

SEWAGE SLUDGE AS FERTILIZER IN THE PRODUCTION OF *Cariniana estrellensis* SEEDLINGS FOR FOREST RESTORATION**ALESSANDRO REINALDO ZABOTTO¹, PATRICK LUAN FERREIRA DOS SANTOS², ARMANDO REIS TAVARES³, IRAÊ AMARAL GUERRINI², FERNANDO BROETTO⁴**

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ABSTRACT: One of the main factors for the production of quality seedlings is fertilization. Some organic materials have been used in substrate mixtures or as fertilizers for the production of seedlings within this context. Sewage sludge is a byproduct of local operation of wastewater-treatment station and has a potential use on agriculture due to the high concentrations of organic matter and nutrients. The objective of this study was to evaluate the growth and development of *Cariniana estrellensis* seedlings fertilized with different doses of sewage sludge supplemented with potassium, planted in pots with 1.7 L in commercial substrate, aiming seedlings production for urban afforestation. The treatments consisted of 0 (control), 30, 60 and 90 g sewage sludge L⁻¹ supplemented with 150 mg K L⁻¹, except for the control treatment. The biometric, physiological and nutritional parameters were evaluated after 120 days of experimentation. The results showed that the addition of sewage sludge to the substrate provided appropriate leaf contents of macro and micronutrients, reflecting higher parameters of height, stem diameter, leaf area and dry biomass of the plants compared to the control treatment. The plants that received the doses of the residue showed higher averages of photosynthetic pigments, positively influencing the gas exchange rates. The application of the dose with 30 g L⁻¹ of the sewage sludge can be indicated for the fertilization of seedlings of the species for urban afforestation. Thus, that is a viable alternative for the destination of the waste.

Keywords: plant nutrition, biosolid, organic fertilizer, urban waste.

LODO DE ESGOTO COMO FERTILIZANTE NA PRODUÇÃO DE MUDAS DE *Cariniana estrellensis* PARA RESTAURAÇÃO FLORESTAL

RESUMO: Um dos principais fatores para a produção de mudas de qualidade é a adubação. Dentro deste contexto, alguns materiais orgânicos têm sido utilizados em misturas de substratos ou como fertilizantes para a produção de mudas. O lodo de esgoto é um subproduto da operação da estação de tratamento de esgoto municipal e tem potencial para uso na agricultura devido às altas concentrações de matéria orgânica e nutrientes. O objetivo deste trabalho foi avaliar o crescimento e desenvolvimento de mudas de *Cariniana estrellensis*, adubadas com diferentes doses de lodo de esgoto suplementado com potássio, plantadas em vasos de 1,7L contendo substrato comercial, visando a produção de mudas para a arborização urbana. Os tratamentos consistiram de 0 (controle), 30, 60 e 90 g de lodo L⁻¹ suplementado com 150 mg K L⁻¹, exceto o tratamento controle. Após 120 dias de experimentação, foram avaliados os parâmetros biométricos, fisiológicos e nutricionais. Os resultados mostraram que a adição de lodo de esgoto ao substrato proporcionou teores foliares adequados de macro e micronutrientes, refletindo maiores parâmetros de altura, diâmetro do caule, área foliar e biomassa seca das plantas em relação ao tratamento controle. As plantas que receberam as doses do resíduo apresentaram maiores médias de pigmentos fotossintéticos, influenciando

positivamente nas taxas de trocas gasosas. A aplicação da dose com 30 g L⁻¹ de lodo de esgoto pode ser indicada para a adubação da espécie na produção de mudas para arborização urbana, sendo alternativa viável para a destinação do resíduo.

Palavras-chave: nutrição de plantas, biossólido, adubo orgânico, resíduos urbanos.

1 INTRODUCTION

Brazil has one of the largest native forest areas in the world, with approximately 485 million hectares (SERVIÇO FLORESTAL BRASILEIRO, 2019). The Atlantic Forest is one of the most devastated among Brazilian biomes, due to illegal logging caused by the urban expansion and economic growth (CARVALHO; MATOS, 2016). Due to the requirement of compensatory environmental measures, there is an intense demand for seedlings to be used to recover degraded areas. However, for successful recovery, during the nursery phase, it is crucial to use high quality native species seedlings, which will directly affect the growth and development of trees after planting in the field (SIQUEIRA *et al.*, 2019).

Sewage sludge is an organic residue generated by the urban wastewater treatment process, whose production increases according to the growth of the urban population. The residue has an average of 40% organic matter, 3% nitrogen, and 2% phosphorus (BETTIOL; CAMARGO, 2006). It contains Ca, Mg, S and micronutrients in its composition, enabling its use as a fertilizer. Consequently, it provides savings in mineral fertilizers (KACPRZAK *et al.*, 2017), and requires only potassium supplementation due to low concentration of this nutrient (ZABOTTO *et al.*, 2020). Nurseries commonly use mineral fertilizers composed mainly of NPK. Thus, the content of nutrients in the substrate is directly related to the quality of the seedlings produced. In this sense, different types of organic fertilizers need to be tested, aiming at replacing or complementing mineral fertilizers, optimizing production at low cost through the use of highly available organic materials, reaching a competitive price in the market (ROWEDER; NASCIMENTO; SILVA, 2015).

The *Cariniana estrellensis* (Raddi) Kuntz (Jequitibá Branco) is a native species to

the Atlantic Forest that makes up the climax forests (GUIDUGLI, 2011). The species is considered of great longevity and is also part of the list of trees at risk of extinction or vulnerable in some Brazilian states. *C. estrellensis* is used in the recovery of degraded areas, and can also be exploited commercially as wood for carpentry, furniture and construction and, to produce good quality cellulose (CARVALHO, 2003).

Therefore, by seeking to combine sustainability in seedlings production, this study aimed to evaluate the growth and development in nursery stage of *C. estrellensis* seedlings fertilized with sewage sludge supplemented with potassium.

2 MATERIAL AND METHODS

The experiment was carried out in an agricultural greenhouse located at Botucatu, São Paulo State, Brazil (22°53'42.4"S - 48°29'36.6"W) and altitude of 815 m. The climate, according to Köppen, is classified as warm temperate (mesothermal), with rainy summer and drought winter. The average temperature in the experimental period was 19.9 °C (14.3 °C minimum and 25.7 °C maximum). The relative humidity of the air was 65.1% (44.8% minimum and 88.1% maximum).

The *Cariniana estrellensis* seedlings were obtained from a commercial nursery in 56 cm³ tubes with 0.15 m height and 3.09 mm stem diameter, in average. Subsequently, the seedlings were transplanted to 1.7 L polyethylene pots containing sphagno peat (70%), roasted rice straw (20%) and perlite (10%) as commercial substrate (Table 1). The electrical conductivity of the substrate (EC_p – dS m⁻¹) and humidity (%) were determined daily, with HH2 Moisture Meter equipment coupled to WET-2 Sensor (DELTA-T DEVICES®).

Table 1. Chemical composition of substrate.

CN	pH	U*	O.M.	C	N	P ₂ O ₅	K ₂ O	Ca	Mg	S	Na	B	Cu	Fe	Mn	Zn
-	-	-					%		mg Kg ⁻¹							
42/1	5.8	8	59	36	0.75	0.40	0.15	1.14	2.55	0.15	1.37	-	24	9.13	138	4

Source: Author (2020). *U – 65 °C

The sewage sludge (SS) was supplied by the sewage treatment station (ETE) located in the city of Botucatu, São Paulo State, Brazil. SS was stored in stalls and revolved daily for approximately 45 days, with temperatures above 55 °C for the disinfection process (Table

2). The treatments used were T1 - Substrate (Control); T2 - Substrate + 30 g L⁻¹ SS + 150 mg L⁻¹ KCl; T3 - Substrate + 60 g L⁻¹ SS + 150 mg L⁻¹ KCl, and T4 - Substrate + 90 g L⁻¹ SS + 150 mg L⁻¹ KCl.

Table 2. Chemical composition of sewage sludge.

C/N	pH	U-65 °C	O.M.	C	N	P ₂ O ₅	K ₂ O	Ca	Mg	S		
-	-	-					%					
7/1	5.8	20	37	26	2.8	3.5	0.1	1.5	0.4	0.4		
Na	B	Cu	Fe	Mn	Zn	As	Cd	Pb	Cr	Hg	Ni	Se
mg Kg ⁻¹												
1.13	145	185	33,79	259	701	<0.1	1.3	26	132	<0.1	17.6	<0.1

Source: Author (2020).

After 120 days of experimentation the variables stem diameter (SD), plant height and total leaf area (LA, Li-3100C, LI-COR[®]) were evaluated. The green leaf color index (LCI), were evaluated with a portable equipment model SPAD-502 PLUS (Konica-Minolta[®]). Sampling was done on the third fully expanded leaf exposed to solar radiation. The contents of photosynthetic pigments were determined in leaf samples (n=3) immersed in 1 mL of Dimethylformamide (DMF) solution and kept for 48 hours in the dark. The extract was analyzed in a spectrophotometer at $\lambda = 649$ nm (chlorophyll *a*), 665 nm (chlorophyll *b*) and 470 nm (carotenoids) and the chlorophylls *a* and *b*, total chlorophyll and carotenoids contents were expressed in $\mu\text{g cm}^{-2}$.

The evaluation of gas exchange was performed with an infrared gas analyzer (IRGA), model LI-6400XT (LI-COR[®]). Readings were taken in the morning on three plants of each treatment, on leaves chosen from the middle third of the plant, fully expanded and mature. After determining the luminous saturation curve of the species, data on net photosynthesis (A) in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, stomatal conductance, (gs) in $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and transpiration (E) in $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ was determined.

The seedlings quality were evaluated using the ratios between height and diameter of the stem (H/SD) and, shoot and root dry mass and root (SDM/RDM) and Dickson quality index - DQI (DICKSON; LEAF; HOSNER, 1960).

$$\text{DQI} = \text{TDM}/(\text{H}/\text{SD}) + (\text{DMAP}/\text{DWS}) \quad (01)$$

Where: TDM = total dry mass, SDM = shoot dry mass, SD = stem dry mass, H = Plant height, and ST = Stem diameter.

The plants were sectioned in leaves, stems and roots and weighed for fresh mass. The plant materials were dried in a forced ventilation oven (65 °C) until reaching constant weight for dry mass. The macro and micronutrient contents of the leaves were analyzed. Phosphorus, potassium, calcium, magnesium and sulfur, copper, iron, manganese and zinc by nitro-perchloric digestion, total nitrogen by sulfuric digestion using the Kjeldahl method and boron digestion by dry method (incineration), according to the methodology proposed by Malavolta, Vitti e Oliveira (1997).

The experimental was a completely randomized design, with 4 treatments and 15

repetitions, totaling 60 plants. The data were subjected to variance analysis (ANOVA) and the means compared by the Tukey test ($p \leq 0.05$). Pearson's linear correlation analysis ($p \leq 0.01$) and ($p \leq 0.05$) was performed between the SPAD, chlorophyll and N contents in leaves. The statistical analyses were performed using the software Sisvar 5.3 Statistical Software (FERREIRA, 2011).

3 RESULTS AND DISCUSSION

3.1 Nutritional contents

The treatment with the highest dose of SS (90 g L⁻¹) shown the highest concentration of N in leaves (Table 3). SS has high N content in its composition (2.8%); thus, can be used as an N source for *C. estrellensis* seedlings. Variations in N levels in plants will depend on each species, but in general, the appropriate concentration can vary between 20 to 50 g kg⁻¹ of dry matter (KERBAUY, 2008). P contents were not significant for treatments with SS (30, 60 and 90 g L⁻¹), with the highest average

observed in the Control treatment. Despite the high concentration of P in the residue, the nutrient is made available to plants slowly, over a longer period of time compared to mineral fertilizers; due to nutrients being in organic forms, dependent on mineralization to become available (LU; HE; STOFFELLA, 2012). K was the second nutrient with highest uptake by *C. estrellensis* (between 10 and 17 g kg⁻¹ in the leaves). K is lost along with the effluent during the wastewater separation process; thus, potassium must be supplemented in SS. The highest Ca and Mg uptake was observed in treatments 60 g L⁻¹ and 30 g L⁻¹ SS, respectively. Ca and Mg are present in the inorganic SS form; therefore, small amounts of the residue can supply the demand for these nutrients in several cultures (TSUTIYA, 2001). S was the lowest nutrient content in the absorption content in *C. estrellensis* leaves, although SS contains approximately 0.4% S. Thus, the decreasing order of the content of macronutrients evaluated in the leaves of *C. estrellensis* was N > K > Ca > Mg > P > S.

Table 3. Concentration of macro and micronutrients in the leaves of *Cariniana estrellensis* submitted to different treatments with sewage sludge.

Treat (g L ⁻¹)	g kg ⁻¹						mg kg ⁻¹				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
0	13 ^C	2.8 ^A	10 ^B	10 ^{AB}	2.9 ^{AB}	2.0 ^A	52 ^A	9 ^A	316 ^A	35 ^D	18 ^A
30	14 ^C	1.7 ^B	16 ^A	10 ^{AB}	3.9 ^A	1.3 ^B	41 ^B	7 ^A	179 ^B	82 ^C	15 ^A
60	21 ^B	1.5 ^C	17 ^A	11 ^A	2.4 ^B	1.5 ^B	45 ^{AB}	7 ^A	185 ^B	174 ^A	17 ^A
90	23 ^A	1.5 ^C	16 ^A	9 ^B	2.4 ^B	1.9 ^A	46 ^{AB}	5 ^A	163 ^B	125 ^B	15 ^A

Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$). **Source:** Author (2020).

Higher concentrations of B in the leaves of *C. estrellensis* (52 mg kg⁻¹) were observed in the control treatment (Table 3), compared to the SS treatments (30, 60 or 90 g L⁻¹). This may occur not only due to the effect of diluting the nutrient on the plant, but also due to one element being able to stimulate or inhibit the absorption of another (antagonism between ions), which are of a physiological nature, during the absorption or translocation of the nutrients in the plants roots to shoots (MARSCHNER, 2012). No statistical differences were observed for Zn, which may be related to the availability of the micronutrient in the substrate, since several factors influence it,

such as: organic matter content, microorganic activity and cation exchange capacity (SOBRINHO et al., 2020) and usually, the highest levels are found in the roots compared to the shoots (MENGEL; KIRKBY, 2001). Cu had the lowest rates of micronutrients absorbed with averages ranging from 5 to 7 mg kg⁻¹. Fe had the highest levels (316 mg kg⁻¹) in the leaves of plants of the control treatment (0 g L⁻¹), with a decrease in concentration as the SS doses increased. SS has considerable levels of Fe in its composition (33,793 mg kg⁻¹), as well as the substrate used (9,133 mg kg⁻¹). Fe concentrations in leaves higher than 131 mg kg⁻¹ can be considered toxic (PREZOTTI;

MARTINS, 2013) plant toxicity symptoms were not observed in all treatments applied. The highest Mn content (174 mg kg^{-1}) was observed in plants that received the dose with 60 g L^{-1} SS. Plant species can differ in the appropriate leaf concentrations of Mn, varying from 30 to 500 mg kg^{-1} of dry mass, with deficiency levels below 20 mg kg^{-1} (MARSCHNER, 2012). Thus, the decreasing order of the micronutrient contents in the leaves of *Cariniana estrellensis* was $\text{Fe} > \text{Mn} > \text{B} > \text{Zn} > \text{Cu}$.

Plants in treatments 30 and 60 g L^{-1} SS showed higher leaf area (LA) than other treatments, with 416.73 and 403.24 cm^2 , respectively. *C. estrellensis* is a climax species and its initial development occurs in shaded areas; therefore, a larger LA may represent greater efficiency in intercepting sunlight. Higher LA of plants (average of 268 cm^2) fertilized with SS is probably produced by the higher content of nutrients present in the residue (SOUSA *et al.*, 2019). Reduced levels of macro-nutrients in *C. estrellensis* cause malformations in cell ultrastructures, such as changes in the quantity of chloroplasts, starch granules, and lipids, which hindered the formation of leaf lamina cells and tissues (ANDRADE; BOARETTO, 2020).

The recommended stem diameter (SD) for seedlings of forest species is between 5 and 10 mm (GONÇALVES *et al.*, 2000). In our study, the doses 30 and 60 g L^{-1} SS were within the recommended average, with 6.22 and 5.82 mm, respectively (Table 4). SD is an important index of good development and survival of the seedlings in the field, avoiding possible tipping that can cause death of the plant. The larger development of the stem diameter occurs in more mature growth phases of the plant

(RAVEN; EVERT; EICHHORN, 2001), especially in this case, a species with long-term growth. Plant height (H) of the plants of treatments with SS (Table 4) were higher than plants of control treatment (0 g L^{-1}), with 39.80 cm on treatment 60 g L^{-1} SS. A minimum of 25 cm in height is recommended for good development in the field of Atlantic Forest tree species (MORAES *et al.*, 2013). Higher production of dry biomass was observed in plants fertilized with 30, 60 or 90 g L^{-1} SS (Table 4). The highest dry leaf mass was observed with the application of 30 and 60 g L^{-1} SS. Doses higher than 60% SS in the composition of substrate stimulate the production of leaves dry mass of seedlings of *Plathymenia reticulata* (SIQUEIRA *et al.*, 2019) and *Tectona grandis* (TRAZZI *et al.*, 2014). The dose 30 g L^{-1} SS positively affected the stem dry mass and root fresh and dry mass. In contrast, higher doses (60 or 90 g L^{-1}) significantly reduced these variables. High doses of nutrients in addition to the appropriate concentration can reduce plant growth due to toxicity (TAIZ; ZEIGER, 2017). Corroborating the data, high doses of SS (above 60 Mg ha^{-1}) in soil had a negative effect on root growth of *Eucalyptus urograndis* (ZABOTTO *et al.*, 2020). Conversely, *Schinus terebinthifolius* produced higher dry mass of shoots and roots when cultivated in SS (no substrate) (ABREU *et al.*, 2017). *C. estrellensis* can reach up to 45 m in height (GATTI *et al.*, 2011), thus, root development is important not only to uptake water and nutrients from the soil, but also in the formation of a structural support system. The total dry mass showed higher values on treatments 30 and 60 g L^{-1} SS, with an average higher than 68% when compared to the control treatment (0 g L^{-1}).

Table 4. Leaf area (LF), height (H), leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM) and total dry mass (TDM) of *Cariniana estrellensis* submitted to different treatments with sewage sludge.

Treatments (g L ⁻¹)	LF cm ²	H cm	SD mm	LDM	SDM	RDM	TDM
----- g -----							
0	83.03 ^C	22.80 ^B	5.06 ^B	0.66 ^C	0.89 ^C	3.64 ^{AB}	5.20 ^C
30	416.73 ^A	38.25 ^A	6.22 ^A	2.98 ^A	1.98 ^A	4.60 ^A	9.58 ^A
60	403.25 ^A	39.80 ^A	5.82 ^A	3.19 ^A	1.77 ^{AB}	3.77 ^{AB}	8.74 ^A
90	271.34 ^B	35.40 ^A	4.93 ^B	2.00 ^B	1.38 ^{BC}	3.43 ^B	6.82 ^B

Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$). Source: Author (2020).

The dose 30 g L⁻¹ provided the higher values for the Dickson Quality Index (DQI) (1.40), and the control treatment (0 g L⁻¹) and 60 and 90 g L⁻¹ SS the lowest values (Table 5). DQI is calculated considering the robustness and balance of the plant's biomass distribution; thus, is a good indicator to evaluate the quality of seedlings (DELARMELINA *et al.*, 2013). Seedlings of *C. estrellensis* have a higher DQI value when fertilized with ammonium nitrate and ammonium sulfate, with seedlings with higher quality and development (GOULART *et al.*, 2021). The ratio between height and stem diameter (H/SD) was positively affected with the application of treatments with SS (Table 5). This relationship suggests a balance in plant

growth, and the value between 5.4 to 8.1 is considered ideal (CARNEIRO, 1995). Plants of the treatments that received the different doses of SS were within the indicated range, with the highest average (7.20) observed in plants fertilized with 90 g L⁻¹ SS. The relationship between the shoot and root dry mass (DMAP/R) showed a higher average (1.40) on treatment 60 g L⁻¹ SS (Table 5). The control treatment (0 g L⁻¹) showed the lowest values for DMAP/R. This index shows the adequate distribution between the shoots and the roots, being lower in the case of nutritional deficiency and indexes around 2.0 must be considered (CALDEIRA *et al.*, 2008).

Table 5. Dickson quality index (DQI), height/stem diameter ratio (H/SD) and dry mass of shoots/roots (DMAP/R) of *Cariniana estrellensis* submitted to different treatments with sewage sludge.

Treatments (g L ⁻¹)	DQI	H/SD	DMAP/R
0	0.96 ^B	4.52 ^C	0.44 ^C
30	1.40 ^A	6.15 ^B	1.08 ^{AB}
60	0.97 ^B	6.86 ^{AB}	1.40 ^A
90	0.88 ^B	7.20 ^A	1.01 ^B

Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$). Source: Author (2020).

3.2 Physiological factors

Chlorophyll *a* were higher in SS treatments compared to control treatment (0 g L⁻¹) (Table 6). Chlorophyll *b*, carotenoids and total chlorophyll contents were higher in plants treated with 60 g L⁻¹ SS (Table 6). High concentrations of chlorophyll can lead to higher photosynthetic parameters of plants (OLIVEIRA *et al.*, 2019), as it has a direct

relationship with the absorption and transfer of light energy (RÊGO; POSSAMAI, 2004). The SPAD index reached 53.46 on treatment 60 g L⁻¹ SS. A similar result was obtained in *Eucalyptus grandis* seedlings with the application of SS biochar, with SPAD indexes above 40 after 30 DAT (GONZAGA *et al.*, 2018). The SPAD index can indirectly estimate the amount of nitrogen present in the leaves, since most of the nitrogen is absorbed by plants

(between 50 and 70 %) (CHAPMAN; BARRETO, 1997) and SPAD indices lower than 40 may indicate N deficiency. In the present study, the SPAD indexes were positively correlated with N, chlorophyll *a*, chlorophyll *b* ($p \leq 0.05$) and total chlorophyll ($p \leq 0.01$) in leaves (table 7). Gas exchange (Table 6) showed higher values in plants fertilized with SS, except for the internal carbon concentration (*Ci*), which obtained the higher values in plants not fertilized (0 g L^{-1}). Plants on the treatment 60 g L^{-1} SS had the highest values liquid photosynthesis (*A*), stomatal conductance (*gs*) and transpiration (*E*). Fertilization with 60 g L^{-1} favored CO_2 assimilation, which were 254% higher than the plants not fertilized (0 g L^{-1}). The management of fertilization, with adequate levels of nutrients can favor different

mechanisms of plant growth, such as photosynthetic performance (MADEIRA *et al.*, 2001). The lowest rates of gas exchange, except for (*E*), were obtained with no fertilization (0 g L^{-1}) and 90 g L^{-1} SS. Commonly, low values of (*A*), *gs* and (*E*) are observed in experiments with plants subjected to water stress, showing the sensitivity of plants to lack of water in the soil, with implications for plant growth (GHOLZ, EWEL, TESKEY, 1990). Sewage sludge has a high-water retention capacity, due to its microporosity and high contents of organic matter which improves water availability to plants (ABAURRE *et al.*, 2021). However, the lack of nutrients also leads to lower gas exchange rates, as observed in plants not fertilized (0 g L^{-1}).

Table 6. SPAD, chlorophyll *a* (Ch *a*), chlorophyll *b* (Ch *b*), carotenoids (Carot), total chlorophyll (Total Ch), liquid photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*) and internal carbon concentration (*Ci*) of *Cariniana estrellensis* submitted to different treatments with sewage sludge.

Treat (g L^{-1})	SPAD	Ch <i>a</i>	Ch <i>b</i>	Carot	Total Ch	(<i>A</i>)	<i>gs</i>	(<i>E</i>)	<i>Ci</i>
0	22.5 ^D	7.0 ^B	2.8 ^B	2.0 ^C	9.8 ^C	4.9 ^D	0.079 ^{AB}	1.9 ^B	277 ^A
30	38.6 ^C	12.4 ^A	4.1 ^{AB}	2.8 ^B	16.4 ^B	11.0 ^B	0.093 ^A	2.4 ^A	187 ^B
60	53.5 ^A	14.4 ^A	8.9 ^A	3.9 ^A	23.2 ^A	12.5 ^A	0.099 ^A	2.6 ^A	174 ^B
90	47.9 ^B	14.7 ^A	5.9 ^{AB}	3.5 ^{AB}	20.6 ^{AB}	7.6 ^C	0.071 ^B	1.8 ^B	185 ^B

Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$). **Source:** Author (2020).

Table 7. Pearson's simple correlation analysis (*r*) between chlorophyll *a* (Ch *a*), chlorophyll *b* (Ch *b*), and total chlorophyll (Total Ch), accumulated N content in leaves and SPAD index of *Cariniana estrellensis* submitted to different treatments with sewage sludge.

	Ch <i>a</i>	Ch <i>b</i>	Total Ch	Nitrogen
Chlorophyll <i>a</i>	-	-	-	-
Chlorophyll <i>b</i>	0.4186 ^{ns}	-	-	-
Total Chlorophyll	0.8705**	0.8114**	-	-
Nitrogen	0.7595**	0.6095*	0.8191**	-
SPAD	0.8658**	0.6912*	0.9318**	0.8558**

ns - not significant, * - significant at 5%, ** - significant at 1%. **Source:** Author (2020).

The electrical conductivity (EC) increased gradually as the concentrations of SS increase (Table 8). The maximum value 2.25 dS.m^{-1} was observed on plants fertilized with 90 g L^{-1} SS. Factors such as the level of salinity, texture, water content and chemical properties can influence electrical conductivity (MOLIN; RABELLO, 2011). Substrates with values

above 1.0 dS.m^{-1} may be inappropriate for seedling production of native forest species, due to the high salinity (GONÇALVES *et al.*, 2000). However, some forest species have shown to be resistant to the high levels of electrical conductivity of substrates formulated with SS (SOUSA *et al.*, 2019). In the present study, root development was reduced on lants

fertilized with the highest dose of SS (90 g L⁻¹), which may be related to the high electrical conductivity of the substrate (KÄMPF, 2005). On the other hand, substrate with electrical conductivity higher than 9.3 dS.m⁻¹ composed with SS for seedling production of *Pinus pinea*, *Cupressus arizonica* and *Cupressus Sempervirens* were adequate for plant development (HERNÁNDEZ-APAOLAZA *et*

al., 2005). In general, even if mineral fertilizers provide nutrients to plants quickly, recycling SS enables the addition of considerable levels of nutrients and organic matter to the substrate with positive impact on plant growth. Waste recycling is of great importance, both from an economic and environmental point of view, with possibilities for cost reduction throughout the plant production chain.

Table 8. Electrical conductivity (EC) of *Cariniana estrellensis* substrates submitted to different treatments with sewage sludge.

Treatments (g L ⁻¹)	EC (dS.m ⁻¹)
0	0.62 ^D
30	1.24 ^C
60	1.72 ^B
90	2.25 ^A

4 CONCLUSIONS

The incorporation of SS on the substrate favored the growth and development of the *Cariniana estrellensis* seedlings and highlighted the fertilizer potential of the residue. Concentrations above 60 g L⁻¹ caused negative effects on the root biomass of the seedlings. The concentration 30 g L⁻¹ SS is recommended for seedlings production of *C. estrellensis* for urban afforestation.

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