

## Nutritional status and physiological parameters of maize cultivated with sewage sludge

Estado nutricional e parâmetros fisiológicos de milho cultivado com lodo de esgoto

Rodrigo Santos Moreira<sup>1</sup> , Guilherme Henrique Expedito Lense<sup>1</sup> , Leonardo Ferreira Fávero<sup>1</sup> ,  
Benedito Majela de Oliveira Junior<sup>1</sup> , Ronaldo Luiz Mincato<sup>1\*</sup> 

<sup>1</sup>Universidade Federal de Alfenas/UNIFAL, Alfenas, MG, Brasil

\*Corresponding author: [ronaldo.mincato@unifal-mg.edu.br](mailto:ronaldo.mincato@unifal-mg.edu.br)

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### ABSTRACT

The use of sewage sludge as a source of nutrients and organic matter for agricultural soils is a well-established practice. However, few reports highlight the effect of the nutrients and potentially toxic elements provided by organic wastes application on the plant physiological parameters, such as photosynthetic activity and stomatal conductivity. We performed a greenhouse experiment with maize exposed to a dystrophic red Latosol amended with mineral fertilizer and different rates of sewage sludge with the following objectives: i) assess the nutrients and metal uptake translocation and distribution in plants and ii) evaluate the relationship between plant physiological parameters and yield indicators under the study conditions. The application of sewage sludge increased the soil organic matter, pH, and the amounts of available Ca, S, and Mg, comparing to the mineral fertilizer treatment. The plants promote a higher translocation of macronutrients to the shoots in the sewage sludge treatments, which results in higher photosynthetic activity, stomatal conductivity, and maize yield parameters. Moreover, the trace elements, which can cause toxicity in small concentrations, were founded mainly in the roots, which indicates a plant defense mechanism.

**Index terms:** Biosolids; *Zea mays*; agricultural production; heavy metals.

### RESUMO

O uso de lodo de esgoto como fonte de nutrientes e matéria orgânica para solos agrícolas é uma prática bem estabelecida. Entretanto, poucos estudos destacam o efeito dos nutrientes e elementos potencialmente tóxicos fornecidos pela aplicação de resíduos orgânicos nos parâmetros fisiológicos da planta, como atividade fotossintética e condutividade estomática. Neste estudo, foi realizado um experimento em casa de vegetação com milho cultivado em um Latossolo Vermelho distrófico com adubação mineral e aplicação de diferentes concentrações de lodo de esgoto, com os seguintes objetivos: i) avaliar a translocação e distribuição de nutrientes e captação de metais em plantas de milho e ii) avaliar a relação entre os parâmetros fisiológicos e indicadores de produção das plantas nas condições do estudo. A aplicação do lodo de esgoto aumentou a matéria orgânica do solo, o pH e as quantidades de Ca, S e Mg disponíveis, em comparação ao tratamento com fertilizantes minerais. As plantas promovem maior translocação de macronutrientes para a parte aérea nos tratamentos com aplicação de lodo de esgoto, o que resulta em maior atividade fotossintética, condutividade estomática e parâmetros de produção de milho. Além disso, os elementos traços, que podem causar toxicidade em pequenas concentrações, foram encontrados principalmente nas raízes, o que indica um mecanismo de defesa das plantas.

**Termos para indexação:** Biossólidos; *Zea mays*; produção agrícola; metais pesados.

## INTRODUCTION

The use of municipal sewage sludge (SS) as a source of nutrients and organic matter for agricultural soils is a well-established practice in many parts of the world, such as England, Australia, the United States of America, and the European Union (Sharma et al., 2017). However, it is estimated that only 10% of the SS produced in Brazil is used for agricultural purposes, while the remainder is disposed of landfills (Borba et al., 2018).

Chemical fertilizers provide nutrients readily available to the plants. However, the availability of nutrients from SS depends on the residue decomposition rate and the interaction with the intrinsic characteristics of the soil-plant system (Antonkiewicz et al., 2019).

The SS characteristics change according to the origin of the solids and the type of processing used by the Wastewater Treatment Plant (Smith, 2009; Kim et al., 2017). Although several studies report positive results from

SS application in several crops (Carbonell et al., 2011; Baioui et al., 2017; Abreu-Junior et al., 2019; Kępka et al., 2016), the heterogeneity of the residue raises doubts regarding their capacity to provide well-balanced nutrients to the plants.

Another factor that limits the use of SS in agriculture is the risk of soil and food contamination by heavy metals (Abreu-Junior et al., 2019; Moreira; Mincato; Santos, 2013). Some metals are essential nutrients required in small contents by the plants. However, in high concentrations, these elements can negatively affect plant physiological parameters, such as photosynthetic activity and stomatal conductivity, thereby reducing crop yields (Bączek-Kwinta, et al., 2019).

In general, SS research is still focused on its effects on plant growth and soil contamination by heavy metals. However, the physiological parameters of plants fertilized with this residue have not been completely clarified yet, and few reports highlight the effects of SS on the distribution of nutrients and potentially toxic elements in different parts of plants (Carbonell, et al., 2011). Thus, the study of the nutritional status and physiological parameters of maize cultivated with SS can help to choose SS rates according to the plant nutrients needs and avoid plant toxicity. In this work, a greenhouse experiment was conducted with maize exposed to a dystrophic red Latosol amended with mineral fertilizer and different rates of SS with the following objectives: i) assess the nutrients and metal uptake translocation and distribution in maize plants and ii) evaluate the relationship between plant physiological parameters and yield indicators under the study conditions.

## MATERIAL AND METHODS

A greenhouse experiment with maize (*Zea mays* L.) was carried out in plastic pots of 25 dm<sup>3</sup> capacity. We collected the soil in an area that has not been fertilized for the last 15 years, located at 21°25' south latitude, and 45°57' long west of Greenwich. The soil was classified as a dystrophic red Latosol, according to the Brazilian Classification System (Embrapa, 2013) and Ferralsol, according to the World Reference Base for Soil Resources

(WRB, 2015). The chemical characterization of soil used in the experiment was performed according to Raij (2001), and are present in Table 1.

The municipal SS was collected in a Wastewater Treatment Plant, located at Municipality of Paraguaçu, Minas Gerais State, Brazil. We collect six samples of the residue used in the experiment for chemical characterization. The pH was determined in a CaCl<sub>2</sub> extract (1:5). The total soil organic carbon (TOC) and total nitrogen (TN) was determined by dry combustion, using an elemental analyzer CN. The other elements were quantified by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Model Optima 7300 DV, Perkin Elmer). The results of SS chemical characterization are present in Table 2.

We validated the precision and accuracy of heavy metals analyses by simultaneous analyses of certified samples of sewage sludge (BCR-144R and BCR-145R) and soil treated with sewage sludge (CRM-143R) obtained in the Institute for Reference Materials and Measurements from the European Commission. We analyzed the samples under the same methodological procedures described and calculated the recovery factor to evaluate the analysis veracity (Brasil, 2011) (Table 3).

The experiment was performed in a completely randomized design with six treatments and four replicates. The treatments consisted of the following sewage sludge rates: 10 (SS1), 20 (SS2), 40 (SS3), 80 (SS4), and 160 (SS5) Mg ha<sup>-1</sup>. The 10 Mg ha<sup>-1</sup> SS rates were defined based on CONAMA Resolution No. 375 (Conama, 2006) considered the nitrogen content of the residue. As a control, a mineral fertilizer treatment (MF) was added, according to Table 4.

Each pot was filled with 25 kg of soil previously mixed with the equivalent amount of SS or mineral fertilizer to reach the selected mentioned application rates. Then, the pots were incubated for 30 days. After the incubation time, four maize seeds were planted manually in the pots, which was previously watered for conditioning purposes. During the experiment, we kept the soil moisture close to 60% of the water-holding capacity. The harvest was performed 131 days after sowing when plants reached physiological maturity.

**Table 1:** Chemical characterization of soil used in the experiment.

pH	V	Al	H+Al	SB	CTC	K	Ca	Mg	P	Mn	Zn	Fe	B	Total C	OM
H <sub>2</sub> O	%	-----				nmol <sub>c</sub> dm <sup>-3</sup>	-----			-----			mg dm <sup>-3</sup>	-----	
5.7	45	0	23	67.9	90.9	6.9	41	20	35	9.1	2.1	36	0.24	18	31

V: base saturation; SB: sum of bases; OM: organic matter.

**Table 2:** Chemical characterization of sewage sludge used in the experiment.

Parameter	Concentration*	CONAMA**
pH	7.20	--
Total carbon (%)	23.6	--
P (g kg <sup>-1</sup> )	6.55	--
K (g kg <sup>-1</sup> )	0.41	--
S (g kg <sup>-1</sup> )	11.02	--
Mg (g kg <sup>-1</sup> )	4.32	--
Ca (g kg <sup>-1</sup> )	40.51	--
B (mg kg <sup>-1</sup> )	119.89	--
Zn (mg kg <sup>-1</sup> )	861.15	2800.00
Mn (mg kg <sup>-1</sup> )	243.45	--
Ni (mg kg <sup>-1</sup> )	16.54	420.00
Pb (mg kg <sup>-1</sup> )	46.16	300.00
Mo (mg kg <sup>-1</sup> )	1.50	50.00
Cd (mg kg <sup>-1</sup> )	0.50	39.00
Cr (mg kg <sup>-1</sup> )	83.46	1000.00
Cu (mg kg <sup>-1</sup> )	134.67	1500.00

\* Average of 6 samples taken from the drying beds of the Wastewater Treatment Plant. \*\*Maximum concentration of heavy metals allowed according to the National Environment Council (CONAMA, 2006).

The leaf photosynthesis rate (A) and stomatal conductance (gs) were measured using a portable photosynthesis system (IRGA, LI-6400XT, Li-Color, Lincoln, NE, USA) and a chamber with an artificial light source (LI-6400-02B RedBlue) at 100 days of cultivation. After harvest, the plants were subdivided into leaves, stem, root, and grain for chemical analysis. We determine the quantities of P, K, Ca, Mg, S, Cr, Cu, B, Ni, and Zn,

according to Malavolta; Vitti and Oliveira (1997). The biomass, harvest index, grain weight, *cob weight*, *cob diameter*, and *cob length* were analyzed to assess plant growth. Finally, soil samples of each pot were collected to analyze the chemical parameters according to Rajj et al. (2001).

The data obtained were submitted to analysis of variance (ANOVA) followed by the Tukey test, considering a 5% significance level. Principal Component Analysis (PCA) was used to verify the interrelationship of maize yield indicators. Statistical analyses were performed using R software (R Development Core Team, 2011).

## RESULTS AND DISCUSSION

### Uptake and translocation of nutrients and potential toxic elements in plants

The soil chemical parameters analyzed are present in Table 5. The SS application increased the amounts of Ca, S, Mg, as well as cation exchange capacity and soil base saturation (Table 4). The pH values in the SS treatments increased compared to the mineral fertilizer application and stayed within the suitable limit, according to Malavolta, Vitti and Oliveira (1997). The residue used in our study was treated with CaO, which contributed to the pH increase, as well as base saturation and Ca contents. However, the amounts of P, B, and Cu were higher in the mineral fertilizer treatment.

The treatments with higher SS application rates presented higher averages compared to the mineral fertilizer treatment. In general, these results indicate that the addition of sewage sludge can be beneficial to soil and provides valuable plant nutrients, which is consistent with several other studies (Abreu-Junior et al., 2017; Melo et al., 2018; Carbonell et al., 2011).

**Table 3:** Certified samples of (BCR-144R and BCR-145R) sewage sludge and soil treated with sewage sludge (CRM-143R).

Metals	BCR 144-R			BCR 145-R			CRM 143-R		
	Certified	Found	frec %	Certified	Found	frec %	Certified	Found	frec %
mg dm <sup>-3</sup>									
Cd	1.84 ± 0.07	1.8	97.8	3.50 ± 0.15	3.5	100	72 ± 1.8	70.1	97.4
Cr	90 ± 6	94	104.4	307 ± 13	294	95.8	426 ± 12	418	98.1
Pb	96 ± 1.6	98	102.1	282 ± 9	285	101.1	174 ± 5	180	103.4
Mn	189 ± 6	193	102.1	156 ± 4	153	98.1	858 ± 11	859	100.1
Ni	44.9 ± 1.5	43	95.8	251 ± 6	255	101.6	1063 ± 16	1051	98.9

f<sub>rec</sub> = Recovery fator.

**Table 4:** Rates and source of nutrients applied in the mineral fertilizer treatment.

Nutrients	Applied rate (mg kg <sup>-1</sup> )	Source
N	300	NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>
P	300	KH <sub>2</sub> PO <sub>4</sub>
K	200	KH <sub>2</sub> PO <sub>4</sub>
S	40	K <sub>2</sub> SO <sub>4</sub>
Mg	46	MgSO <sub>4</sub> ·7H <sub>2</sub> O
B	2.5	H <sub>3</sub> BO <sub>3</sub>
Cu	7.5	CuSO <sub>4</sub> ·5H <sub>2</sub> O
Mo	0.5	(NH <sub>4</sub> ) <sub>6</sub> MO <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O
Zn	2.5	ZnSO <sub>4</sub> ·7H <sub>2</sub> O

Source: Novais, Neves and Barros et al. (1991).

Treatments with SS and mineral fertilizer exhibited low levels of P in leaves, which was below the suitable limits (2.5 - 3.5 g kg<sup>-1</sup>), according to the standard established by Malavolta, Vitti and Oliveira (1997). Although the high levels of P in the sewage sludge composition, its available to the plants depends on the mineralization kinetics of

the organic matter, which releases the nutrient gradually (Øgaard; Brod, 2016). For this reason, the soil P content in the MF treatment was significantly higher than the SS treatments (Table 4). Despite this, the higher SS rates (SS4 and SS5) showed higher levels of P in the leaves, which can be explained by the increase of the soil organic matter found in these treatments (Figure 1). According to Souza et al. (2006), management practices that increase soil organic matter content improve the mineral P availability since the carboxylic and phenolic functional groups present in the organic matter are responsible for blocking the positively charged sites of Fe and Al oxides, reducing the P adsorption.

There was a low K translocation for the grains, which concentrated mainly in maize stalk and leaves (Figure 1). This result was expected once the K is associated with maize stalk structure and strength and is responsible for activating enzymes that act in the processes of photosynthesis and respiration (Hasanuzzaman et al., 2018). The SS presents low levels of K due to its high water solubility, which makes that much of the nutrient be leaching in the drying beds of the Wastewater Treatment Plant (Paglia et al., 2007). However, there was no significant difference to K content in leaves between SS4 and MF treatment.

**Table 5:** Effect of mineral fertilizer and different sewage sludge rates on the soil chemical parameters.

Soil parameters	Treatments						
	MF	SS1	SS2	SS3	SS4	SS5	CV%
pH	5.0c	5.8b	5.9b	5.9b	5.8b	6.3a	4.2
SOM (g dm <sup>-3</sup> )	30.0b	30.7b	34.1a	31.6ab	32.8a	33.6a	15.3
P (mg dm <sup>-3</sup> )	75.1a	12.9e	18.5ed	21.2dc	26.2c	34.5b	19.3
K (mmol <sub>c</sub> dm <sup>-3</sup> )	6.0a	4.8ab	5.2a	5.0a	4.2ab	2.9b	34.5
Ca (mmol <sub>c</sub> dm <sup>-3</sup> )	38.7f	43.9e	50.0d	56.0c	67.9b	95.4a	7.0
S (mg dm <sup>-3</sup> )	2.0a	22.1d	25.4d	57.7c	129.5b	290.4a	17.8
Mg (mmol <sub>c</sub> dm <sup>-3</sup> )	15.1d	16.5d	18.4c	18.0c	21.3b	23.6a	6.4
Zn (mg dm <sup>-3</sup> )	3.9bc	2.4d	3.6c	4.1bc	4.7b	6.8a	18.9
Ni (mg kg <sup>-1</sup> )	0.21a	0.15c	0.18b	0.17b	0.20a	0.15c	7.3
Pb (mg kg <sup>-1</sup> )	0.80a	0.76a	0.74a	0.78a	0.69a	0.53b	14.8
B (mg kg <sup>-1</sup> )	0.54a	0.37cd	0.32de	0.43bc	0.49ab	0.26e	18.9
Cr	0.02a	0.02a	0.02a	0.02a	0.02a	0.005b	31.6
Cu	2.30a	1.18c	1.44b	1.38b	1.40b	1.20c	6.8
CEC (mmol <sub>c</sub> dm <sup>-3</sup> )	93.1d	86.0e	96.5cd	102.0c	116.6b	137.5a	5.0
Base saturation	64.4d	75.8c	76.9bc	78.4bc	80.3b	88.4a	3.8

\* Means followed by the same letters do not differ statistically according to the t-test at P ≤ 0.05. CV% = coefficient of variation. SOM = soil organic matter; CEC = cation exchange capacity; MF = Mineral fertilizer; SS1 = 10 Mg ha<sup>-1</sup>; SS2 = 20 Mg ha<sup>-1</sup>; SS3 = 40 Mg ha<sup>-1</sup>; SS4 = 80 Mg ha<sup>-1</sup>; SS5 = 160 Mg ha<sup>-1</sup>.

In general, treatments with higher SS rates showed higher levels of Ca in plant tissue, which was concentrated mainly in leaves and roots, presenting low translocation to the grains (Figure 1). Similar to Ca, the increase of SS rates incremented the levels of Mg and S tissues (Figure 1), which indicates the potential of residue to provide these nutrients to the plants.

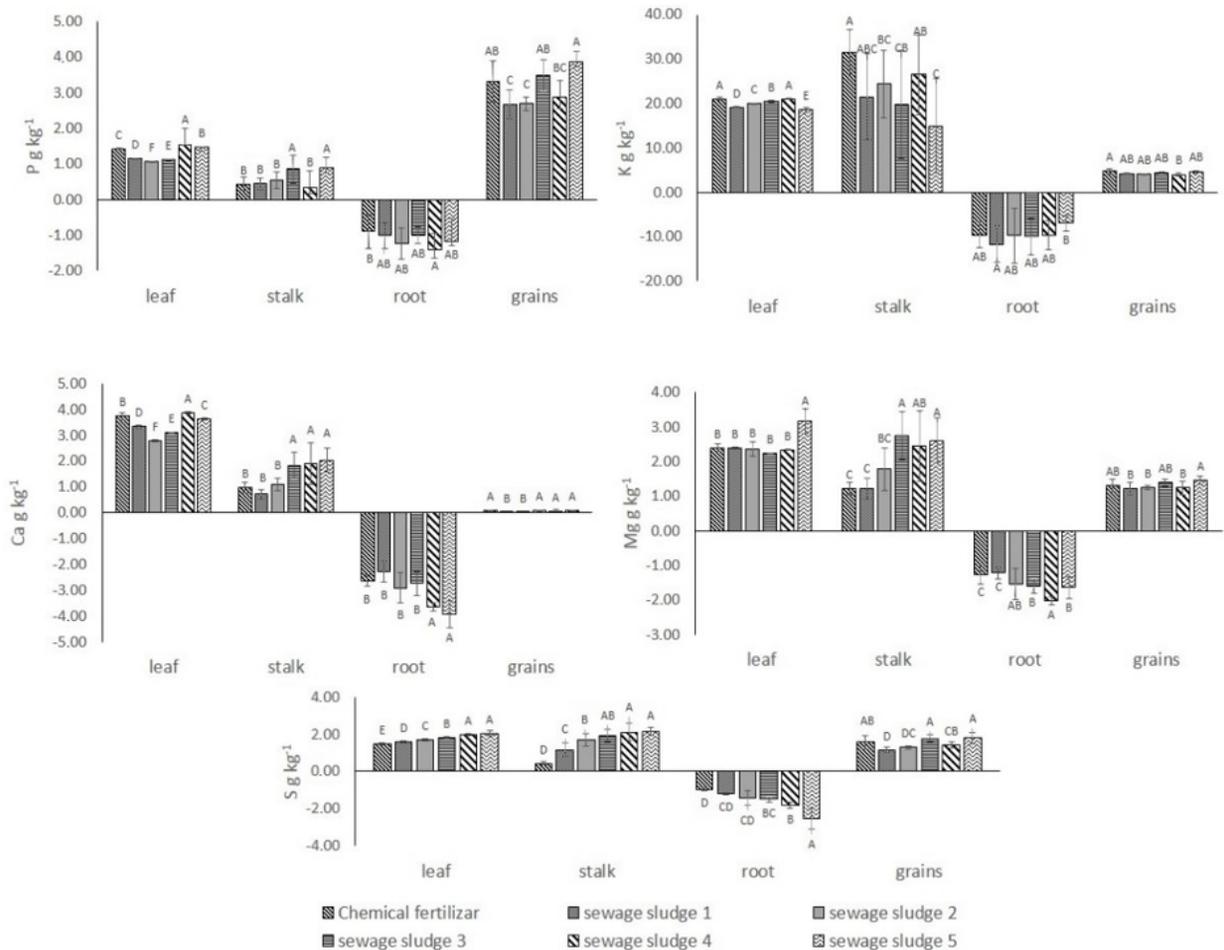
Regarding trace elements, higher concentrations of Cr, B, Cu, Ni, and Mn were found mainly in the roots (Figure 2), which can be a defense mechanism of the plants, as high concentrations of these elements can cause toxicity and a decline in the photosynthesis rates (Marco et al., 2016). The Cd contents in the plant tissues were below the *ICP-OES* limit detection.

These results are in agreement with Carbonell et al. (2011) and Bay et al. (2017), who observed that the

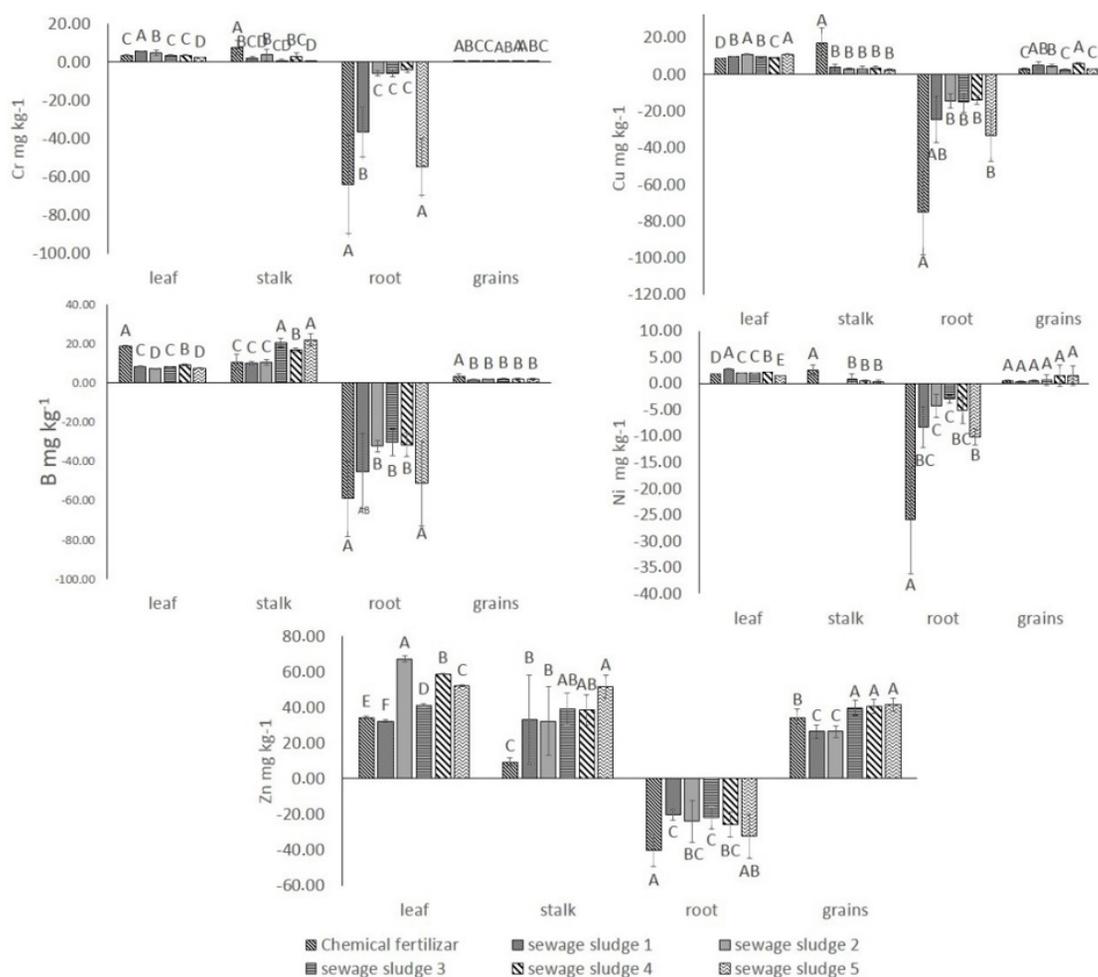
root system acts as a barrier to the uptake of heavy metals generating low concentrations in the shoots.

Although the increase of the SS rates promoted an increment of Zn in the soil (Table 5), the SS2 treatment presented higher Zn content in the leaves (Figure 2). This result can be explained by the increase in soil organic matter content, which can complex this element, reducing its availability to plants (Moreira; Mincato; Santos, 2013).

We found 67.2 mg kg<sup>-1</sup> Zn in the SS2 treatment, which is higher than the suitable range of 15.0 – 50.0 mg kg<sup>-1</sup> (Malavolta; Vitti; Oliveira, 1997), but smaller than 500 mg kg<sup>-1</sup>, considered the limit to the element reaches the toxic level (Kabata-Pendias; Pendias, 2001). From the point of view of grain quality for human nutrition, Zn contents in maize grains are of great interest. Zn deficiency in humans



**Figure 1:** Effect of mineral fertilizer and sewage sludge rates on the distribution of macronutrients in different parts of maize plants.



**Figure 2:** Effect of mineral fertilizer and sewage sludge rates on the distribution of trace elements in different parts of maize plants.

is a malnutrition problem worldwide (Roohani et al., 2013) and, it is more widespread in areas of high cereal and low animal food consumption (Mackowiak et al., 2011). Thus, SS application to soil seems to be a practical approach to improving grain Zn concentrations in staple foods, like maize.

### Physiological parameters and plant growth

Sewage sludge application promoted a significant increase of maize photosynthetic activity and stomatal conductivity compared to mineral fertilizer (Table 6). According to Baioui et al. (2017), there is a strong relationship between photosynthetic activity and adequate nutrient supply provided by organic wastes.

Several studies showed that the photosynthetic physiology activities of the plant were inhibited markedly by heavy metals stress. However, this not seemed to be our

case because the heavy metals supplied by the application of SS did not negatively affect the photosynthetic activity of plants. These results probably happened due to the low translocation of these elements to the shoots, since most metals accumulated in the roots.

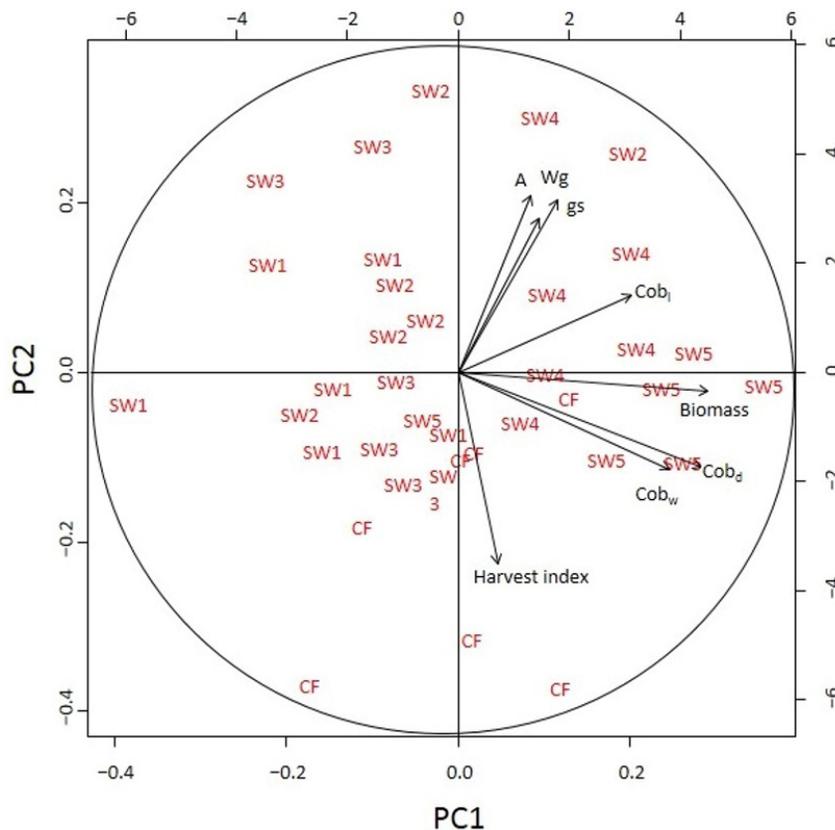
The improvement of physiological parameters of plants promoted a higher biomass production, once the photosynthesis is the way of plants accumulating organic matter and produce biomass. The SS5 treatment provided an increase of 21% in the biomass production compared to the MF treatment. These results are in agreement with Bai et al. (2017) that found a higher biomass production of maize cultivated with SS. The treatments with higher SS rates also provided an increase in cob length, weight, and diameter. However, the harvest index presented a slight variation among treatments.

The results of the principal component analysis for the maize yield indicators analyzed in this study are present in Figure 3. The interaction between the two main components demonstrated that there is a close relationship

**Table 6:** Yield indicators of maize cultivated with different sewage sludge rates and mineral fertilizers.

Treat.	Wg	A	gs	Biomass	Cob <sub>w</sub>	Cob <sub>l</sub>	Cob <sub>d</sub>	hi
	g kg <sup>-1</sup>	μmol m <sup>-2</sup> s <sup>-1</sup>	mol m <sup>2</sup> s <sup>-1</sup>	-----g-----	-----cm-----	---		
MF	21.0b	42.8b	0.2b	162.7cb	115.8b	140.7ab	43.0ab	38.1a
SS1	20.1c	69.5a	0.4ab	136.0d	85.9c	122.0c	40.6b	34.7ab
SS2	20.3c	74.6a	0.6a	158.5bc	95.5c	133.0abc	41.0b	33.4b
SS3	20.9b	76.8a	0.6a	142.7cd	93.1c	129.2bc	41.3b	35.4ab
SS4	22.8a	83.1a	0.6a	179.6ab	119.1ab	145.2a	42.8ab	36.2ab
SS5	23.1a	75.2a	0.5a	197.0a	134.0a	144.5a	46.0a	36.4ab
CV%	1.5	16.6	26.8	11.2	12.1	7.6	7.0	8.5

Means followed by the same later doo not differ according to t-test at p<0.05. Treat. = treatment; MF = mineral fertilizer; SS1 = 10 Mg ha<sup>-1</sup>; SS2 = 20 Mg ha<sup>-1</sup>; SS3 = 40 Mg ha<sup>-1</sup>; SS4 = 80 Mg ha<sup>-1</sup>; SS5 = 160 Mg ha<sup>-1</sup>. Wg = weight of 1000 grains; A = photosynthetic activity; gs = stomatal conductivity; Cob<sub>w</sub> = cob weight; Cob<sub>l</sub> = cob length; Cob<sub>d</sub> = cob diameter; hi = harvest index; CV% = coefficient of variation.



**Figure 3:** Principal components analyses of maize yield indicators cultivated with chemical fertilizer and different sewage sludge rates. MF = mineral fertilizer; SS1 = 10 Mg ha<sup>-1</sup>; SS2 = 20 Mg ha<sup>-1</sup>; SS3 = 40 Mg ha<sup>-1</sup>; SS4 = 80 Mg ha<sup>-1</sup>; SS5 = 160 Mg ha<sup>-1</sup>. Wg = weight of 1000 grains; A = photosynthetic activity; gs = stomatal conductivity; Cob<sub>w</sub> = cob weight; Cob<sub>l</sub> = cob length; Cob<sub>d</sub> = cob diameter.

between the yield maize components. For analyzing the first principal component in the *axis 1*, there is a clear separation between treatments. On the right are the treatments with higher SS rates (SS4 and SS5), which showed a good correlation with yield indicators. On the left are the treatments with the lowest SS rates that, in general, presented the lowest yield indicators values. The mineral fertilizer treatment was distributed between the two groups, demonstrating an intermediary position considering the SS rates.

The positive effect of the SS application on the photosynthetic activity and stomatal conductivity with consequent increases in biomass and other maize yield indicators is due to the higher soil nutrient availability and the additional benefits generated by the soil-plant system, such as higher water retention and improved soil biological activity, which are benefits promoted by the SS application widely documented in the literature (Glab, et al. 2020; Vieira; Pazianotto, 2016).

## CONCLUSIONS

The application of sewage sludge improved soil properties compared to mineral fertilizer, promoting an increase in organic matter, nutrients, and pH, with better results found in the highest applied rates. The plants promote a higher translocation of macronutrients to the shoots in the sewage sludge treatments, which results in higher photosynthetic activity, stomatal conductivity, and maize yield parameters. Moreover, the trace elements, which can cause toxicity in small concentrations, were founded mainly in the roots, which indicates a plant defense mechanism.

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