

# Impact of treated sewage effluent on soil fertility, salinization, and heavy metal content

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**ABSTRACT:** A long-term experiment (2013 to 2017) of treated sewage effluent (TSE) levels via fertigation in a humid tropical region was installed in Jaboticabal, state of São Paulo, Southeast of Brazil, to evaluate the impact of TSE on soil fertility and the potential for salinization and heavy metal contamination of an Oxisol. The area was cultivated with *Urochloa brizantha* under the application of four TSE levels at the irrigation depth (0, 11, 60, and 100% TSE) during the four years of the experiment. The TSE chemical composition was monitored throughout the experimental period. The heavy metal levels and soil fertility were evaluated in the 0-0.10 and 0.10-0.20 m layers at the end of the four years of the experiment. Fertigation using TSE did not increase the concentration of potentially toxic elements (As, Cd, Cu, Hg, Mo, Ni, Pb, Se, and Zn) in the soil due to their low concentration in the effluent. A higher Na concentration was also observed at high TSE levels (60 and 100%). However, the sodium adsorption rate in the TSE was low, generating no potential for soil salinization and sodification. Fertigation with TSE levels increased soil fertility, increasing by more than 10% the P content and base saturation in the soil compared to control. According to the chemical attributes evaluated in the soil and effluent, this long-term study showed that TSE application via fertigation in humid tropical regions on clayey soils cultivated with forage grasses is recommended.

**Key words:** water reuse, environmental contamination, soil chemical analysis, potentially toxic elements, wastewater.

## INTRODUCTION

The increasing demand for food and water and the environmental pollution as a result of constant population growth are important global challenges facing humanity nowadays. Water consumption has increased in recent years at twice the rate of population growth and will continue to grow at a similar rate in the near future (Lavrnić et al. 2017). The agricultural sector is the most affected by water scarcity, as it accounts for 70% of global freshwater withdrawals for irrigation and over 90% of consumption (Norton-Brandão et al. 2013). On the other hand, the concentration of the human population in urban areas has significantly increased the production of sewage wastewater, which causes detrimental environmental impacts if appropriate actions are not taken.

In this context, wastewater can be an important component of an integrated water management policy (Van Lier and Huibers 2010; Andrews et al. 2016). Treated domestic wastewater is a widely available sustainable hydric source that can be used to meet the growing demand for agricultural irrigation (Santos et al. 2017). Thus, in recent years, wastewater recycling has gained importance as part of agricultural water sources in many countries facing water scarcity (Coelho et al. 2020).

The application of treated sewage effluent (TSE) in agricultural areas may be a viable alternative for nutrient recycling, reducing the water footprint in agriculture and increasing soil fertility. Therefore, it is necessary to examine the quality of the effluent to be used for this purpose. The application of TSE in agriculture provides essential nutrients to crops, especially

nitrogen, phosphorus, and potassium, making it possible to increase soil fertility and crop yield while concurrently reducing production costs (Fonseca et al. 2007). Another advantage of reusing TSE in agriculture is the reduction in the inappropriate disposal of effluent in water courses, diminishing environmental pollution (Coelho et al. 2020).

Long-term application of TSE with harmful contaminants in the effluent can cause chemical, physical, and biological degradation of the soil (Qadir et al. 2010). Wastewater reuse should be applied in a controlled and monitored way to avoid the risk of toxic and pathogenic contamination of the soil, plant, and groundwater (Qadir et al. 2010). TSE may contain pollutants such as salts, potentially toxic elements or heavy metals, organic compounds, bacteria, and viruses. The increase of potentially toxic elements such as cadmium, copper, iron, manganese, lead, and zinc in fertigated soils causes problems for agricultural production, such as plant toxicity, and contamination of soil and groundwater (Singh et al. 2004). The regional climate is paramount in the groundwater contamination potential, as it is a factor that alters soil depth and, consequently, groundwater depth. Thus, the contamination potential of groundwater due to TSE application in arid and semi-arid climates may be higher than the potential in humid climates, given the higher soil and groundwater depth under the latter situation (Singh et al. 2004; Coelho et al. 2020).

Therefore, it is important not only to demonstrate the technical and economic feasibility of TSE fertigation in crops, but also to quantify the potential negative impacts on the plant, soil, and groundwater and to identify possible health problems arising from this practice. With the hypothesis that the long-term application of high amounts of TSE would increase soil fertility, but could promote heavy metal contamination and soil salinization and sodification, this study aimed to evaluate the effects of TSE application on soil fertility and the potential for salinization, sodification, and heavy metal contamination of an Oxisol after a long-term (four years) use in a humid tropical region.

## MATERIAL AND METHODS

The study was conducted at Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil (latitude 21°15'S, longitude 48°18'W, and altitude of 595 m) for four years (2013 to 2017). According to Köppen's classification, the region climate is Aw, that is, a tropical climate with average annual rainfall of 1,400 mm, temperature of 22.7°C, dry and mild winter, and hot and rainy summer (Alvares et al. 2013). The soil is classified as an Oxisol (Soil Survey Staff 2014). It has a very clayey texture, with 625 g·kg<sup>-1</sup> of clay, 220 g·kg<sup>-1</sup> of silt, and 155 g·kg<sup>-1</sup> of sand in the 0-0.30 m layer (Gee and Or 2002).

In July 2012, before the experiment installation, the soil chemical characterization (Raij et al., 2001) was carried out, obtaining the results: hydrogen potential (pH) (CaCl<sub>2</sub>) = 5.4; organic matter (OM) = 30 g·dm<sup>-3</sup>; P<sub>resin</sub> = 69.5 mg·dm<sup>-3</sup>; K = 6.7 mmol<sub>c</sub>·dm<sup>-3</sup>; Ca = 36 mmol<sub>c</sub>·dm<sup>-3</sup>; Mg = 18.8 mmol<sub>c</sub>·dm<sup>-3</sup>; H+Al = 31.8 mmol<sub>c</sub>·dm<sup>-3</sup>; sum of bases (SB) = 61.5 mmol<sub>c</sub>·dm<sup>-3</sup>; cation exchange capacity (CEC) = 94 mmol<sub>c</sub>·dm<sup>-3</sup>; and base saturation (V) = 66%. Before experiment initiation, limestone with a relative total neutralization power (RTNP) of 90 was applied at the rate of 1.5 Mg·ha<sup>-1</sup> and incorporated into the 0-0.20 m soil layer through two heavy harrows and one light harrow to level the soil surface. In March 2016, 1 Mg·ha<sup>-1</sup> limestone with RTNP of 90 was applied to the soil surface.

The experiment consists of data collected during the period from 2013 to 2017 (Santos et al. 2017; Dantas et al. 2020). The plot size was 2.4 m wide and 6 m long. The experimental design consisted of banded blocks, with four replications. The treatments consisted of four TSE levels with water (E0 = 0% or water only, E1 = 11%, E3 = 60%, and E5 = 100% or effluent only). These treatments were chosen because they represented the most discrepant TSE levels and an average value (60%), in addition to the control without TSE application. The treatments were established by the application of fertigation with a line-source sprinkler system: three parallel lateral lines spaced at 12 m from each other, with the center line applying TSE and the external lines applying water. Sprinklers on each line were spaced at 6 m from each other. The TSE levels were defined using the TSE application equation for sprinklers (Santos et al. 2017). The applied water depth was uniform, while TSE concentrations varied gradually and perpendicularly to the sprinkler line, with high concentrations near the line applying TSE, but very low concentrations near the lines spraying only water.

The grass *Urochloa brizantha* Marandu was grown in the study area. TSE levels E0, E1, E3, and E5 corresponded to the treatments with no TSE application (E0) and low (E1), medium (E3), and high (E5) TSE levels, as established by Santos et al. (2017) and Dantas et al. (2020).

The TSE fertigation management was defined by criteria that aimed to meet the water or nutritional crop demands in the reference treatment (E3), considering the highest in each 28-day harvest cycle. The criterion of nutritional demand was assumed to be equal to 15 kg·ha<sup>-1</sup> of N per Mg of biomass (Vilela et al. 1998) produced in the E3 treatment, which was taken as a reference during the forage harvest intervals. Thus, irrigations with TSE were applied until reaching the amount of nitrogen required as a function of forage yield harvested in the E3 treatment in the previous harvest. The water demand was met by the application of an irrigation depth corresponding to the crop evapotranspiration (ET<sub>c</sub>), assumed to be equal to the reference evapotranspiration (ET<sub>o</sub>), as the crop coefficient (K<sub>c</sub>) of *U. brizantha* is equal to 1 (Allen et al. 1998). Daily meteorological data from the weather station nearby the experimental area was used to calculate ET<sub>o</sub> by the Penman-Monteith equation (Allen et al. 1998). If the amount of effluent to meet the nitrogen demand by the *U. brizantha* were greater than the amount of water required by the crop in a period of 28 days, the application of effluent would be based on nutritional demand, otherwise the water demand was used. For further details, check the studies by Santos et al. (2017) and Santos et al. (2019).

Fertilizers were applied to supplement phosphorus and potassium requirements in all treatments at doses of 3.5 and 18 kg K<sub>2</sub>O·ha<sup>-1</sup> per Mg of biomass produced in the E3 treatment during the forage harvest intervals.

Irrigation plus precipitation was higher than the evapotranspiration during all years of experimentation, which may have resulted in the leaching of water and TSE. Rainfall reached 1,393, 721, 2,014, and 1,430 mm, while the irrigation depths reached 853, 2,253, 1,567, and 1,110 mm in 2013, 2014, 2015, and 2016, respectively. The high irrigation depth was a consequence of the criterion used to meet the water or nutritional demand of the crop, as described in the previous section.

The amount of N and Na supplied by TSE and mineral N varied over the years because of the criteria to meet the water or nutritional demand and the resulting fertigation application. Nitrogen fertilization in *U. brizantha* was carried out both due to the TSE application and mineral fertilizer (mineral N). The total amounts of N applied by TSE was defined by the effluent level in each treatment. For treatments E0, E1, E3, and E5, 0, 351, 1,935, and 3,202 kg·ha<sup>-1</sup> of N were applied by TSE, respectively. Through fertilization with mineral fertilizer (urea), 2,191, 362, 362, and 362 kg·ha<sup>-1</sup> of N were applied for treatments E0, E1, E3, and E5, respectively. For study purposes, treatment E0 received an amount of mineral N similar to the N total amount applied (TSE + mineral N) in treatment E3.

The amount of P and K applied in the treatments was equal, with values of 322 kg P·ha<sup>-1</sup> and 1,768 kg K·ha<sup>-1</sup> in the sum of the four years. Amounts of 0, 389, 2,129, and 3,552 kg Na·ha<sup>-1</sup> were applied in the sum of the four years for treatments E0, E1, E3, and E5, respectively. Therefore, higher fertigation applied for treatment E5 in the dry year (2014) resulted in higher N (1,132 kg·ha<sup>-1</sup>) and Na amounts (1,256 kg·ha<sup>-1</sup>) compared to the wet year (2015), when the same treatment received only 786 kg N·ha<sup>-1</sup> and 674 kg Na·ha<sup>-1</sup>.

Treated sewage effluent was collected from the sewage treatment plant (STP) of Jaboticabal county and pumped through a polyvinyl chloride (PVC) pipe to the experimental area, 1.5 km apart, and stored in a reservoir. Jaboticabal has 71,662 inhabitants in an area of 707 km<sup>2</sup> and population density of 101.4 inhabitants km<sup>-2</sup>. The average sewage discharge of 200 dm<sup>3</sup>·inhabitant<sup>-1</sup> goes through several phases in the STP: a preliminary phase, in which the gross material is separated by a mechanical grid, followed by a mixed system (aerobic and anaerobic) composed of an up-flow anaerobic digester, and facultative lagoons.

TSE samples were collected in 2013, 2014, and 2017 from the reservoir and analyzed in accordance with American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF) for pH, electrical conductivity, nitrogen (nitrate, nitrite, ammoniacal nitrogen, and organic nitrogen), total iron, potassium, phosphorus, calcium, magnesium, manganese, sodium, and some potentially toxic elements (As, Hg, Mo, Ni, Pb, Se, and Zn) (APHA, AWWA and WEF 2005). The sodium adsorption ratio was calculated using Eq. 1. In 2013 and 2014, effluent analyses were performed three times throughout the period. In 2017, the collection was carried out only once throughout the period.

$$\text{SAR} = \frac{[\text{Na}^+]}{\sqrt{\frac{[\text{Ca}^{2+}] + [\text{Mg}^{2+}]}{2}}} \quad (1)$$

In which:

SAR: the sodium adsorption ratio ( $\text{mmol}_c \cdot \text{dm}^{-3})^{1/2}$ ;

$[\text{Na}^+]$ : the sodium concentration ( $\text{mmol} \cdot \text{dm}^{-3}$ );

$[\text{Ca}^{2+}]$ : the calcium concentration ( $\text{mmol} \cdot \text{dm}^{-3}$ );

$[\text{Mg}^{2+}]$ : the magnesium concentration ( $\text{mmol} \cdot \text{dm}^{-3}$ ).

Total nitrogen (TN), nitrate ( $\text{NO}_3^-$ -N) and nitrite ( $\text{NO}_2^-$ -N) were determined using kits from Alfacit®. The methodologies adopted by the kits followed APHA, AWWA and WEF (2005). Ammoniacal N ( $\text{NH}_4^+$ -N) and Kjeldahl N (KN) were determined by the distillation method using a steam distiller (APHA, AWWA and WEF 2005). Organic N (ON) was calculated using Eq. 2.

$$\text{ON} = (\text{NH}_4^+ - \text{N}) - \text{KN} \quad (2)$$

The total phosphorus (TP) content was determined by molecular spectroscopy in the visible region (4500PE method) after nitro-sulfuric digestion of the samples (APHA, AWWA and WEF 2005).

Analyses of soil potentially toxic elements were performed before the experiment was set up (2012) in the 0-0.10 m layer and at the end of the experiment (2017) in the 0-0.10 and 0.10-0.20 m layers for the treatments E0, E1, E3, and E5. For heavy metals, soil samples were collected in each replication of each treatment, generating a composite sample for each TSE level and layer for analysis.

Sampling and analysis for soil variables: pH, P, K, Ca, Mg, H+Al, OM, SB, cation exchange capacity (T) and V were carried out in February 2017 in the 0-0.10 and 0.10-0.20 m layers for the treatments E0, E1, E3, and E5. Soil samples were collected from the four replicates of the experiment, forming a composite sample for each repetition. These samples were air-dried and passed through a 2-mm opening sieve. For soil total N (NT) and Na, sampling and analysis were carried out in March and December 2014 and February 2017 in the 0-0.10 and 0.10-0.20 m layers for the treatments E0, E1, E3, and E5. For the NT and Na variables, samples were collected in each repetition, which later formed a pooled sample composed by treatment.

The contents of the potentially toxic elements (Zn, Cd, Cr, Cu, Ni, As, Hg, Se, and Mo) and Na were determined by the United States Environmental Protection Agency (USEPA) method 3050B (USEPA 1984). The TN concentration was determined by the distillation method (Silva 2009). The percentage of exchangeable sodium (PES) was calculated according to Richards (1970).

In addition, pH, OM, available phosphorus (P), K, Ca, Mg, and potential acidity (H+Al) were analyzed, and, subsequently, T, SB, and V were calculated (Raij et al. 2001).

Soil solution samples were collected and analyzed throughout the experimental period (2013 to 2017) to determine the pH and electrical conductivity in treatments E0, E3, and E5 at depths of 0.15, 0.30, and 0.55 m. The pH and electrical conductivity (EC) of the soil solution were measured in the field in each treatment replication using a pH meter and a conductivity meter, as previously described in the TSE analysis. The soil solution was collected by solution extractors installed at the predetermined sampling depths. The extractors consisted of a PVC tube closed by a rubber cap at one end and a porous ceramic capsule at the other end. A hand pump was used the day before sampling to produce a vacuum of 80 kPa into the tube to suck the soil solution through the ceramic capsule.

The attributes soil pH, OM, P, Ca, Mg, H+Al, SB, T, V, soil solution pH and EC were subjected to analysis of variance ( $p < 0.05$ ) and, when necessary, to the Tukey's honestly significant difference (HSD) test ( $p < 0.05$ ) in order to compare

the evaluated TSE levels and the soil layers. For the total N and Na soil contents, graphs were plotted to characterize the temporal variation of their values at each TSE level evaluated. Regarding heavy metals, the average contents determined at each TSE level and soil layer at the end of the experiment in 2017 were compared to the maximum limit established by legislation (CETESB 2014).

## RESULTS AND DISCUSSION

The pH values in TSE are between the upper and lower limits, and EC is below the limits established by Resolution No. 430/2011 of the Brazilian National Council for the Environment (CONAMA) (Brasil 2011) and the Food and Agriculture Organization (FAO) (Ayers and Westcot 1985) (Tab. 1). The values measured in February 2017 were similar to those obtained in the summers of 2013 and 2014. It indicates that the chemical characteristics of TSE were maintained throughout the four years of study, which can be attributed to the composition stability of the TSE received from the Jaboticabal treatment plant. EC ( $0.44 \text{ dS}\cdot\text{m}^{-1}$ ) and SAR [ $5.6 (\text{mmol}_c\cdot\text{dm}^{-3})^{1/2}$ ] classify TSE as C2S1, according to Richards (1954), corresponding to medium risk of salinization and low risk of sodification.

The chemical characteristics of TSE are comparable to those of other regions in Brazil and worldwide (Leal et al. 2009; Gharaibeh et al. 2016). However, depending on the region, the sodium concentration in TSE can be very high ( $> 100 \text{ mg}\cdot\text{dm}^{-3}$ ) (Fonseca et al. 2007), which may cause a higher potential for soil degradation.

Potentially toxic elements in TSE were found within the limits established by Resolution No. 430/2011 of CONAMA (Brasil 2011) for disposal in water courses, except for Cr, Cu, Mo, and Zn in 2013, whose values exceeded the concentration limit proposed in this resolution (Tab. 2). With the exception of Cr, the other elements (Cu, Mo and Zn) are plant micronutrients. As the TSE application in the present study was on the soil and not in the water courses, the presence of these elements can be beneficial to increase the soil micronutrients availability and promote greater crops growth. It is noteworthy that, due

**Table 1.** Chemical characteristics of the treated sewage effluent (TSE) of Jaboticabal, SP, Brazil, in the summers of 2013 and 2014 and February 2017.

Variable	Unit	2013	2014	2017	Limit value*
pH	–	$7.2 \pm 0.2$	$7.0 \pm 0.4$	7.4	5-9
EC	$\text{dS}\cdot\text{m}^{-1}$	$0.45 \pm 0.01$	$0.46 \pm 0.09$	0.44	<3
TOC	$\text{mg}\cdot\text{dm}^{-3}$	$36.3 \pm 9.6$	$59.3 \pm 19.1$	–	–
$\text{NO}_3^-$ -N	$\text{mg}\cdot\text{dm}^{-3}$	$4.4 \pm 0.9$	$4.6 \pm 3.2$	< 0,10	< 10
$\text{NO}_2^-$ -N	$\text{mg}\cdot\text{dm}^{-3}$	$0.04 \pm 0.0$	$0.41 \pm 1.1$	< 0,01	< 0.02
$\text{NH}_4^+$ -N	$\text{mg}\cdot\text{dm}^{-3}$	$38.8 \pm 12.2$	$29.2 \pm 17.7$	38.33	1–40
TN	$\text{mg}\cdot\text{dm}^{-3}$	$53.7 \pm 7.6$	$52.4 \pm 7.1$	44.36	–
ON	$\text{mg}\cdot\text{dm}^{-3}$	$11.3 \pm 11.0$	$19.9 \pm 15.9$	2.33	–
TKN	$\text{mg}\cdot\text{dm}^{-3}$	$49.2 \pm 8.0$	$49.1 \pm 8.1$	40.66	–
TFe	$\text{mg}\cdot\text{dm}^{-3}$	$0.42 \pm 0.1$	$0.62 \pm 0.4$	0.39	< 5
K	$\text{mg}\cdot\text{dm}^{-3}$	$16.6 \pm 2.1$	$23.1 \pm 8.8$	15.3	10–40
TP	$\text{mg}\cdot\text{dm}^{-3}$	$0.9 \pm 0.3$	$1.2 \pm 0.4$	2.2	< 2
Ca	$\text{mg}\cdot\text{dm}^{-3}$	$14.0 \pm 1.6$	$16.7 \pm 8.8$	12.0	20–120
Mg	$\text{mg}\cdot\text{dm}^{-3}$	$5.0 \pm 1.0$	$7.1 \pm 3.2$	2.72	10–50
Mn	$\text{mg}\cdot\text{dm}^{-3}$	$0.1 \pm 0.1$	$0.11 \pm 0.1$	0.15	1.0
Na	$\text{mg}\cdot\text{dm}^{-3}$	$54.8 \pm 9.5$	$61.8 \pm 8.0$	58.5	50–250
SAR	$(\text{mmol}_c\cdot\text{dm}^{-3})^{1/2}$	$3.2 \pm 0.6$	$3.3 \pm 0.6$	5.59	4.5–7.5

\*Limits established by Ayers and Westcot (1985), Feigin et al. (1991) and Brasil (2011); pH: hydrogen potential; EC: electrical conductivity; TOC: total organic carbon;  $\text{NO}_3^-$ -N: nitrate;  $\text{NO}_2^-$ -N: nitrite;  $\text{NH}_4^+$ -N: ammonium; TN: total nitrogen; ON: organic nitrogen; TKN: total Kjeldahl nitrogen; TFe: total iron; K: potassium; TP: total phosphorus; Ca: calcium; Mg: magnesium; Mn: manganese; Zn: zinc; Na: sodium; SAR: sodium adsorption ratio.

**Table 2.** Contents of potentially toxic elements in the effluent of the Jaboticabal, SP, Brazil, sewage treatment plant in 2013, 2014, and February 2017.

Potentially toxic element	Unit	2013	2014	2017	Limit value*
As	mg·dm <sup>-3</sup>	< 0.01	< 0.001	< 0.086	0.5
Cd	mg·dm <sup>-3</sup>	0.2	0.03	0	0.2
Cr	mg·dm <sup>-3</sup>	0.22	0.02	0	0.1
Cu	mg·dm <sup>-3</sup>	5.58	0.024	0.01	1.0
Hg	mg·dm <sup>-3</sup>	0.01	< 0.001	< 1.5	0.01
Mo	mg·dm <sup>-3</sup>	0.38	–	4.38	0.01
Ni	mg·dm <sup>-3</sup>	0.25	< 0.01	0	2.0
Pb	mg·dm <sup>-3</sup>	0.16	< 0.01	0.1	0.5
Se	mg·dm <sup>-3</sup>	< 0.02	–	< 2.0	0.3
Zn	mg·dm <sup>-3</sup>	6.5	0.2	0.05	5

\*Brasil (2011).

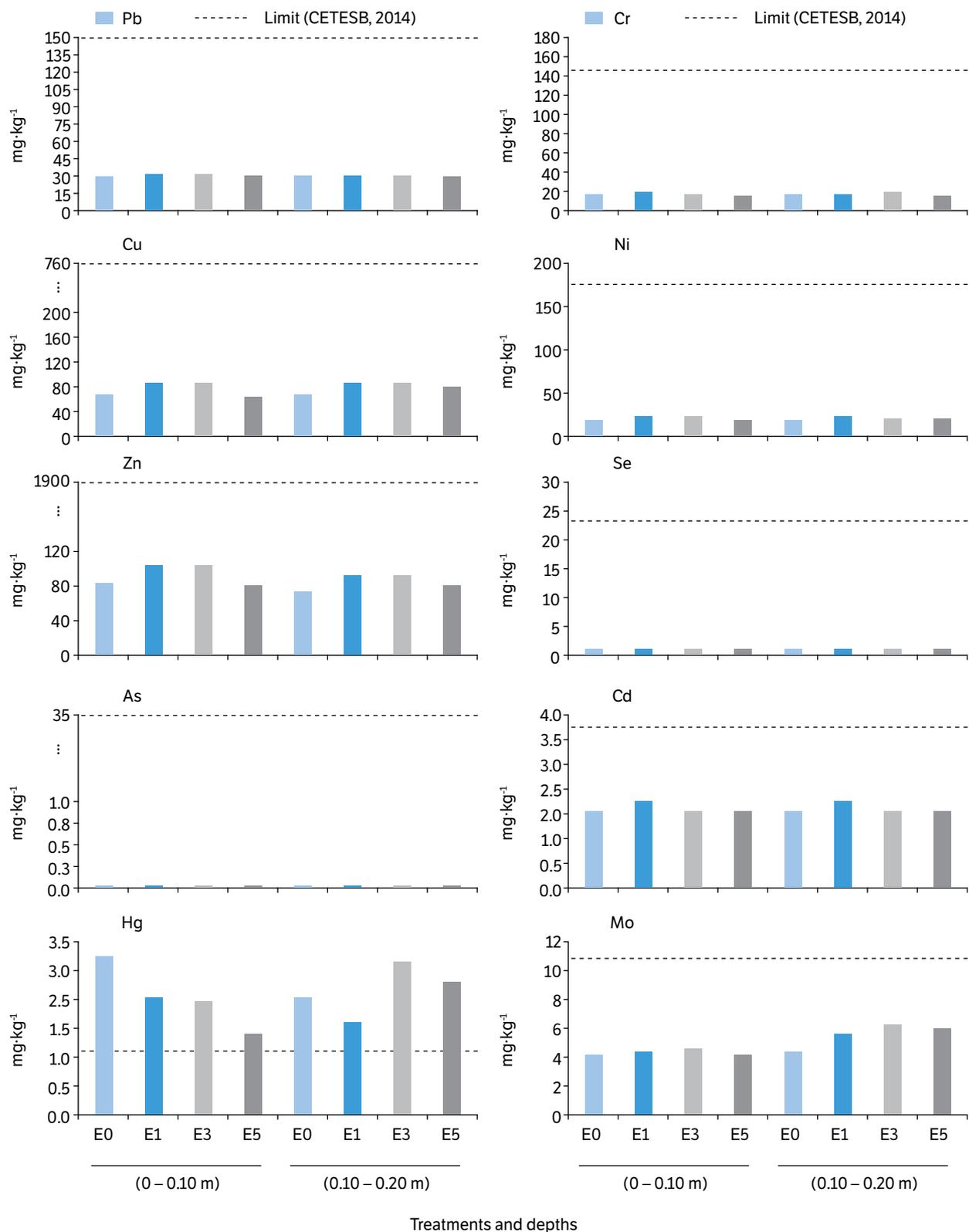
to the high weathering degree of the soil evaluated in this study (Oxisol), TSE fertigation may be an important source for the supply of these micronutrients. Santos et al. (2017) carried out a study in the same experimental area in March 2013 and found low to high concentrations of potentially toxic elements. However, the analysis carried out in December 2014 showed reduction in the load of these elements in the TSE, with concentrations below the established levels for disposal in water courses (Brasil 2011) (Tab. 2).

The concentrations of potentially toxic elements in the Oxisol were below the limit values for soil and groundwater in the state of São Paulo (CETESB 2014) during the entire period, except for Hg, which had concentrations above the established standard in all treatments (Fig. 1). Treatment E0 (irrigation with water) also presented a high soil concentration of Hg despite its low TSE concentration, indicating that this element does not come from the effluent, but from the soil formation (geology). Furthermore, in 2012, before experiment initiation, the soil Hg content determined in the 0-0.10 m layer was 1.49 mg·kg<sup>-1</sup>, which is above the maximum allowed limit. Treatments with a lower TSE level showed higher Hg availability, especially in the 0-0.10 m soil layer, and lower soil pH (Fig. 2). Increased Hg availability occurs because the lower the soil pH, the higher the availability of metallic elements, such as Hg (Raij et al. 2001). For the 0.10-0.20 m layer, there were no relevant differences in the Hg content between the treatments, with only E3 standing out with a higher value than E0 and E1.

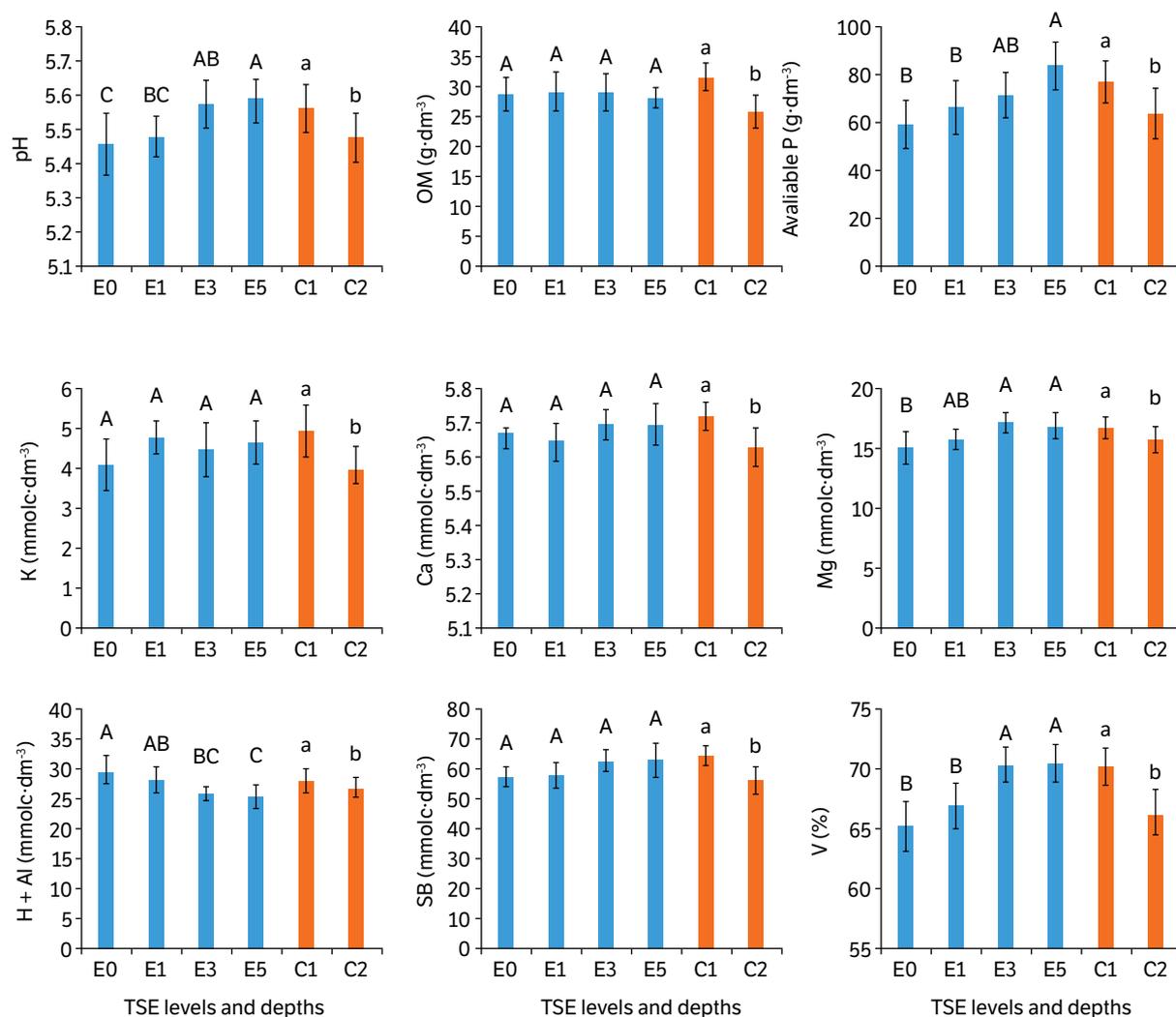
Khan et al. (2008) researched the risk of soil contamination by potentially toxic metals (Cd, Cr, Cu, Ni, Pb, and Zn) using wastewater in China and found a significant difference in their concentrations for the treatment in which wastewater was used, although their levels were within legally established standards. Studies have indicated that wastewater contaminates the soil with potentially toxic elements (Liu et al. 2005). However, in the present study, the concentrations of these elements in TSE were low (Tab. 2), indicating a low risk of soil contamination by heavy metals.

Significant effects of TSE and depth were observed on soil pH (Tab. 3). A higher pH was observed for treatments with higher TSE levels (E5 and E3) compared to the lower pH value found for treatments with lower (E1) or no effluent application (E0) (Fig. 2). Similarly, Abegunrin et al. (2016) analyzed physicochemical parameters in soil irrigated with TSE and also found that the wastewater application increased the soil pH value. In contrast, Mojiri (2011) used wastewater in irrigation and observed a decrease in soil pH, which was attributed as per Smith and Giller (1992) to the effluent characteristics (mainly acidity). Some soils have buffering power that allows them to keep their pH unchanged when irrigated with wastewater.

Significant effects of TSE levels and depths were also observed on the available P, Mg, and V (Tab. 3), with higher means in the E5 level than in the E0 level and 0-0.10 m depth than in the 0.10-0.20 m depth (Fig. 2). The means of K, Ca, and SB were similar for TSE levels, but differed for depths (Tab. 3 and Fig. 2), with higher values in the 0-0.10 m depth due to the higher OM content in this soil layer, resulting in a higher number of charges for retaining nutrients. The higher macronutrient concentration in the surface layer can be attributed to TSE and fertilizer applications on the soil surface. Similarly, a higher OM concentration was found in the 0-0.10 m layer, but with no difference between treatments. Erthal et al. (2010) also



**Figure 1.** Concentrations of potentially toxic elements in the soil according to the soil layer and treated sewage effluent treatments (E0 = 0%, E1 = 11%, E3 = 60%, and E5 = 100% of effluent) in February 2017. The dashed line indicates the concentration limits according to the guiding values for soil and groundwater in the state of São Paulo, Brazil (CETESB 2014).



pH: hydrogen potential; OM: organic matter; TSE: treated sewage effluent; SB: sum of bases; V: base saturation; \*uppercase letters compare effluent levels and lowercase letters compare soil layers according to Tukey's multiple comparison test ( $p < 0.05$ ).

**Figure 2.** Comparison of means test for soil pH and soil macronutrients as a function of treated sewage effluent levels and soil layers\*. E5 = 100%, E3 = 60%, E1 = 11%, and E0 = 0; C1 = 0-0.10 m, and C2 = 0.10-0.20 m.

found no variation in the soil OM content using TSE in the irrigation and associated the low soil OM concentration with the low total organic carbon content in the effluent.

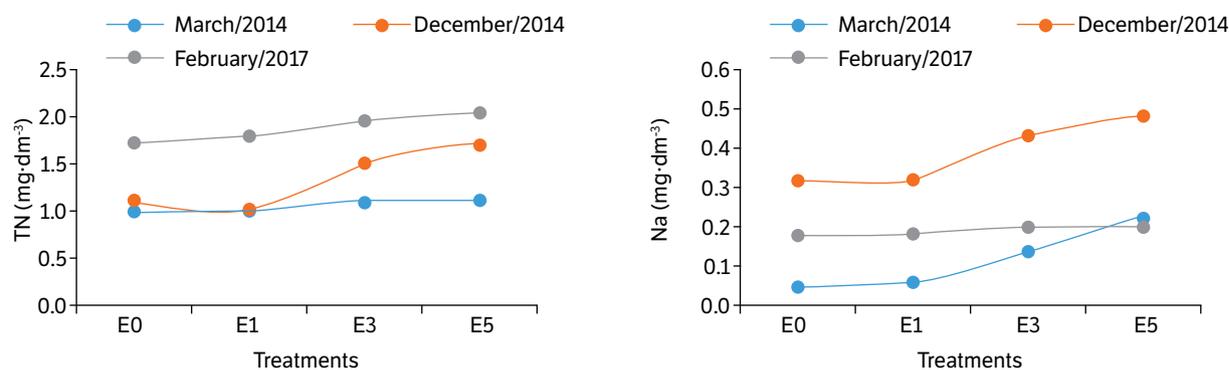
However, it is noteworthy that the OM content went from  $30 \text{ g}\cdot\text{dm}^{-3}$  in the 0-0.10 m soil layer in the experimental area before the experiment started, in 2012, to a value close to  $35 \text{ g}\cdot\text{dm}^{-3}$  in the same layer in 2017. The increased OM can be caused by the *U. brizantha* biomass accumulation over time (Figueiredo et al. 2013). These results indicate that the organic matter increased due to the *U. brizantha* biomass decomposition from medium to long term, which might be beneficial for the microbial biodiversity, improving the soil physical, chemical, and biological characteristics (Silva and Mendonça 2007). Gharaibeh et al. (2016) used TSE and observed an increase in OM content from 2.77% at the beginning of the measurements to 4.37% after two years and 7.19% after five years. Other studies have shown increases in the OM concentration over the years due to wastewater application (Galavi et al. 2010; Gharaibeh et al. 2016). In addition, the high clay content of the soil can physically protect OM from decomposition.

The analysis of TN showed, at the end of the experiment, in February 2017, that TSE application at the highest levels, such as in the treatments E3 and E5, increased the TN content compared to the treatments E0 and E1 (Fig. 3). The same pattern was observed in March and December 2014, when the TN content increased in the surface layer from 1.1 to  $1.7 \text{ mg}\cdot\text{dm}^{-3}$

**Table 3.** Analysis of variance for the effects of treated sewage effluent (TSE) levels (E5 = 100%, E3 = 60%, E1 = 11%, and E0 = 0%) and depths (0–0.10 and 0.10–0.20 m) on the soil macronutrients, soil solution pH, and electric conductivity from 2013 to 2017.

Source of variation	TSE	Depth	TSE × Depth	CV%
Soil pH	**	**	NS	1.48
OM	NS	**	NS	10.09
Available P	**	**	NS	15.48
K	NS	**	NS	14.06
Ca	NS	**	NS	9.24
Mg	**	*	NS	7.19
H+Al	**	*	NS	7.74
SB	*	**	NS	7.73
T	NS	**	NS	6.17
V	**	**	NS	2.94
Soil solution pH	NS	**	NS	2.39
EC	**	NS	NS	14.70

TSE: treated sewage effluent; CV: coefficient of variation; pH: hydrogen potential; OM: organic matter; SB: sum of bases; T: cation exchange ratio; V: base saturation; EC: electrical conductivity; \*significant at a 5% probability level ( $p < 0.05$ ); \*\*significant at a 1% probability level ( $p < 0.01$ ); NS: not significant ( $p \geq 0.05$ ).

**Figure 3.** Total nitrogen (TN) and sodium (Na) concentration in the soil as a function of treated sewage effluent levels (E0 = 0%, E1 = 11%, E3 = 60%, and E5 = 100%) in the 0-0.20 m layer in March and December 2014 and February 2017 in Jaboticabal, SP, Brazil.

for the treatments E3 and E5, respectively, compared to E0 and E1 (Fig. 3). The higher TN concentration in the surface layer for treatments with higher TSE levels was due to the nutrient supply by TSE fertigation, in which TN effluent average content was  $50.2 \text{ mg}\cdot\text{dm}^{-3}$ . Santos et al. (2019) observed that one of the factors that promoted an increase in *U. brizantha* yield in treatments with a higher TSE level (E3 and E5) was the great availability of N in the soil. The authors observed that the amount of N extracted by *U. brizantha* in the E5 treatment were  $1,045 \text{ kg}\cdot\text{ha}^{-1}$  in 2013 and  $1,501 \text{ kg}\cdot\text{ha}^{-1}$  in 2014, while for the E1 treatment the extraction was only 491 and  $404 \text{ kg}\cdot\text{ha}^{-1}$ , respectively. Even with 184% greater N extraction in the E5 treatment compared to E1, the soil TN was higher for the management with higher TSE levels in 2017, demonstrating the high capacity of this wastewater in providing N to the soil.

Sodium concentration also varied between treatments, with higher levels in the treatments E3 and E5 (Fig. 3). A gradual increase in soil sodium concentration was observed from March to December 2014 with an increase in TSE levels (E5 and E3). In February 2017, sodium concentration was lower relative to December 2014, which may be related to climate variations and to extraction by plants.

Increased sodium concentration is one of the main concerns when using TSE in fertigation (Azevedo et al. 2013). However, the values measured in this experiment (Fig. 3) do not present soil sodification hazard since the calculated

exchangeable sodium percentage (ESP) values ( $< 1\%$ ) were lower than the critical level (15%) delineated by the Richards' classification (Richards 1954).

The experimental area showed no risk of soil sodification even with the application of high rates of Na by TSE over the four years, which can be explained by the extraction of part of the Na present in the soil by *U. brizantha* and the climate of the study region. Santos et al. (2017) observed that *U. brizantha* presented a leaf Na content in the E5 treatment of  $2 \text{ g}\cdot\text{kg}^{-1}$  during the first two years of this study. The annual Na extraction by *U. brizantha* was  $120 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ , considering its average dry matter production per year, which is equivalent to  $480 \text{ kg}\cdot\text{ha}^{-1}$  during the four years of the experiment. Thus, Na extraction by the crop likely mitigated the harmful effects of this element in the soil. Furthermore, it is highlighted that future research on the use of different crops to mitigate the soil sodification potential due to TSE application would be of great scientific interest, especially in regions with annual precipitation similar or lower than the evapotranspiration.

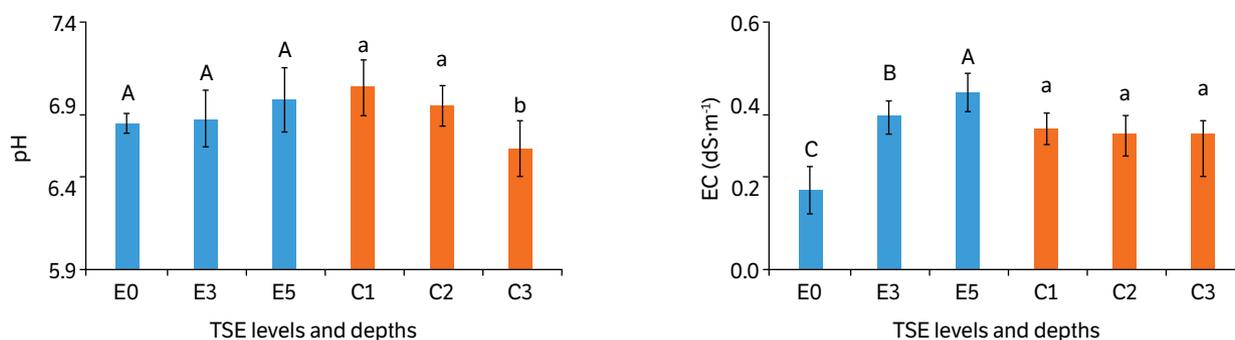
In addition, the study was conducted in a humid tropical region, with normal annual rainfall close to 1,400 mm, which is higher than the accumulated evapotranspiration per year. This fact prevents and/or minimizes Na accumulation in most soil surface layers, avoiding possible soil sodification or salinization. Regions with annual rainfall below the evapotranspiration accumulate salts on the soil surface, resulting in reduction of soil physical quality (Bardhan et al. 2016). In this study, in addition to not observing a potential for soil salinization and sodification, the soil physical quality was not changed due to TSE application levels (Coelho et al. 2020).

Soil solution pH was significantly affected by depth, but not by TSE levels. The pH was close to neutral at depths of 0.15 and 0.30 m and decreased significantly at 0.55 m, becoming acidic with increasing depth (Fig. 4). The higher pH in shallow layers reflects the liming that was applied to correct acidity over the experimental period. TSE pH was close to 7. Therefore, fertigation did not change the pH of the soil solution.

EC was affected significantly by TSE levels, while no effect of depth was observed. The comparison of means revealed significant increases in EC for E5, followed by E3 (Fig. 4). Although increasing EC in the soil solution, the magnitude of the resulting EC ( $< 0.5 \text{ dS m}^{-1}$ ) for the highest TSE level is not important, as it is almost one-tenth of the critical limit established by Richards (1954), that is,  $4 \text{ dS m}^{-1}$ . Therefore, there was no salinity risk due to TSE application in this soil.

Azevedo et al. (2013) evaluated fertigation with domestic wastewater on chili pepper (*Capsicum frutescens* L.) and found increased EC value of the soil saturation extract due to the treatment, which was associated with the presence of high salt loads in the effluent, thus increasing soil EC.

The effect of applying TSE levels on the yield, nutrient extraction, and quality of *U. brizantha* (Santos et al. 2017; Santos et al. 2019; Dantas et al. 2020) and soil physical quality (Coelho et al. 2020) was studied over the four years of experiments. We also evaluated the effects of long-term TSE application on soil fertility and the potential for soil salinization, sodification, and contamination by heavy metals. The experiments mentioned showed that TSE application increased *U. brizantha* yield and quality and did not change soil physical attributes.



\*Uppercase letters compare effluent levels and lowercase letters compare soil layers according to Tukey's multiple comparison test ( $p < 0.05$ ).

**Figure 4.** Comparison of means test for hydrogen potential (pH) and electric conductivity (EC) in the soil solution as a function of treated sewage effluent levels (E5 = 100%, E3 = 60%, and E0 = 0) and soil layers (C1 = 0.15, C2 = 0.30, and C3 = 0.55 m)\*.

## CONCLUSION

Here, we found that TSE application over long term increased soil fertility and did not increase the potential for soil salinization, sodification, and heavy metal contamination. Soil pH, EC, and sodium concentration increased, but they remained within the permissible limits. On the other hand, TSE application increased the concentration of soil macronutrients. The increased levels of EC and sodium concentration due to TSE application presented no risk of soil sodification. Therefore, there was no apparent risk of soil contamination by TSE application due to the characteristics of the studied effluent since the content of potentially toxic elements in the soil showed no increase with fertigation.

The long-term study showed that fertigation using TSE is recommended in humid tropical regions on clayey soils cultivated with forage grasses, as it generated no high potential for soil salinization, sodification, and heavy metal contamination, as long as the effluent has similar characteristics, as in the present study. Furthermore, the use of TSE fertigation in agricultural areas mitigates possible contamination by the direct discharge of these wastes into surface water courses. For even more robust results, studies are needed contemplating an even longer period (> 10 years) to confirm the TSE fertigation effects on soil attributes, as the results can be influenced by the climatic conditions, crops cultivated, management applied, and the soil type.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Barbosa A. M. S.; Faria R. T. and Saran L. M.; **Methodology:** Barbosa A. M. S.; Faria R. T.; Saran L. M.; Santos G. O. and Dantas, G. F.; **Investigation:** Barbosa A. M. S.; Santos G. O. and Dantas G. O.; **Writing – Original Draft:** Barbosa A. M. S. and Coelho A. P.; **Writing – Review and Editing:** Faria R. T. and Saran L. M.; **Funding Acquisition:** Barbosa A. M. S., Faria R. T. and Saran L. M.; **Resources:** Faria R. T. and Saran L. M.; **Supervision:** Faria R. T. and Saran L. M.

## DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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**ERRATA**

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