumulação e translocação do cobre, no crescimento inicial das mudas de milho, foi submetido à toxicidade do cobre. As sementes foram embebidas por 48 horas em papel Germitest utilizando solução contendo nitroprussiato de sódio $Na_2[Fe(CN)_5NO]2H_2O$ como doador de óxido nítrico, ferrocianeto de sódio $Na_4Fe(CN)_6$ como compensador e água deionizada (controle). O experimento foi conduzido em esquema fatorial inteiramente casualizado 4 x 3, com 12 tratamentos e 8 repetições, totalizando 96 bandejas contendo 25 sementes por repetição. Os resultados mostraram que as doses de óxido nítrico não foram suficientes para atenuar a toxicidade do cobre, evidenciando o acúmulo do metal nas raízes. As doses de nitroprussiato de sódio e ferrocianeto de sódio proporcionaram toxicidade, alterando o equilíbrio mineral na mobilização de macro e micronutrientes catiônicos e sua translocação para a parte aérea de mudas de milho K9606VIP3 no crescimento inicial.

Received 7 Oct, 2023 • Accepted 10 Jun, 2024 • Published 11 Jul, 2024

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SECTION EDITOR IN CHARGE

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INTRODUCTION

The maize crop is highlighted in the worldwide and Brazilian agribusiness, presenting itself as a raw material for both human and animal nutrition. For the 2023/24 harvest, the cultivated area is estimated at 3,995.4 thousand hectares, 10.1% lower than that recorded in the last harvest. The expected production is 23,490 thousand tons, 14.2% lower than that obtained in the last cultivation cycle (Conab, 2024).

The exploitation of minerals can cause changes in the natural content of heavy metals in the environment, the practice of mining can result in tailings (Mikula *et al*., 2020). The development of anthropic activities such as industry, mining, agriculture, urban waste disposal has been the main actions that result in increased contamination by heavy metals in soil, water and air. One of the most harmful metals resulted from these activities is cadmium, and even at low concentrations it is very toxic, especially in plant structures (Nogueira *et al*., 2022; Machado *et al*., 2024).

Heavy metals are elements that have an atomic density greater than 5 g. cm^{-3} and are associated with environmental pollution and toxicity to living beings. These heavy metals can also bind to the cell wall of plants due to the presence of functional groups such as carboxyl (-COOH), hydroxyl (-OH) and thiol (–SH), which are present in the wall components, competing for binding sulfhydryl (-SH) groups, , or replacing Mg^{2+}, Zn^{2+} or Fe^{2+} in chloroplast proteins (Ameh & Sayes, 2019).

The establishment of the maize seedling results in intense changes in morphology, cell structure and mobilization of reserves predominant in the endosperm, starch, cell wall and storage proteins. These proteins are mobilized by the action of hydrolytic enzymes and secreted into the starchy endosperm in the maize grain, the macro and micronutrient reserves are necessary to start seedling development (Fritsche Neto & Borém, 2015).

The heavy metal can cause oxidative stress and interfere in the activity of enzymes such as α-amylase and β-amylase, enzymes responsible for the degradation of starch. The inhibition of these enzymes may be indicative of the mechanisms of copper toxicity (Asati *et al*., 2016). Copper (Cu) is important due to its vital and indispensable role in plant growth, due to its ability to lose and gain electrons easily. Cu acts as cofactor in several enzymes, such as cytochrome c oxidase, polyphenol oxidase, Cu Zn^{-1} superoxide dismutase and plastocyanin (Nazir *et al*., 2019).

The presence of Cu in toxic amounts in plant tissues (20 to 100 mg kg^{-1}) can block water absorption by interfering with the germination process, affecting enzymatic activity. Cu catalyzes the production of reactive oxygen species, such as hydrogen peroxide (H_2O_2), which is harmful to cellular components such as DNA, proteins and lipids, reducing development and causing tissue damage (Zhang *et al*., 2019).

Plants grown in soils with a high copper content show a reduction in photosynthetic rate and respiration, a reduction in root length, biomass, and a decline in nutrient absorption (Marques *et al*., 2018). Root length is an important parameter since the radicle is the first seedling organ that comes into contact with the contaminated solution (Feng *et al*. 2016).

Some attenuators, such as Nitric oxide (NO), are used to mitigate the negative effect of heavy metals. The Nitric oxide (NO) is a gaseous molecule with a simple molecular structure, which occurs naturally in plant cells, has hydrophobic properties, being able to diffuse freely through membranes (De Marco *et al*., 2017). It is an endogenous or exogenously supplied molecule through donor compounds. The application of exogenous Nitric oxide in the form of sodium nitroprusside (NPS) improves plant tolerance to stress caused by heavy metals (Xu *et al*., 2017). Research has shown that treatment with low Nitric oxide concentrations can decrease oxidative damage and stimulate the synthesis and activity of antioxidant enzymes when the plant is under various types of stress (Del Rio, 2015).

In the literature, few studies have been carried out to verify the attenuating effect of sodium nitroprusside-NPS and sodium ferrocyanide-FCS on copper toxicity in maize seedlings of the variety K9606VIP3. Therefore, this study aimed to evaluate the effect of nitric oxide on the levels of macronutrients and cationic micronutrients, such as magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn), and the bioaccumulation and translocation factor of copper in the initial growth of maize seedlings subjected to Cu toxicity.

MATERIAL AND METHODS

The experiment was carried out in the seed laboratory of the group of Studies on Biodiversity in Higher Plants (EBPS) located at the Institute of Agricultural Sciences- ICA belonging to UFRA (Federal Rural University of the Amazon) - Belém, with geographic coordinates of 01º 27' 21" S, 48º 30'16" W and an average altitude of 10 m. According to Koppen, the regional climate is classified as Af (equatorial) (Lopes *et al*., 2018).

Hybrid maize seeds of the variety K9606 VIP3 from the company KWS SAAT SE & Co. KgaA were used. This hybrid was chosen for the work because it has greater production stability and greater tolerance to pests and diseases.

The seeds were soaked for 48 hours in Germitest paper using a solution containing sodium nitroprusside $Na_2[Fe(CN)_5NO]2H_2O$ as a donor of nitric oxide, sodium ferrocyanide $Na_4Fe\ (CN)_6$ as compensator and deionized water (control). The seeds were placed in trays with washed sand, autoclaved and dried in a 70ºC oven, containing concentrations of 0 µM, 100 µM and 200 µM $CuSO_4.5H_2O$ and deionized water (control) at 60% of the field capacity, with a photoperiod of eight hours (8 hours of light and 16 hours of darkness) and temperature of 25 ± 2°C (RAS), remaining for seven days. Concentrations of copper and nitroprusside that had not been used in previous research with this early-growing maize hydride were used.

The design consisted of a completely randomized design arranged in a 4 x 3 factorial scheme. The seeds were soaked for 48 hours in the treatments that contained deonized water (control), 0 µM-NPS-sodium nitroprusside + 150 µM-FSC-sodium ferrocyanide, 75 µM-NPS-sodium nitroprusside + 75 µM-FCS-Sodium ferrocyanide, 150 µM-NPS-sodium nitroprusside + 0 µM-FCS-sodium ferrocyanide, seeded in trays with sand containing the three concentrations of CuSO4. 5H2O (0. 100 and 200 µM), making up 12 treatments with 8 repetitions, totaling 96 trays, containing 25 seeds per repetition. The plants were kept for a period of 7 days to measure their growth at 60% of field capacity (data not shown), with an eight-hour photoperiod (8 hours of light and 16 hours of dark) at 25 ± 2°C (Seed Analysis Rules -RAS).

After seven days, the seedlings were removed, and the determination of aerial part dry mass (MSPA) and root dry mass (MSR) were carried out by the forced air oven method at 70ºC until reaching constant weight (Nakagawa, 1999). After weighing, the dry matter was ground and stored in Falcon tubes to later be taken to the Museu Paraense Emílio Goeldi (MPEG) for analysis of the levels of copper and cationic micro and macronutrients in the roots and aerial parts of the seedlings.

The analyses of macro and micronutrient, such as magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) were determined according to methodology described by Miyazawa *et al*. (2009). An amount of 500 mg of the crushed material was weighed, transferred to a digester tube, and 8 mL of HNO_3:$HClO_4$ solution (3:1) was added and left at room temperature overnight in a digester block. Subsequently, the temperature of the digester block was raised to 120ºC until the brown steam was completely released. Thereafter, the temperature was increased to 200ºC until the white steam was completely released, and allowed to cool. After digestion, the tube solution was filtered and the volume was completed to 25 mL with deionized water. The mineral composition of this solution was determined at the Chemical Analysis Laboratory of the Museu Paraense Emílio Goeldi, using a thermal flame absorption spectrometer (Thermo, model ICE3000). The procedure was performed in duplicate and the analytical blank was prepared by the same method without adding the sample.

The bioconcentration factor (FBC) and the translocation factor (FT) were determined using the equations described below by Wang *et al*. (2018).

$$\text{FBC} = \frac{\text{Cup (mg kg}^{-1})}{\text{Cusn (mg L}^{-1})} \tag{1}$$

where: Cup represents the Cu concentration in the seedling and Cusn represents the Cu concentration in the nutrient solution.

$$\text{FT} = \frac{\text{Cupa (mg kg}^{-1})}{\text{Cur (mg kg}^{-1})} \tag{2}$$

where: Cupa represents the concentration of Cu in the aerial part and Cur represents the concentration of Cu in the roots.

The data were subjected to analysis of variance (ANOVA), using the Tukey test with 5% probability in the SISVAR program (Ferreira, 2019).

RESULTS AND DISCUSSION

The study's findings indicate that the main effect of the increase in copper concentrations was the accumulation of this nutrient in the roots, interfering with the mobilization of nutritional reserves, causing a reduction in the growth of the roots and aerial parts of the seedlings. Machado *et al*, (2024), reported a reduction in the size of roots and aerial parts of Mahogany seedlings that were subjected to high doses of heavy metal, corroborating the results found in the present study.

The doses of sodium nitroprusside-NPS and sodium ferrocyanide-FCS did not attenuate the harmful effects of Cu, causing toxicity in the concentration of macro and micronutrients in the roots and aerial parts. This effect may be attributed to increased copper accumulation in roots due to its affinity with carboxylic groups in this organ which is directly in contact with the metal.

The treatment with 200 µM Cu concentration showed the highest copper concentration in roots, with an average of 159.5 mg kg-1 copper in the NPS (150 µM) + FCS (0 µM) treatment, a 7,787.2% increase from the control treatment's 2.0 mg kg-1. Regarding the copper content in the aerial part, the highest average of 15.3 mg kg^{-1} was found in the seedlings that received the dose of 200 µM of Cu in the treatment with NPS (75 µM) + FCS (75 µM), which means an increase of 1562.7% when compared to the control treatment, which presented an average of 0.9 mg kg^{-1} (Tables 1 and 2).

Table 1. Nutrient contents in the roots of maize seedlings (*Zea mays L.*) treated with sodium nitroprusside and sodium ferrocyanide submitted to copper toxicity

Treatment	Cu	Mg	Ca	Mn	Cu	Zn	Fe
	(µM)	-----	-----	-----	(mg kg^{-1})	-----	-----
Water	0	137.4 ±4.3 Cc	36.5 ± 1.0 Cb	7.2 ± 0.2 Aa	2.0 ± 0.2 Cc	16.5 ± 0.5Bb	576.4 ± 26.6 Aa
	100	366.6 ±18.3 Bb	86.7 ± 3.4 Ba	2.8 ± 0.3 Bb	71.6 ± 1.0 Cb	30.6 ± 2.0Aa	131.8 ±5.4 Ab
	200	467.6 ±26.5 Aba	84.9 ± 11.9 Ba	3.1 ± 0.4 Ab	144.0 ± 0.7 ABa	38.0 ± 3.9Aa	113.1 ±2.5 Ac
NPS (0 µM) + FCS (150 µM)	0	190.3 ±10.1 Bc	59.4 ±5.1 Bc	7.5 ± 0.5 Aa	4.1 ± 0.1 Bc	21.8 ± 0.6Ab	493.0 ±25.9 ABa
	100	398.2 ±8.5 Abb	74.8 ± 5.1 Bb	2.8 ± 0.6 Bb	83.0 ± 1.6 ABb	29.2 ± 2.5Aa	109.0 ±5.3 Bb
	200	487.8 ±3.9 Aba	151.7 ± 11.4 Aa	1.5 ± 0.2 Bc	139.4 ± 7.2 Ba	24.9 ± 0.8Cab	115.7 ± 3.1 Ab
NPS (75 µM) + FCS (75 µM)	0	250.1 ±3.3 Ab	134.7 ± 4.9Ab	4.8 ± 0.4 Ba	5.5 ±0.2 Ac	17.6 ± 0.7Bb	437.9 ±24.6 Ba
	100	456.4 ±22.1 Aa	216.7 ± 16.7Aa	4.3 ± 0.2 Aa	85.4 ± 1.9 Ab	29.0 ± 1.2Aa	142.2 ±6.5 Ab
	200	515.3 ±22.4 Aa	165.9 ±3.3Ab	4.1 ± 0.3 Aa	139.6 ± 4.4 ABa	28.8 ± 0.8Bca	120.8 ±1.7 Ac
NPS (150 µM) + FCS (0 µM)	0	146.4 ±6.4 Cc	66.3 ± 0.8 Ba	3.3 ± 0.1 Ca	5.1 ± 0.1 Ac	20.3 ± 0.4Abc	323.8 ±5.5 Ca
	100	347.6 ±27.2 Bb	71.2 ± 1.6 Ba	3.7 ± 0.3 Aba	76.2 ± 3.9 BCb	24.6 ± 0.8Ab	152.6 ±10.0 Ab
	200	442.8 ±10.6 Ba	82.7 ± 3.7 Ba	4.3 ± 0.2 Aa	159.5 ± 4.2 Aa	35.1 ± 4.1Aba	126.6 ±2.4 Ac

Columns with different uppercase letters between treatments (water, NPS (0 µM) + FCS (150 µM), NPS (75 µM) + FCS (75 µM), NPS (150 µM) + FCS (0 µM) under the same copper concentration) and lowercase letters between copper concentrations (0, 100 and 200 µM under the same treatment) indicate significant differences by the Tukey test at the 5% probability level. Values correspond to the means and standard deviations of five repetitions

Table 2. Nutrient contents in the aerial part of maize seedlings (*Zea mays L.*) treated with sodium nitroprusside and sodium ferrocyanide submitted to copper toxicity

Treatment	Cu	Mg	Ca	Cu	Zn	Fe
	(µM)	-------------	-------------	(mg kg^{-1})	-------------	-------------
Water	0	365.8 ± 1.7 Ca	135.3 ± 22.9 Ba	0.9 ± 0.3 Bc	33.4 ± 1.8 Ba	79.1 ± 1.5 Ca
	100	262.6 ± 1.8 Bb	69.3 ± 5.6 Ab	4.1 ± 0.1 Bb	31.4 ± 0.9 Ba	80.6 ± 1.3 Ca
	200	359.9 ± 11.2 Aa	116.9 ± 6.6 Ba	11.4 ± 0.4 ABa	35.9 ± 0.8 Aa	75.1 ± 0.6 Ba
NPS (0 µM) + FCS (150 µM)	0	414.2 ± 10.9 Ba	218.9 ± 11.0 Aa	0.7 ± 0.1 Bc	38.1 ± 1.2 Aba	97.9 ± 1.7 Ba
	100	355.8 ± 4.3 Ab	74.5 ± 9.0 Ab	5.8 ± 0.2 Ab	31.4 ± 2.1 Bb	71.7 ± 1.0 Db
	200	306.6 ± 3.6 Bc	186.5 ± 26.3 Aa	10.5 ± 0.8 Ba	33.6 ± 1.7 Abab	77.9 ± 3.4 Bb
NPS (75 µM) + FCS (75 µM)	0	464.1 ± 6.8 Aa	189.0 ± 4.8 Aa	1.1 ± 0.2 Bc	42.2 ± 1.8 Aa	100.9 ± 0.5 Ba
	100	284.9 ± 6.7 Bb	28.7 ± 9.7 Bc	5.5 ± 0.1 Ab	40.3 ± 3.9 Aa	89.1 ± 1.3 Bb
	200	245.4 ± 13.6 Cc	72.8 ± 6.4 Cb	15.3 ± 1.3 Aa	29.7 ± 1.1 Bb	88.3 ± 2.8 Ab
NPS (150 µM) + FCS (0 µM)	0	377.9 ± 6.0 Ca	170.9 ± 2.4 ABa	2.3 ± 0.3 Ac	43.4 ± 1.5 Aa	114.9 ± 1.9 Aa
	100	265.6 ± 3.0 Bc	107.6 ± 2.9 Ab	5.0 ± 0.2 ABb	33.7 ± 0.3 Abb	108.5 ± 5.1 Aa
	200	300.1 ± 11.2 Bb	34.6 ± 1.4 Dc	10.1 ± 0.7 Ba	30.3 ± 0.6 Abb	92.4 ± 1.4 Ab

Columns with different uppercase letters between treatments (water, NPS (0 µM) + FCS (150 µM), NPS (75 µM) + FCS (75 µM), NPS (150 µM) + FCS (0 µM) under the same copper concentration and lowercase letters between copper concentrations (0, 100 and 200 µM under the same treatment) indicate significant differences by the Tukey test at the 5% probability level. Values correspond to the means and standard deviation of five repetitions.

The treatments with NPS and FCS did not alleviate the toxic effects of Cu, which provided an increase in the calcium concentration in the roots. The highest calcium content was found in the roots that received the dosage of 100 µM Cu, with the highest average of 216.7 mg kg^{-1} in the treatment with NPS (75 µM) + FCS (75 µM), indicating an increase of 493.0% when compared to the control treatment that presented an average of 36.5 mg kg^{-1}. The treatments increased the calcium concentration in the aerial parts. The highest average of 218.9 mg kg^{-1} calcium was found in seedlings that received the doses of 0 µM Cu in the treatment with SPL (0 µM) + FCS (150 µM), indicating an increase of 61.8% when compared to the control treatment that presented average 135.3 mg kg^{-1}.

The treatments with NPS and FCS did not attenuate the copper toxicity, which provided an increase in the magnesium content in the roots. The treatments with NPS (75 µM) and NPS (0 µM) provided an increase in magnesium concentration in the roots and aerial parts. The roots that received a dose of 200 µM Cu showed the highest average of 515.3 mg kg^{-1} in the treatment with SPN (75 µM) + FCS (75 µM), which means an increase of 275.1% when compared to the control treatment that presented an average of 137.4 mg kg^{-1}. Concerning the magnesium concentration in the aerial part, the highest average of 464.1 mg kg^{-1} was found in seedlings that received a dose of 0 µM Cu in the treatment with NPS (75 µM) + FCS (75 µM), indicating 26.9% when compared to the control treatment that presented an average of 365.8 mg kg^{-1}.

Araujo *et al*, (2024) observed that fertigation with synthetic sewage allowed greater productivity of cowpea grains, which must be related to the greater extraction of P, K, Ca and Mg due to the lowest final concentration of these elements in the soil in each layer studied. However, the dosage of sewage applied is very important to prevent crop toxicity.

The treatments with NPS and FCS did not mitigate the effects of Cu, verifying an increase in zinc concentration in the roots. The treatments with NPS (0 µM) and NPS (150 µM) provided an increase in zinc concentration in the roots. In the roots that received a dose of 200 µM of Cu, higher levels of zinc were observed, with a higher average of 38.0 mg kg^{-1} in the treatment with deionized water, indicating an increase of 131% when compared to the control treatment that presented an average of 16.5 mg kg^{-1}. The treatments provided an increase in the zinc content in the aerial parts. The highest average of 42.2 mg kg^{-1} was observed in seedlings that received a dose of 0 µM Cu in the treatment with NPS (75 µM) + FCS (75 µM),showing an increase of 26.5% when compared to the control treatment that presented an average of 33.4 mg kg^{-1}.

The treatments with NPS and FCS did not attenuated the toxicity of Cu, which caused a reduction in the iron concentration in the roots. The highest levels of iron in the roots were found in the control plants and those that received NPS (0 µM). The treatments provided an increase in the iron concentration in the aerial parts. The lowest iron levels were found in the roots and aerial parts of the seedlings that received the dose of 100 µM Cu. The lowest averages of 109.0 mg kg^{-1} and 71.7 mg kg^{-1} were observed in the treatment with NPS (0 µM) + FCS (150 µM), which means a reduction of 81.1% and 9.4% when compared to the control treatments that presented an average of 576.4 mg kg^{-1} and 79.1 mg kg^{-1} in the roots and aerial parts, respectively.

The treatments with NPS and FCS did not attenuate the toxic effects of Cu, which reduced the manganese concentration in the roots. The NPS (75 µM) and NPS (150 µM) treatments provided a reduction in manganese contents, with the lowest manganese concentration being found in the roots that received the dose of 100 µM Cu. The lowest average of 2.8 mg kg^{-1} manganese was observed in the treatments with deionized water and NPS (0 µM) + FCS (150 µM), indicating an reduction of 61.2% when compared to the control treatment that presented an average of 7.2 mg kg^{-1} (Table 1). The manganese concentration in the aerial part did not present a detectable limit in the nutritional analysis.

Gatti *et al*. (2023) concluded in a study with maize that the root dry mass production showed that calcium omission caused a significant reduction in root growth. Plants subjected to calcium deficiency had shortened roots and dark brown coloration, along with initial signs of chlorosis in the young leaves. These characteristics evolved into necrosis, leading to plant death if the deficiency persist. These results corroborate the fact that there is a need for more studies with nutrient concentrations in maize crops.

The highest averages of the bioaccumulation factor-FBC were 13.44 and 12.55, verified in the roots of the seedlings of the treatments NPS (75 µM) + FCS (75 µM) with 100 µM Cu and NPS (150 µM) + FCS (0 µM) with 200 µM of Cu, respectively (Table 3). Regarding the aerial part, there was a lower average of 0.65 in the treatment with deionized water with 100 µM of Cu. A greater translocation factor-FT of 0.4809 was observed in the treatment NPS (150 µM) + FCS (0 µM) with 0 µM Cu (Table 4).

Table 3. Bioaccumulation factor (FBC) of maize seedlings (*Zea mays L.*) treated with sodium nitroprusside sodium ferrocyanide submitted to copper toxicity

Treatment	Cu	FBC	
	(µM)	Aerial part	Root
Water	100	0.65 ± 0.01 Bb	11.27 ± 0.16 Ca
	200	0.90 ± 0.03 Aa	11.33 ± 0.06 ABa
NPS (0 µM) + FCS (150 µM)	100	0.91 ± 0.03 Aa	13.06 ± 0.25 ABa
	200	0.82 ± 0.06 Aa	10.97 ± 0.57 Bb
NPS (75 µM) + FCS (75 µM)	100	0.87 ± 0.01 Aa	13.44 ± 0.30 Aa
	200	1.20 ± 0.10 Aa	10.98 ± 0.35 Bb
NPS (150 µM) + FCS (0 µM)	100	0.79 ± 0.03 Aba	11.99 ± 0.61 BCa
	200	0.84 ± 0.05 Aa	12.55 ± 0.33 Aa

Columns with different uppercase letters between treatments (water, NPS (0 µM) + FCS (150 µM), NPS (75 µM) + FCS (75 µM), NPS (150 µM) + FCS (0 µM) under copper concentration) and lowercase letters between the copper concentrations (100 and 200 µM under the same treatment) indicate differences by the Tukey test at the level of 5% of probability. Values correspond to the means and standard deviations of five repetitions

Table 4. Translocation factor (FT) of maize seedlings (*Zea mays L.*) treated with sodium nitroprusside and sodium ferrocyanide submitted to copper toxicity

Treatment	Cu (μM)		
	0	100	200
	FT		
Water	0.455 ± 0.213 ABa	0.057 ± 0.001 Ab	0.082 ± 0.001 Abb
NPS (0 μM) + FCS (150 μM)	0.180 ± 0.012 ABa	0.073 ± 0.001 Ab	0.076 ± 0.006 Bb
NPS (75 μM) + FCS (75 μM)	0.205 ± 0.047 Ba	0.065 ± 0.002 Ac	0.111 ± 0.012 Ab
NPS (150 μM) + FCS (0 μM)	0.481 ± 0.069 Aa	0.069 ± 0.006 Ab	0.064 ± 0.006 Bb

Different uppercase letters in the column and different lowercase letters in the row indicate significant differences by the Tukey test at the 5% probability level. Values correspond to the means and standard deviations of five repetitions

Phytate is a molecule that has an extreme affinity for chelating positively charged components such as divalent mineral nutrients (Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+} and Cu^{2+}), trace elements, proteins and carbohydrates (Kumar *et al*., 2016). In this study we observed an increase in the levels of Ca^{2+}, Mg^{2+}, Zn^{2+} in the roots of copper treatments. This result may be occurred because during germination there is an increase in phytase activity and reductions in phytic acid levels, increasing the bioavailability of several minerals during germination (Chandra & Kumar, 2017). This phenomenon could justify the increase of some nutrients in the seedling roots.

Cu induces changes in membrane properties and function of carriers and ion channels and may be responsible for imbalances in nutrient concentrations. Cu has a greater affinity for hard binders present in the roots. The cell walls have a strong capacity to accumulate metallic cations, being as efficient barriers in the translocation of most metals (Chandra & Kumar, 2017). This property can provide the accumulation of metallic cations mainly in the roots, causing an imbalance in nutrient mobility from roots to shoots.

In the plasma membrane (MP) are located the type H^+-ATPases P, which couple the hydrolysis of ATP to the transport of H^+ out of the cell (from the cytoplasm to the apoplast), with their physiological role associated with nutrition and cell expansion (Chandra & Kumar, 2017). An increase in Cu concentration can cause an imbalance in the transport of nutrients, causing accumulation and low mobility of some macro and micronutrients from the root to the shoot. Plants have a complex network of transporters involved in intracellular balance to prevent the accumulation of Cu. Other types of Cu transporters are heavy metal P-type ATPases (Kumar *et al*., 2016). Zancheta *et al*. (2011) researching copper phytoextraction per millet (*Pennisetum glaucum* cv. BRS 1501) and sorghum (*Sorghum bicolour* cv. Vassoura) verified that Cu was accumulated preferentially in the root system and, therefore, translocated in a low proportion to the aerial part.

Root length is an important parameter for evaluating the effect of the metal, since the root is the first seedling organ that comes into contact with the contaminated solution. Cu has greater affinity for groups existing in the roots and for this reason it accumulates in greater concentration in this organ, disturbing the root elongation and preventing the redistribution of auxin due to a shortage of nutrients to the embryo due to the low mobilization of cotyledon reserves (Feng *et al*., 2016).

The direct role of Nitric oxide in hydrolytic enzymes has been demonstrated in *Eleusine coracana* plants, observing an increase in amylase activity. It can interfere with the transport and mobilization of nutrients, causing an increase in their concentrations. Nitric oxide helps in the development of lateral roots and adventitious roots. Its regulation and stress responses depends on its concentration, and its effects depend on chemical changes in proteins. Therefore, nitric oxide act through cellular interactions utilizing oxide-reduction wherein biological conditions and, in the presence of O_2, the nitric oxide radical (NO) is susceptible to oxidation and reduction. Thus, it can be transformed into other reactive nitrogen species, such as the reduction of an electron of the NO radical that gives rise to the NO^- nitroxyl anion, whose half-life is short, as it reacts quickly with O_2, giving rise to the peroxynitrite (ONOO) (Kotapati *et al*., 2017).

Nitric oxide acts in interference with calcium ions ($Ca^{2+)}$ to form a complex signalling network in response to abiotic stresses (Gohari *et al*., 2020). The same authors reported that in high concentrations, Cu leads to the activation of Ca^{2+} influx channels, thus activating programmed cell death, inhibiting root elongation. The physiological response of plants to stress is an increase in Ca concentrations in the cytoplasm, which is involved in the regulation of physiological processes of the plant and the adaptation to stress. Ca^{2+} also promotes the accumulation of organic solutes, such as proline (Hasanuzzaman *et al*., 2018), which can interfere with the transport and concentration of nutrients in the roots and aerial parts.

Zhu *et al*. (2017) explained that Mg^{+2} has a strong influence on the filling of grains, has a fundamental role for the growth and development of plants, essential for the synthesis of chlorophylls. According to Bucker-Neto *et al*. (2017), under heavy metal stress conditions, plants show a rapid increase in ethylene production and reduce plant growth and development. There seems to be a positive correlation between Nitric oxide and ethylene, where Nitric oxide stimulates the synthesis of ethylene, increasing the levels of auxin in roots, affecting the transport of Mg^{+2} (Liu *et al*., 2018). This phenomenon could justify the increase in concentration.

According to Iwai *et al*. (2012), after the beginning of the germination of maize seeds, high levels of Zn were found in the root and in the coleoptile (200 mg kg^{-1}), which points to great remobilization of this element at this stage. The authors also verified in Rice seeds that a part of Zn was linked to phytic acid and can also be found organic acids, such as malate or citrate. Buet *et al*., (2014) verified a high Zn accumulation in meristematic tissues of wheat seedlings. Nitric oxide can reduce the accumulation of Zn. However, different results were verified in this study.

A correlation was found between the inhibition of root growth and the concentration of Fe in the roots. The root and leaves growth in a substrate with a toxic Cu level is commonly linked to a decrease in the Fe content in these organs, due to the Fe being replaced by Cu (Asati *et al*., 2016).

The accumulation of Nitric oxide in *Arabidopsis* led to the release of Fe from cell walls. Nitric oxide can play an important role in the internal homeostasis of Fe when the levels of available Fe is increased (Zhu *et al*., 2018). The presence of low molecular weight complexes between Fe and Nitric oxide in plants can influence the availability and internal delivery of Fe (Zhu *et al*., 2018).

Bücker-Neto *et al*. (2017) stated that ions can compete directly for transport, and this competition is influenced by the properties of transport and the difference in the concentration of ions in the solution. Therefore, can infer that the increase in the concentration of Cu led to a reduction in the content of Fe and Mn in seedlings, establishing an antagonistic relationship between these nutrients.

The greater the bioaccumulation factor-FBC, the greater the accumulation of heavy metals in the plant. The translocation factor-FT indicates the ability of a plant to translocate or transfer metals from its roots to its shoot. When the translocation factor value is less than 1, this indicates that the metal has accumulated in plant roots. Furthermore, higher translocation factor values indicate translocation of the metal to the aerial part of the plant (Zvobgo *et al*., 2018).

When plants are stressed by heavy metals, they can actively regulate the concentration of these elements (Fernández *et al*., 2017). The negative effect of Cu toxicity is mainly on root growth and morphology, where the Cu is absorbed and accumulated in the roots. Cu is strongly linked to the cell walls of the roots, and its translocation to the aerial part is prevented (Rehman *et al*., 2019). Thus, greater accumulation of this element is observed in the roots in relation to the aerial part.

CONCLUSION

- There was a greater accumulation of Cu in the maize roots, verifying that the application of nitric oxide in the form of sodium nitroprusside-NPS did not attenuate Cu toxicity on nutrient levels in early-growing maize seedlings.
- Copper had a negative effect on the nutritional balance of macro and micronutrients, since it

was verified a reduction in roots and aerial part growth of maize seedlings.

- The doses of sodium nitroprusside-NPS and sodium ferrocyanide-FCS caused a toxic effect on maize seedlings, changing the mineral balance and the mobilization of nutrient concentration from the roots to the aerial part.

ACKNOWLEDGMENT

We are grateful to Capes - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, for the granting of the scholarship.

UFMS – Universidade Federal de Mato Grosso do Sul.

UFRA – Universidade Federal Rural da Amazônia, to the group of Biodiversity Studies in Higher Plants - EBPS and the Museu Paraense Emílio Goeldi for nutritional analyzes.

AUTHORSHIP CONTRIBUTION STATEMENT

BRITO, A. E. A.: Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing; **SILVA, P. A.:** Data curation, Project administration, Writing – review & editing; **OLIVEIRA, J. T.:** Formal Analysis, Methodology, Software, Supervision, Writing – review & editing; **ROQUE, C. G.:** Funding acquisition, Methodology, Writing – review & editing; **CASTRO, T. R.:** Formal Analysis, Funding acquisition, Writing – review & editing; **OLIVEIRA NETO, C. F.:** Data curation, Supervision, Visualization, Writing – review & editing.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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