Nitric Oxide as Attenuator Changes Ecophysiological in Maize Plants (*Zea mays* L.) Subjected to Copper Toxicity

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Abstract

The objective of this work was to evaluate the attenuating effect of the nitric oxide donor (sodium nitroprusside -NPS) on the ecophysiological responses of corn plants (*Zea mays* L.) submitted to copper toxicity. Corn seeds of the K9606 VIP3 variety were soaked for 48 hours in Germitest paper with a solution containing treatment with sodium nitroprusside $Na_2[Fe(CN)_5NO] 2H_2O$ (0, 200 and 300μM), sodium ferrocyanide $Na_4Fe(CN)_6$ (300, 100 and 0 μM) respectively and deionized water (control), sown in buckets with 15 kg of soil incubated for 50 days containing copper concentrations $CuSO_4.5H_2O$ (0, 60 and 200 mg kg^{-1}). The design consisted of randomized blocks with 12 treatments and 4 repetitions, making a total of 48 plants. The data were submitted to ANOVA, using the Tukey test at 5% using SISVAR. There was no effect of treatments on height, leaf area and number of leaves, observing an increase in stem diameter of plants that received copper concentrations. There was no effect of treatments on the chlorophyll content measured by the SPAD index and gas exchange. The concentrations of 60 mg kg^{-1} and 200mg kg^{-1} of copper did not negatively affect the fluorescence variables of chlorophyll *a*, acting as a micronutrient within favorable limits. The variable chlorophyll fluorescence of the demonstrated the protective effect of nitric oxide present in nitroprusside sodium cyanide and sodium ferrocyanide present in the corn plants of the variety subjected to K9606VIP3 are copper concentrations.

Keywords: phytotoxicity, ecophysiological variables, contamination

1. Introduction

Corn is a fundamental product for Brazilian agriculture, grown in all regions of the country, being the second crop with the highest grain production in the national territory with a large share in exports (USDA, 2019), being the most produced and consumed cereal in the country (Contini et al., 2019), with national average productivity of 5,719 kg ha^{-1} and 5,682 kg ha^{-1} in the off-season (CONAB, 2020).

Abiotic stresses are the main environmental problems that negatively influence plant growth and development (Dresselhaus and Hückelhoven 2018). The phytotoxic effect of heavy metals can be characterized by a decrease in the total phytomass of the plants, in lengths of the aerial part and root and a decrease in the concentration of chlorophyll (Pinto, 2017). The increase in agricultural production raised the need for adding inputs, increasing the levels of heavy metals in soils (Komárek et al., 2010; Ali et al., 2013; Sarwar et al., 2017).

Copper (Cu) is classified as a heavy metal, however it is considered an essential element in plants (Yruela , 2009), which can become toxic in high concentrations (Rodrigues et al., 2016), causing disturbances in the structure of proteins and inhibition of cell stretching (Yruela , 2013). The excess of Cu can cause damage to the photosynthetic apparatus (González- mendoza et al., 2013), and alterations in photosynthesis can represent the physiological state of the plant (Kalaji et al., 2016).

Environmental stresses can interfere with the efficiency of photosynthesis and inactivate PS II-photosystem II (P680) and the electron transport chain for the production of ATP and NADPH2 (Costa et al., 2003). Fluorescence analysis has advantages for studies of electron transport during photosynthesis and it is possible to record electron transport in photosystem II (Franco, 2015).

The application of exogenous nitric oxide-NO can mitigate the decrease in photosynthetic capacity in plants, caused by a variety of abiotic stresses (Wang et al. 2014), is able to diffuse through membranes (Lamattina & García Mata, 2016), it is a molecule that acts as a signaler involved in the regulation of plant growth and development, defense against pathogens and responses to abiotic stress (Sanz et al., 2015).

Thus, the aim of this study was to assess the dampening effect of nitric oxide (NO) in biometrics, chlorophylls, gas exchange and chlorophyll fluorescence of *the* corn plants variety K9606 VIP3 undergoing s copper concentrations.

2. Material and Methods

The experiment was conducted in a greenhouse located at the Institute of Agricultural Sciences-ICA belonging to UFRA (Federal Rural University of the Amazon) - Belém, with geographical coordinates of 01º 27 '21 "S, 48º 30' 16" W and average altitude of 10 m. The chemical characteristics of the collected soil, in the 0-20 cm layer were: pH ($CaCl_2$): 5.1, pH Buffer (SMP): 6.25, Organic Matter: 19 g / dm^3; Calcium: 35 mmolc dm^{-3}; Magnesium: 9 mmolc dm^{-3}; Potassium: 0.8 mmolc dm^{-3}; Sodium: 0.3 mmolc dm^{-3}; Phosphorus: 20 mg dm^{-3}; Total Organic Carbon: 11 g dm^{-3}; Sulfur: 11 mg dm^{-3}; Manganese: 22.5 mg dm^{-3}; Iron: 20 mg dm^{-3}; Copper: 0.4 mg dm^{-3}; Zinc: 5.3 mg dm^{-3}; Boron: 0.54 mg dm^{-3}. Capac. of cation exchange: 78.1 mmol dm^{-3}; Sum of bases: 45.1 mmolc dm^{-3}; Saturation by Al: 2.17% and exchangeable acidity: 1 mmolc dm^{-3}. The physical characteristics of the soil: Clay: 98 g kg^{-1}; Silt: 120 g kg^{-1}; Total Sand: 782 g kg^{-1}; Type texture: Medium.

The soil was removed at UFRA/Campus Belém, sieved and placed in 15 kg buckets of soil containing the concentrations of $CuSO_4 .5H_2O$ (0, 60 and 200 mg kg^{-1}), allowing to incubate for a period of 50 days wrapped in plastic bags and irrigated daily, maintaining a 60% field capacity.

The seed corn variety K9606VIP3 of KWS SAAT company SE & Co. KGaA seeds were soaked for 48 hours in Germitest paper with solution containing the treatments with sodium nitroprusside $Na_2 [Fe (CN)_5 NO] 2H_2O$ (0, 200 and 300 µM) and the sodium ferrocyanide compensator $Na_4 Fe (CN)_6$ (300, 100 and 0 µM) respectively, deionized water (control). The seeds were sown in 15 kg buckets containing soil incubated for 50 days with $CuSO_4 .5H_2O$ at concentrations 0, 60 and 200 mg kg^{-1}. The experiment lasted 93 days until the ears were removed.

2.1 Gas Exchange and Fluorescence of Chlorophyll a

The gas exchange measurements net photosynthetic rate (A), stomatal conductance (gs), sweating (E) and fluorescence of chlorophyll *a* was performed with the portable gas analyzer infrared (IRGA, LI-COR 6400-XT, Lincoln , USA) with fluorescence chamber (6400-40) with an area of 2 cm^2.

They were performed at 55º and 56º DAS in blocks 1 and 2 and 3 and 4, respectively. Being measured under favorable environmental conditions, between 9:00 am and 11:00 am, PARi 1000.41 µmol $m^{2} s^{-1}$ One leaf per plant was always inserted in the equipment chamber in the middle region of the leaf (6th leaf fully expanded), the same used to analyze the chlorophyll content through the SPAD index. The relationship between intracellular carbon and environment (Ci/Ca), internal CO_2 concentration (Ci), water use efficiency (EUA) and leaf temperature (Tleaf) were also quantified.

After reading gas exchanges, aluminum foil was placed on the same sheets to keep them in the dark for at least 30 minutes to carry out the chlorophyll a fluorescence reading.

2.2 Biometric Measurements

At the VT stage (weighing) (Ritchie et al., 1993), the following biometric characteristics were evaluated: height (cm) using a graduated ruler, being measured from the neck to the apex of the flag leaf; stem diameter (mm) at 8-10 centimeters from the stem of the plant with the aid of a caliper; and, for number of leaves, all those present in the plant were counted.

2.3 Chlorophyll Content

To determine the chlorophyll content, the Minolta SPAD-502 chlorophyll meter (Soil Plant Analysis Development) was used. The evaluations were carried out at 53º DAS, when more than 50% of the plants were in the VT-Pendoamento phase, where the last branch of the tassel is visible at the top of the plant. The style stigmas ("hair") of corn did not yet appear in all plants at this stage. 10 readings were made on the 6th fully expanded sheet.

3. Results and Discussions

3.1 Gas Exchange and Chlorophyll Fluorescence a

The measurements of gas exchange and fluorescence of chlorophyll *a* can serve as tools to verify the integrity of the photosynthetic apparatus in the face of environmental adversities.

Regarding gas exchange, there was no interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with copper concentrations or the isolated effect of treatments (Table 1).

The copper excess can cause stress abiotic causing damage to photosystems, resulting in the decline of photosynthesis (Küpper & Andresen , 2016) and may impact on the concentration of chlorophyll (Cambrollé et al., 2015), decrease in activity carboxilative of Rubisco (Siedlecka & Krupa , 2004), competition with other metal ions such as Fe, Ni and Zn and increased lipid peroxidation (Küpper & Andresen , 2016).

Different results were verified in this work, in which the dosages of 60 mg kg^{-1} and 200 mg kg^{-1} of copper were not enough to alter the gas exchange variables in corn plants, possibly Cu acted as a micronutrient within the limits favorable (Souza et al., 2014), with direct participation in electron transport (Dalcorso et al., 2014), between cytochrome b6f and photosystem I (PSI) in the photochemical phase (Yruela, 2013).

Mateos-Naranjo et al. (2008) researching the effect of copper on the growth and photosynthesis of *Spartina densiflora* Brongn, describe that the quantum efficiency of FSII, the rate of liquid photosynthesis, stomatal conductance and pigment concentration decreased with increasing Cu concentration.

Table 1. Summary of analysis of variance of photosynthesis (A), stomatal conductance (gs), internal CO_2 concentration (Ci), transpiration (E), water use efficiency (EUA), leaf temperature (Tleaf), relation internal CO_2 concentration/external CO_2 concentration (Ci / Ca) of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

Causes of variation	A	g_s	Ci	E	EUA	Tleaf	Ci/Ca
	(µmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})			(µmol mmol^{-1})	(° C)	(mol mol^{-1})
	Medium square						
Nitroprusside	4.58 ns	0.56 ns	6095.00 ns	1.81 ns	0.69 ns	0.10 ns	0.20 ns
Copper	5.58 ns	0.14 ns	4141.10 ns	0.04 ns	0.18 ns	0.00 ns	0.13 ns
Nitroprusside x Copper	7.86 ns	0.22 ns	1608.60 ns	0.52 ns	0.34 ns	0.10 ns	0.05 ns
Blocks	41.67 *	4.02 *	15054.00 *	21.56 *	10.4 *	1.30 *	0.51 *
Residue	10.05	0.44	4070.70	0.76	0.99 ns	0.20	0.13
Average	12.80	0.14	208.15	4.00	3.53	36.10	0.53
CV (%)	25.6	6.2	23.8	17. 2	35.8	0.4	25.0

CV: Coefficient of variation;

* Significant at the 5% probability level by the Tukey test

ns not significant at the 5% probability level by the Tukey test

Regarding chlorophyll fluorescence *a* it was found that there was no significant effect on the parameters Fs (steady state fluorescence), Fm '(maximum fluorescence adapted to the light), ΦPSII (effective quantum efficiency), NPQ (non-photochemical dissipation), Fo' (minimum fluorescence of the sheet adapted to the light), Fv'/Fm' (Genty parameter), ETR (electron transport rate) (Tables 2 and 3).

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Table 2. Summary of analysis of variance of F0 (initial fluorescence), Fm (maximum fluorescence), Fv(variable fluorescence), Fv/Fm (PSII potential quantum efficiency), Fs (steady state fluorescence), Fm '(fluorescence maximum adapted to the clear), ΦPSII (effective quantum efficiency), NPQ (non-photochemical dissipation), Fo/Fm'(basal quantum efficiency of PS II) from corn plants (*Zea mays* L.) of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

Causes of variation	Fo	Fm	Fv	Fv / Fm	Fs	Fm ' (µmol e $^{-}$ m $^{-2}$ s ^{1})	ΦPSII	NPQ	Fo/ Fm '
	Medium square								
Nitroprusside	1.36 ^{ns}	3.96 *	0.08 ^{ns}	0.30 ^{ns}	0.12 ^{ns}	0.29 ^{ns}	8.68 ^{ns}	0.50 ^{ns}	0.69 ^{ns}
Copper	3.42 *	2.13 ^{ns}	0.17 *	0.38 *	0.14 ^{ns}	0.04 ^{ns}	4.91 ^{ns}	0.27 ^{ns}	1.66 *
Nitroprusside x Copper	0.56 ^{ns}	0.84 ^{ns}	0.08 ^{ns}	0.06 ^{ns}	0.14 ^{ns}	0.27 ^{ns}	9.00 ^{ns}	1.28 ^{ns}	0.56 ^{ns}
Blocks	1.49 ^{ns}	0.70 ^{ns}	0.03 ^{ns}	0.14 ^{ns}	0.05 ^{ns}	0.26 ^{ns}	3.08 ^{ns}	0.57 ^{ns}	0.59 ^{ns}
Residue	6.23	1.00	0.04	0.10	0.20	0.25	5.12	0.79	0.24
Average	187.48	1154.15	976.11	0.84	371.02	523.55	0.30	1.18	0.35
CV (%)	5.8	0.7	0.6	24.1	2.4	2.5	20.7	6.19	17.7

CV: Coefficient of variation;

* Significant at 5% probability by F

ns not significant at 5% probability by F

Table 3. Summary of analysis of variance of Fo '(minimum leaf fluorescence adapted to light), qP (photochemical dissipation), Fv'/Fm '(Genty parameter), ETR (electron transport rate), qL (coefficient of photoinhibition) of corn plants (*Zea mays* L.) of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

Causes of variation	Fo'	qP	Fv '/ Fm'	ETR (µmol e $^{-}$ m $^{-2}$ s $^{-1}$)	qL
	Medium square				
Nitroprusside	1.07 ^{ns}	1.06 *	0.76 ^{ns}	1.25 ^{ns}	0.49 *
Copper	3.43 ^{ns}	0.68 *	0.47 ^{ns}	0.91 ^{ns}	1.01 *
Nitroprusside x Copper	0.95 ^{ns}	0.22 ^{ns}	0.09 ^{ns}	1.30 ^{ns}	0.28 ^{ns}
Blocks	1.86 ^{ns}	0.31 ^{ns}	0.15 ^{ns}	0.30 ^{ns}	0.32 ^{ns}
Residue	1.20	0.18	0.28	1.00	0.13
Average	155.39	0.44	0.67	60.37	1.11
CV (%)	8.9	23.7	28.1	1.9	18.9

CV: Coefficient of variation;

* Significant at the 5% probability level by F

ns not significant at 5% probability by F

F0 (initial fluorescence) is the minimum constant fluorescence in the dark when all reaction centers are open, it is one of the parameters of chlorophyll fluorescence used to assess plant stress to abiotic factors, for example, toxic metals (Yadav et al., 2018). The increase in F0 (initial fluorescence) indicates impairment in the reactions of PS II- photosystem II (Paunov et al., 2018) or decrease in the capacity of transferring the excitation energy from the antenna to the reaction center (Baker & Rosenqvist, 2004) , which was not observed in the present study, verifying that the treatment with 60 mg kg $^{-1}$ of copper provided an average of 163.42, reducing the F0 by 16.53% (initial fluorescence) when compared to the treatment with 0 mg kg^{-1} of copper which obtained an average of 195.78 (Table 4).

Table 4. Effect of copper concentrations on stem diameter and chlorophyll a fluorescence parameters of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

Copper (mg kg $^{-1}$)	Diameter (mm)	Fo	Fv	Fv/Fm	Fo/Fm'	qP	qL
0	10.27 ± 1.14 b	195.78 ± 11.24 a	972.87 ± 36.95 ab	0.84 ± 0.01 ab	0.33 ± 0.03 b	0.44 ± 0.02 ab	1.14 ± 0.08 a
60	13.62 ± 1.25 a	163.42 ± 13.09 b	1047.68 ± 44.63 a	0.86 ± 0.01 a	0.31 ± 0.03 b	0.41 ± 0.01 b	1.20 ± 0.08 a
200	11.43 ± 1.48 ab	204.96 ± 13.21 a	907.56 ± 30.45 b	0.81 ± 0.01 b	0.41 ± 0.03 a	0.46 ± 0.02 a	1.00 ± 0.04 b

Means followed by the same letter in the column do not differ by Tukey 's test at the 5% probability level. Match values described are means of four replicates and DP.

The fluorescence of chlorophyll *a* is a tool for detecting damage to the photosynthetic apparatus caused by abiotic stresses (Stirbet et al., 2018).

In the present study it was found that there was no effect of the interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations in the parameter Fv (variable fluorescence) verifying the effect of the treatment with 200 mg kg $^{-1}$ copper which had an average of 907.56 with a reduction of 6.71% compared to treatment with 0 mg kg $^{-1}$ of copper which had an average of 972.87. This reduction may be due to the replacement of Mg by Cu in the chlorophyll molecule, causing structural changes in the photosynthetic pigments of the PSII (Zvezdanovic et al., 2007).

The higher the Fv (variable fluorescence) the greater the plant's ability to transfer the energy of the electrons ejected from the pigment molecules to produce ATP, NADPH and reduced ferrodoxin (Fdr) (Baker, 2008).

The maximum potential quantum yield of PSII is given by the Fv/Fm ratio measured in material adapted to the dark, verifying the effect of the treatment of 200 mg kg^{-1} of copper, which presented an average of 0.81 providing a reduction of 2.92% in relation to to treatment with 0 mg of copper, which presented an average of 0.84 (Table 4).

The decline in Fv/Fm values (PSII potential quantum efficiency) is a good parameter to monitor the inactivation of PSII reaction centers (Schansker et al., 2014), values below 0.75 may indicate that the plant is suffering damage caused by some type of stress, indicating a reduction in the proportion of open reaction centers (Maxwell & Johnson, 2000).

Cambrollé *et al.* (2015) observed a reduction in the maximum quantum yield of PSII (Fv/Fm) and in the effective quantum efficiency of PSII (Y (II)) due to the phytointoxication caused by Cu, which causes the degradation of the internal content of the chloroplast and the replacement of Mg by Cu in chlorophyll. Different results were found in this work, verifying that the Fv/Fm parameter, even reduced, was within acceptable limits for normal plants.

There was no effect of the interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations in the parameter F0/Fm'(basal quantum production of the non-photochemical process in PSII) verifying the effect of the treatment of 200 mg kg $^{-1}$ of copper, which presented an average of 0.41 providing an increase of 24.54% when compared to treatment with 0 mg kg- ^{1} of copper, which presented an average of 0.33 (Table 4), with the relationship between F0 (minimum or initial fluorescence of chlorophyll *a* in the dark-adapted state) and Fm '(maximum leaf fluorescence adapted to the light). Several authors cite the increase in this relationship as indicative of stress, suggesting normal values, that is, values recommended as standard, between 0.14 and 0.20 (Rohácek , 2002).

There was no effect of the interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations in the parameter qP (photochemical *quenching*) verifying the effect of the treatment of 200 mg kg $^{-1}$ of copper, which presented an average of 0.46 providing an increase of 3.66% in relation to the treatment with 0 mg kg-1 of copper, which presented an average of 0.44 (Table 4).

The qP parameter (photochemical *quenching*) indicates the proportion of reaction centers open in the PSII (Minagawa , 2008), it is the dissipation caused by the photochemical process, that is, it is caused by the use of energy to reduce NADP, being directly related to the redox state of plastoquinone and decreases in proportion to the closing of the reaction centers (Sousa, 2012).

Cu acts as cofactors in various enzymes such as cytochrome *c* oxidase, Cu/Zn superoxide dismutase and plastocyanine (Nazir et al., 2019 ; Zhang & Li, 2019), which transports electrons in primary photosynthetic reactions (Dalcorso et al., 2014; Yruela , 2013).

There was no effect of the interaction between the measurements of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with copper concentrations in the parameter qL (*quenching* related to photosynthesis photoinhibition), verifying the treatment effect of 200 mg kg $^{-1}$ of copper that presented an average of 1.00 providing a reduction of 12.43% when compared to the treatment with 0 mg kg $^{-1}$ of copper that presented an average of 1.14 (Table 4). This parameter is a protection mechanism that allows the excess thermal energy to be dissipated. This dissipation of energy can prevent the formation of reactive oxygen species (ROSs), which can irreversibly damage proteins, lipids and pigments in photosynthetic membranes (Horton & Ruban, 2004).

With respect to the chlorophyll fluorescence parameters *to* which were significant in relation to dosages of nitroprusside sodium (NPS) and sodium ferrocyanide (FCS) showed that treatment with (300 mM) nitroprusside sodium + (0 mM) ferricyanide of sodium showed an average of 1122.76 providing a reduction of 2.81% in the parameter Fm (maximum fluorescence) when compared to the control treatment (deionized water) which presented an average of 1155.26 (Table 4).

The parameter Fm (maximum fluorescence) indicates the maximum fluorescence intensity that occurs when practically all quinone A (QA) is reduced and the reaction centers reach their maximum capacity (Baker & Rosenqvist, 2004). This decrease may be associated with the inactivation of PSII in the membranes of the thylakoid directly affecting the flow of electrons between the photosystems (Strasser et al., 2004).

NO can function as a positive and negative regulator of responses to stress depending on its local concentration (Mur et al. 2012), its effects depend on chemical changes in proteins, such as S- nitrosylation (Malik et al., 2011; Fungillo et al., 2014).

Misra et al. (2014) suggested that chloroplast proteins like the proteins of the PSII reaction center D1 and D2, are targets of NO, which may prevent electron transfer and consequent inactivation of the PSII reaction center (Yamamoto et al., 2008).

Table 5. Effect of sodium nitroprusside (NPS) and sodium ferrocyanide (FCS) on the chlorophyll a fluorescence parameters of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

Treatment	Fm	qP	qL
Water	1155.26 ± 19.22 a	0.40 ± 0.02 b	1.12 ± 0.05 a
SPL (0 µM) + FCS (300 µM)	1157.97 ± 20.53 a	0.47 ± 0.02 a	1.21 ± 0.06 a
SPL (200 µM) + FCS (100 µM)	1177.84 ± 15.55 a	0.47 ± 0.02 a	0.96 ± 0.03 b
SPL (300 µM) + FCS (0 µM)	1122.76 ± 9.97 b	0.43 ± 0.02 ab	1.15 ± 0.07 a

Means followed by the same letter in the column do not differ by Tukey 's test at the 5% probability level. Match values described are means of four replicates and DP.

Treatment with (300 mM) nitroprusside sodium + (0 mM) sodium ferrocyanide (200 mM) nitroprusside sodium + (100 mM) sodium ferrocyanide and (0µM) nitroprusside sodium + (300 mM) sodium ferrocyanide presented averages of 0.43, 0.47 and 0.47 respectively, in the qP parameter (photochemical *quenching*), providing an increase of 7.55%, 20% and 17.70% when compared to the control treatment (deionized water) which showed an average 0.40 (Table 5).

The slightly protective effect of NO in relation to the maximum activity of photosystem II, can reflect on a good functioning of the electron transport chain, generating ATP and NADPH (Silveira et al., 2016).

The release of cyanide can reduce the photochemical activity of photosystem II-PS II, affecting plant productivity. The nitroprusside sodium (SNP) and sodium ferrocyanide (FSC) release cyanide during their photolysis reducing the photochemical activity of PSII (Wodala et al, (2010). Different results were obtained in this study, where the dosages nitroprusside of sodium and sodium ferrocyanide did not have a negative effect on the qP parameter (photochemical *quenching*).

According to Silva (2015), NO can react quickly with O_2^- producing peroxynitrite (ONOO-) (Popova & Tuan, 2010), being able to act in increasing the synthesis and/or activity of antioxidant enzymes, such as peroxidases from ascorbate (Del Rio, 2015), resulting in the activation of defense mechanisms (Saxena & Shekhawa, 2013).

The treatment with (200 µM) sodium nitroprusside + (100 µM) sodium ferrocyanide showed an average of 0.96 providing a reduction of 14.14% in the parameter qL (*quenching* related to photosynthesis photoinhibition) when compared to the control treatment (deionized water) that obtained an average of 1.12 (Table 5).

NO through S- nitrosylation triggers changes in enzyme dynamics (Farnese et al., 2016), being an important process in the response of plants to abiotic stresses, regulating a wide range of cellular functions and signaling events (Sevilla et al., 2015), being able to control protein activity (Yu et al., 2014).

Cyanide has the capacity to generate an electrical potential by inhibiting electrogenic ionic transport, increasing the pH of the external environment (Cardoso et al., 2013). The energy in NADH is dissipated in the form of heat. The oxidation of cyanide-resistant NADH is a characteristic feature of this electron transport pathway in plants (Lenhinger, 2006). According to Gerivani et al. (2016) due to cyanide being less toxic to plants due to the functioning of the alternative oxidase and its ability to metabolize.

3.2 Biometric Measurements

There was no interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations for the biometric variables: height and number of leaves and these were not affected by the isolated effect of the treatments (Table 6).

Table 6. Summary of the analysis of variance of the SPAD index, height, leaf area, number of leaves, stem diameter of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

Causes of variation	SPAD	Height (m)	Leaf area (cm^2)	Number of sheets	Diameter (mm)
	Medium square				
Sodium nitroprusside	624.92 ns	70.99 ns	131.48 ns	0.41 ns	36.69 ns
Copper	76.76 ns	8.94 ns	109.96 ns	0.15 ns	366.46 *
Nitroprusside x Copper	409.21 ns	28.03 ns	88.01 ns	0.80 ns	99.74 ns
Blocks	789.58 ns	536.84 *	77.11 ns	1.56 ns	39.88 ns
Residue	816.92	114.17	95.81	0.69	62.97
Average	24.44	118.70	1856.44	14.52	116.89
CV (%)	15.8	24.8	10.1	2.8	17.9

CV: Coefficient of variation;

* Significant at the 5% probability level by the Tukey test

ns not significant at the 5% probability level by the Tukey test

In the present study, no visible symptoms of Cu toxicity were observed, such as chlorosis caused by damage to thylakoid membranes (Adrees et al. 2015). According to Yuan et al. (2013) observed that in higher concentrations of Cu there was inhibition of cell division and elongation in *Arabidopsis thaliana* , reduced growth due to biochemical and metabolic changes (Wang et al., 2017) as an increase in the concentration of ROSs (Ravet & Pilon , 2013) .

The effect of copper measurements on the stem diameter variable was observed, showing averages of 13.62 cm and 11.43 cm, increasing 32.66% and 11.29% in treatments with 60 mg kg^{-1} and 200 mg kg^{-1} copper, respectively, compared to treatment with 0 mg kg^{-1} of copper, which averaged 10.27 cm (Table 4).

Copper is an essential element in vegetables (Yruela, 2009), participating as a cofactor in several enzymes (Zhang & Li, 2019), acting as a micronutrient within favorable limits (Souza et al., 2014). Most of the inorganic Cu in the soil occurs as oxides and sulfides which are insoluble at low bioavailability (Mihaljevic et al., 2019). According to Kumpiene et al. (2008), organic matter competes for metals with the oxide exchange sites, presenting a mitigating effect of copper toxicity.

Berton et al. (1997) found that corn plants with a content of 335 mg kg^{-1} of Cu in the leaves, did not manifest symptoms of toxicity. According to Benimeli et al. (2009), corn plants can tolerate and accumulate high concentrations of Cu without showing visible morphological changes, as verified in the present work. The accumulation of copper in the root system may represent a strategy of tolerance of the plant to the excess of heavy metals in the soil (Cambrollé et al., 2013).

According to Silva (2019) working with sorghum, he found that for the variables stem diameter, plant height and number of tillers, doses above 160 (mg of copper kg^{-1} of soil) begin to affect negatively.

3.3 Chlorophyll Content

There was no interaction between the measurements of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with copper concentrations nor the isolated effect of treatments on the determination of chlorophyll content measured by the SPAD index (Table 6). The Minolta SPAD-502 chlorophyll meter has been investigated as an instrument for rapid diagnosis of the nutritional status of different cultures, adding advantages such as simplicity of use, in addition to enabling a non-destructive assessment of leaf tissue (Argenta et al., 2001).

According to Tiecher et al. (2017) a reduction in the content of chlorophyll *a*, chlorophyll *b* and carotenoids is generally observed in plants grown in soils with high levels of Cu, this may be due to the fact that excess Cu^{2+} can replace Mg^{2+} in the structure of the chlorophyll, in addition to the antagonism between the elements Fe and Cu, causing a reduction in chlorophyll, PSII becomes more susceptible to photoinhibition (Yruela, 2009). Different results were found in this study in which no significant effects of the applied doses were observed in relation to the chlorophyll content measured by the SPAD index.

4. Conclusion

The dosages of nitric oxide and copper concentrations did not influence the gas exchange variables, the chlorophyll content measured by the SPAD index and the biometric variables (height, leaf area, number of leaves), observing an increase in the stem diameter of the plants that received copper concentrations. The concentrations of 60 mg kg^{-1} and 200 mg kg^{-1} of copper did not negatively affect the fluorescence variables of chlorophyll a, acting as a micronutrient within favorable limits. The chlorophyll a fluorescence variables demonstrated the protective effect of nitric oxide present in sodium nitroprusside and cyanide present in sodium ferrocyanide in corn plants of the K9606VIP3 variety.

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References

Adrees, M., Ali, S., Muhammad, R., Muhammada, I., Farhat, A., Mujahid, F., … Saima, A. B. (2015). The effect of excess copper on growth and physiology of important food crops: a review. *Environmental Science and Pollution Research, 22,* 8148-8162. https://doi.org/10.1007/s11356-015-4496-5

Argenta, G., Silva, P. R. F., Bartolini, C. G., Forsthofer, E. L., & Strieder, M. L. (2001). Relationship of the chlorophyll meter reading with the levels of extractable chlorophyll and nitrogen in the corn leaf. *Brazilian Journal of Plant Physiology, 13,* 158-167. https://doi.org/10.1590 / S0103-31312001000200005

Arunakumara, K. K. I. U., Walpola, B. C., & Yoon, M. H. (2013). Agricultural methods for toxicity alleviation in metal contaminated soils: a review. *Korean Journal of Soil Science* & *Fertilizer's*, *46*, 73-80. https://doi.org/10.7745/KJSSF.2013.46.2.073

Baker, B. (2008). Chlorophyll Fluorescence: a probe of photosynthesis in vivo. *Annual Review of Plant Biology, 59*, 89-113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Baker, N. R., & Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany, United Kingdom, 55,* 1607-1621. https://doi.org/10.1093/jxb/erh196.

Cambrollé, J., García, J. L., Figueroa, M. E., & Cantos, M. (2015). Evaluating wild grapevine tolerance to copper toxicity. *Chemosphere, 120,* 171-178. https://doi.org/10.1016/j.chemosphere.2014.06.044

Cambrollé, J., Mancilla-Leytón, L. M., Munõez-Vallés, S., Figueroa-Luque, E., Luque, T., & Figueroa, M. E. (2013). Effects of copper sulfate on growth and physiological responses of *Limoniastrum monopetalum, Environmental Science and Pollution Research, 20*, 8839-8847. https://doi.org/10.1007 / s11356-013-1833-4

Cardoso, C. A. (2013). *Demonstration of bioelectric activity in Marandu grass (Brachiaria brizantha cv. Marandu)* (Master's Dissertation). Faculty of Zootechnics and Food Engineering - University of São Paulo.

Contini, E., Mota, M. M., Marra, R., Borghi, E., Miranda, R. A., Silva, A. F., & Mendes, S. M. (2019). Corn: characterization and technological challenges. Brasília: Embrapa. (Challenges of Brazilian Agribusiness, 2).

Dalcorso, G., Manara, A., Piasentin, S., & Furini, A. (2014). Nutrient metal elements in plants. *Metallomics, 6, 1770 - 1788.* https://doi.org/10.1039/C4MT00173G

Del Rio, L. A. (2015). ROS and RNS in plant physiology: an overview. *Journal of Experimental Botany*, *66*, 2827-2837. https://doi.org/10.1093/jxb/erv099.

Dresselhaus, T., & Hückelhoven, R. (2018). Biotic and abiotic stress responses in crop plants. *Agronomy, 8,* 267. https://doi.org/10.3390/agronomy8110267.

Farnese , F. S., Menezes-Silva, P. E., Gusman, G. S., & Oliveira, J. A. (2016). When the bad guys become the good ones: the key role of reactive oxygen species and nitric oxide in the plant responses of abiotic stress. *Plant Science, 7,* 471. 10.3389 / fpls. 2016.00471

Franco, M. H. R., Lemos, V. T., Aguiar, L. M., France, A. C., Ferreira, E. A., & Silva, D. V. (2015). Physiological characteristics of common bean cultivated in soils after phytoremediation of picloram. *Brazilian Journal of Herbicides, 14,* 315-325. https://doi.org/10.7824/rbh.v14i4.454

Fungillo, L., Skelly, M. J., Loake, G. J., Spoel, S. H., & Salgado, I. (2014). Snitrosothiols regulate nitric oxide production and storage in plants through the nitrogen assimilation pathway. *Nat. Commun, 5,* 5401. https://doi.org/10.1038 / ncomms6401

Gerivani, Z., Vashaee, E., Sadeghipour, H. R., Aghdasi, M., Shobbar, Z. S., & Azimmohseni, M. (2016). Short versus long term effects of cyanide on sugar metabolism and transport in dormantwalnutkernels. *Plant Science, 252,* 193-204. https://doi.org/10.1016/j.plantsci.2016.07.016

Gonzalez-Mendoza, D., Gil FE Escoboza -Garcia, F., Santamaria, J. M., & Zapata- Perez, O. (2013). Copper stress on photosynthesis of black mangle (*Avicennia germinans*). *Proceedings of the Brazilian Academy of Sciences, 85,* 665-670. https://doi.org/10.1590/S0001-37652013000200013

Horton, P., & Ruban, A. (2004). Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. *Journal of Experimental Botany, 56,* 365-373. https://doi.org/10.1093/jxb/eri023

Kalaji, H. M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., & Samborska, I. A. (2016). Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum, 38, 102.* https://doi.org/10.1007/s11738-016-2113-y

Kumpiene, J., Lagerkvist, A., & Maurice, C. (2008). Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments-A review. *Waste Management, 28,* 215-225. https://doi.org/10.1016/j.wasman.2006.12.012

Küpper, H., & Andresen, E. (2016). Mechanisms of metal toxicity in plants. *Metallomics, 8,* 269-285. https://doi.org/10.1039/C5MT00244C

Lamattina, L. E., & García Mata, C. (2016). *Gasotransmitters in plants: the emergence of a new paradigm in cell signaling.* Geneva: Springer International Publishing.

Lehninger, A. L. (2006). *Principles of biochemistry* (4th ed). São Paulo: Sarvier.

Malik, S. I., Hussain, A., Yun, B. W., Spoel, S. H., & Loake, G. J. (2011). GSNOR mediated de-nitrosylation in the plant defense response. *Plant Science, 181,* 540-544. https://doi.org/10.1016/j.plantsci.2011.04.004.

Mateos-Naranjo, E. , Redondo-Gómez, S., Cambrollé , J., & Figueroa, M. H. (2008). Growth and photosynthetic responses to copper stress of an invasive cordgrass, *Spartina densiflora*. *Marine Environmental Research, 66,* 459-465. https://doi.org/10.1016/j.marenvres.2008.07.007

Maxwell, K., & Johnson, G. N. (2000). Chorophyll fluorescence- a practical guide. *Journal of Experimental Botany, 51,* 659-668. https://doi.org/10.1093/jexbot/51.345.659

Mihaljevič, M., Baieta, R., Ettler, V., Vaněk, A., Kříbek, B., Penížek, V., … Mapani BS (2019). Tracing the metal dynamics in semi-arid soils near mine tailings using stable Cu and Pb isotopes. *Chemical Geology.*, *515,* 61-76. https://doi.org/10.1016/j.chemgeo.2019.03.026

Minagawa, J. (2008). Fluorescence quenching analysis. In: Stern, D.; Witman, G. (Ed). *The Chlamydomonas Sourcebook*, 2nd ed., V. 3.

Misra, A. N., Vladkova, R., Singh Misra, M., Dobrikova, A. G., & Apostolova, E. L. (2014). Action and target sites of nitric oxide in chloroplasts. *Nitric Oxide, 39,* 35-45. https://doi.org/10.1016/j.niox.2014.04.003

Mur, L. A. J., Mandon, J., Persijn, S., Cristescu, S. M., Moshkov, I. E., Novikova, G. V., & Gupta, K. J. (2012). Nitric oxide in plants: an assessment of the current state of knowledge. *AoB Plants 5*, 1-17. https://doi.org/10.1093/aobpla/pls052

Murchie, E. H., Ali, A., & Herman, T. (2015). Photoprotection as a Trait for Rice Yield Improvement: Status and Prospects. *Rice, 8,* 31-39. https://doi.org/10.1186/s12284-015-0065-2

Oracz, K., El-Maarouf-Bouteau, H., Kranner, I., Bogatek, R., Corbineau, F., & Bailly, C. (2009). The mechanisms involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signaling during germination. *Plant Physiology*, *150,* 494-505. https://doi.org/10.1104/pp.109.138107

Paunov, M., Koleva, L., Vassilev, A., Vangronsveld, J., & Goltsev, V. (2018). Effects of different metals on photosynthesis: cadmium and zinc affect chlorophyll fluorescence in durum wheat. *International Journal of Molecular Sciences, Basel, 19,* 787. https://doi.org/10.3390/ijms19030787

Pinto, F. R. (2017). *Characterization of accumulation and tolerance to Cd- induced stress in spinach plants* (*Spinacea oleracea* L.). (Doctoral thesis in Environmental Engineering). University of Lisbon, Portugal.

Popova, L., & Tuan, T. (2010). Nitric oxide in plant: properties, biosynthesis and physiological functions. *Iranian journal of science & tecnology , transaction A, 34,* 245-255.

Ritchie, S. W., Hanway, J. J., & Benson. G. O. (1993). *How a corn plant develops.* Ames: Iowa State University of Science and Technology, Cooperative Extension Service. 21p.

Rodrigues, A. C. D., Santos, A. M., Santos, F. S., Pereira, A. C. C., & Sobrinho, N. M. B. A. (2016). Mechanisms of Response of Plants to Pollution by Heavy Metals: Possibility of Using Macrophytes for Remediation of Contaminated Aquatic Environments. *Revista Virtual de Quimica, 8,* 262-276.

Rohácek, K. (2002). Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica, 40,* 3-29. https://doi.org/10.1023/A.1020125719386

Roháček, K., Soukupová, J., & Barták, M. (2008). Chlorophyll fluorescence: a wonderful tool to study plant physiology and stress. *Res Signpost*, *37*(661), 41-104.

S ouza, V. L., Almeida, A. A. F., Jones, J. S., Mangabeira, P. A., Jesus, R. M., Pirovani, C. P., ... Louguercio, L. L. (2014). Altered physiology, cell structure, and gene expression of *Theobroma cacao* seedlings subjected to Cu toxicity. *Environmental Science and Pollution Research, 21,* 1217-1230. https://doi.org/10.1007/s11356-013-1983-4

Sanz, L., Albertos , P., Mateos , I. , Sánchez-Vicente, I., Lechón , T., Fernández-Marcos, M., & Lorenzo, O. (2015). Nitric oxide (NO) and phytohormones crosstalk during early plant development. *Journal of Experimental Botany, 66,* 2857-2868.

Saxena, I., & Shekhawat, G. S. (2013). Nitric oxide (NO) inalleviation of heavy metal induced phytotoxicity and its roleinproteinnitration. *Nitric Oxide, 32,* 13-20. https://doi.org/10.1016/j.niox.2013.03.004

Schansker, G., Tóth, S. Z., Holzwarth, A. R., & Garab, G. (2014). Chlorophyll *a* fluorescence: beyond the limits of the *Q* A -model. *Photosynth Res., 120,* 43-58. https://doi.org/10.1007/s11120-013-9806-5

Sevilla, F., Camejo, D., Ortiz- Espín, A., Calderón, A., Lázaro, J. J., & Jiménez, A. (2015). The thioredoxin / peroxiredoxin / sulfiredox in system: current overview of its redox function in plants and regulation by reactive oxygen and nitrogen species. *J. Exp. Robot.*, 66, 2945-2955.

Siedlecka, A., & Krupa, Z. (2004). Rubisco activity maintenance in environmental stress conditions-how many strategies. *Cellular and Molecular Biology Letters, 9,* 56-57.

Silva, A. L. (2015). *Effect of sodium nitroprusside (SNP) on the germination of Senna macranthera seeds (DC. Ex Collad) HS Irwin & Baneby under salt stress*. Federal University of Viçosa.

Silva, Juliano Cesar da (2019). Development and phytoextraction capacity of agricultural plants grown in soil with different textures and copper contents / Juliano Cesar da Silva. 86 p.

Silveira, N. M., Frungillo, L., Marcos, F. C. C., Pelegrino, M. T., Miranda, M. T., Seabra, A. B., Salgado, I., Machado, E. C., & Ribeiro, R. V. (2016). Exogenous nitric oxide improves sugarcane growth and photosynthesis under water deficit. *Plant, 244,* 181-190. https://doi.org/10.1007/s00425-016-2501-y

Sousa, C. P. (2012). *Herbicide action on the photosynthetic activity of plants with C3 and C4 metabolism* (PhD in Plant Physiology). Federal University of Pelotas.

Stirbet, A., Lazár D., Kromdijk, J., & Govindjee G. (2018). Chlorophyll a fluorescence induction: can just a one-second measurement be used to quantify abiotic stress responses? *Photosynthetica, 56,* 86-104. https://doi.org/10.1007/s11099-018-0770-3

Strasser, R. J., Michael, M. T., & Srivastava, A. (2004). Analysis of the Chlorophyll A Fluorescence Transient. In: Govindje. GP (Ed.). *Advances in photosynthesis and respiration* *chlorophyll fluorescence a signature of photosynthesis.* Kluwer Academic Publishers, pp. 321-362.

Tiecher, T. L., Tiecher, T., Ceretta, C. A., Ferreira, P. A. A., Nicoloso, F. T., Soriani, H. H., … Brunetto, G. (2017). Tolerance and translocation of heavy metals in young grapevine (*Vitis vinifera*) grown in sandy acidic soil with interaction of high doses of copper and zinc. *Scientia Horticulturae, 222,* 203-212. https://doi.org/10.1016/j.scienta.2017.05.026

USDA (2019). United States Department of Agriculture. *Grain*: world markets and trade. Available at: < https : // www.fas.usda.gov/data/grain-world-markets-and-trade >. Accessed on: 29 oct. 2019.

Wang, L. H., Wang, W., Zhou, Q., & Huang, X. H. (2014). Combined effects of lanthanum (III) chloride and acid rain on photosynthetic parameters in rice. *Chemosphere, 112,* 355-361. https://doi.org/10.1016/j.chemosphere.2014.04.069

Wodala, B., Ordog, A., & Horváth, F. (2010). The cost and risk of using sodium nitroprusside as a NO donor in chlorophyll fluorescence experiments *Journal of Plant Physiology, 167,* 1109-1111. https://doi.org/10.1016/j.jplph.2010.03.013

Yadav, S; Shankar, A; Rai, S; Rai, R; Singh, S; & Rai, LC (2018) Chlorophyll fluorescence and photosynthesis under abiotic stress. Environment and Photosynthesis a Future Prospect, India, 287-304.

Yruela, I. (2009). Copper in plants: Acquisition. transport and interactions. *Functional Plant Biology, 36,* 409-430. https://doi.org/10.1071/FP08288

Yruela, I. (2013). Transition metals in plant photosynthesis. *Metallomics*, *5,* 1090-1109. https://doi.org/10.1039/C3MT00086A.

Yu, J., Sun, R., Zhao, Z., & Wang, Y. (2014). Auricularia polytricha polysaccharides induce cell cycle arrest andapoptosis in human lung cancer A549 cells. *International Journal of Biological Macromolecules*, *68,* 67-71. https://doi.org/10.1016/j.ijbiomac.2014.04.018

Zvezdanovic, J., Markovic, D., & Nikolic, G. (2007). Different possibilities for the formation of complexes of copper and zinc with chlorophyll inside photosynthetic organelles: chloroplasts and thylakoids. *Journal of the Serbian Chemical Society*, *72,* 1053-1062. https://doi.org/10.2298/JSC0711053Z