



## Nutritional diagnosis and seedling quality of *Psidium cattleianum* var. *cattleianum* Sabine in different substrates<sup>1</sup>

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**Abstract:** The objective of the research was to evaluate the effect of different substrates with sewage sludge on nutrients concentration in biomass of *Psidium cattleianum* var. *cattleianum* Sabine seedlings. The treatments are different substrate compositions containing sewage sludge. After 180 days of seeding, biometric characteristics were measured: aerial part height, root system length, stem base diameter, number of leaves, fresh and dry aerial biomass, root and total biomass. Dickson's Quality Index was calculated and nutrients concentration was determined in root system, stem and leaves. It was observed that macronutrients accumulation in root followed the order: N > Ca > Mg > P > K > S; in stem: Ca > N > K > Mg > P > S and in leaves: N > Ca > K > Mg > P > S, where sulfur was less concentrated in three vegetative structures. Micronutrients accumulation in root followed the order: Fe > Zn > Mn > Cu > B > Mo, and in stem and leaves: Fe > Zn > B > Mn > Cu > Mo. It is noted that in three vegetative structures, iron and zinc were more concentrated, and molybdenum less concentrated. Sewage sludge use as a substrate proved to be effective in viable seedlings production for planting in field.

**Keywords:** Nutrients; Forest nutrition; Red strawberry-guavas

## Diagnose nutricional e qualidade de mudas de *Psidium cattleianum* var. *cattleianum* Sabine em diferentes substratos

**Resumo:** O objetivo da pesquisa foi avaliar o efeito de diferentes substratos, que continham lodo de esgoto, no teor de nutrientes na biomassa de mudas de *Psidium cattleianum* var. *cattleianum* Sabine. Os tratamentos consistiram em diferentes composições de substrato contendo lodo de esgoto. Decorridos 180 dias após a semeadura foram mensuradas as características biométricas: altura da parte aérea e comprimento do sistema radicular, diâmetro do colo, número de folhas, biomassa fresca e seca da parte aérea, biomassa radicular e total. Foi calculado o Índice de Qualidade de Dickson e também foi quantificado o teor de macro e micronutrientes do sistema radicular, do caule e das folhas. Observou-se que o acúmulo dos macronutrientes no sistema radicular seguiu a ordem de N > Ca > Mg > P > K > S; no caule Ca > N > K > Mg > P > S e nas folhas N > Ca > K > Mg > P > S, sendo que nas três estruturas vegetativas o enxofre esteve menos concentrado. Para os micronutrientes, o acúmulo no sistema radicular se deu com Fe > Zn > Mn > Cu > B > Mo, no caule e nas folhas Fe > Zn > B > Mn > Cu > Mo. Nota-se que nas três estruturas vegetativas analisadas, ferro e zinco estiveram mais concentrados e molibdênio menos concentrado. O uso de lodo de esgoto como substrato se mostrou efetivo na produção de mudas viáveis para plantio em campo.

**Palavras – chave:** Nutrientes; Nutrição florestal; Araçá-vermelho

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## Introduction

Seedling quality is one of the most important factors in forest stands implantation for wood production or mixed stands for environmental preservation and/or degraded areas restoration (GONÇALVES et al., 2014). Among the factors that contribute to quality seedlings production, there are the substrates. However, diversity of options for this material is great, and there is no perfect substrate for all conditions and species. Thus, it is preferable to use substrate components in mixture form, because when isolated can favor, or not, the seedling development (WENDLING and GATTO, 2002).

One of the organic compounds possible to be used in substrate formulation for seedlings production is sewage sludge (SS), whose production has been increasing due to high demographic concentration and urbanization expansion, which implies a greater generation of this residue that is sent to sewage treatment stations. SS is a material that, depending on its final destination, can generate ecological and sanitary problems, given that most of this residue generated in Brazil is still deposited in landfills which, in addition to high costs, can cause social and environmental problems (PÉREZ et al., 2011). Among alternatives for SS final disposal, agricultural and forestry purposes presents itself as one of most convenient, since SS is an organic material that contains essential nutrients for plants, it is recommended its application as a soil conditioner and/or fertilizer (CAMARGO et al., 2013).

The SS use in agriculture as organic fertilizer is currently seen as the most promising alternative for final disposal of this residue. In experiments carried out by Campos and Alves (2008) in *Eucalyptus* silviculture, SS has potential to replace mineral fertilizers. Faustino et al. (2005), proved the viability of SS as a substrate component for *Senna siamea* Lam seedlings production, concluding that SS use can be a viable alternative for its final disposal, in addition to be used for seedlings

production for urban afforestation and degraded areas recovery.

The specie red strawberry-guavas (*Psidium cattleianum* var. *cattleianum* Sabine) has a potential to form mixed or pure stands for wood production, environmental preservation and/or degraded areas restoration, using SS, *Psidium cattleianum* var. *cattleianum* belonging to Myrtaceae family, occurrence an extensive area on Brazilian Atlantic coast, from Bahia state to northeast of Uruguay (MARCHIORI and SOBRAL, 1997). This species can be successfully implanted for erosion control, slopes and dunes containment and, along channels, highways, railways, and as a hedge in internal divisions or limits of rural properties. The objective of the research were to evaluate the sewage sludge (SS) potential as an organic material and nutrients source for red strawberry-guavas (*Psidium cattleianum* var. *cattleianum* Sabine) seedlings production and nutrient content analyze in seedlings biomass.

## Material and methods

The experiment was conducted in a greenhouse with 256 m<sup>2</sup> area (8 m width x 32 m length x 4 m height), covered with 100 µm low density polyethylene (PeBD), 50% shade and automatic micro sprinkler irrigation, located at *Universidade Federal do Pampa - São Gabriel* campus (30°20'11" S and 54°19'11" W of central geographical coordinates and 114 m of altitude), São Gabriel, Rio Grande do Sul state, Brazil.

*P. cattleianum* var. *cattleianum* fruits were collected in São Gabriel, Rio Grande do Sul state, Brazil. Later, they were taken to Laboratory of Botany at *Universidade Federal do Pampa - São Gabriel* campus, where they were manually pulped, followed by washing in a mesh under current water, for separated the seeds from fruits, with seeds being placed to dry in shade and paper filter, eliminating immature, damaged or damaged seeds by insects.



The SS used was obtained from *Estação de Tratamento de Esgoto São Gabriel Saneamento*, located in São Gabriel, Rio Grande do Sul state, Brazil, and they were previously cleaned by solarization process, during 40 days. This process results in biosolid production with a better sanitary profile, in order to promote prior disinfection and disinfestation of pathogens and, less restrictions for agricultural use (FAUSTINO et al., 2005; CALDEIRA et al., 2014).

Treatments were composed by T1 = 50% of commercial substrate Plantmax® + 50% of

organic compost from horse bedding (OC), T2 = 20% of sewage sludge (SS) + 80% OC, T3 = 40% SS + 60% OC, and T4 = 60% SS + 40% OC. Before seeding, in each treatment were contents analyzed of nitrogen (N), sulfur (S), calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), zinc (Zn), copper (Cu), boron (B), iron (Fe), manganese (Mn) and sodium (Na), in Soil Analysis Laboratory of *Universidade Federal de Santa Maria*, Santa Maria, Rio Grande do Sul state, Brazil (Table 1).

**Table 1** - Nutrients concentrations on substrates used for *Psidium cattleianum* var. *cattleianum* Sabine seeding.

**Tabela 1** - Teores de nutrientes nos substratos utilizados para sementeira de *Psidium cattleianum* var. *cattleianum* Sabine.

	N	Ca	Mg	K	P	Zn	Cu	S	B	Fe	Mn	Na
	%	$cmol_c L^{-1}$				$mg L^{-1}$						
<b>T1</b>	1.00	10.64	7.48	628.32	383.57	24.49	0.34	79.05	0.13	2,907.50	10.87	88.78
<b>T2</b>	1.19	12.61	7.81	564.36	309.02	43.72	3.61	82.22	0.18	6,343.74	19.05	72.69
<b>T3</b>	1.37	8.40	4.67	360.45	309.09	61.11	17.48	94.91	0.27	8,697.52	18.91	44.58
<b>T4</b>	1.46	7.71	3.86	312.08	309.08	61.30	22.13	87.37	0.12	10,792.40	22.18	36.75

Where: Treatments T1 = 50% of commercial substrate Plantmax® + 50% of organic compost from horse bedding (OC), T2 = 20% of sewage sludge (SS) + 80% OC, T3 = 40% SS + 60% OC, and T4 = 60% SS + 40% OC.

Seeding was carried out in polyethylene tubes with 50 cells of 200 cm<sup>3</sup> each, containing one seed per tube, arranged on metal benches suspended at 100 cm from the soil level. Irrigation was carried out daily by system above mentioned, for substrates humidity maintaining, contributing to germination, and later seedlings emergence.

The experiment was a completely randomized design, with four treatments (T1, T2, T3 and T4) with four repetitions for each treatment, and 50 repetitions each. After 180 days of seeding, seedlings biometric characteristics were measured: aerial part height (H) and root system length (RSL), stem base diameter (SBD), and number of leaves (NL),

counted manually. After measurements, seedlings were collected, fractionated in aerial part (DMAP) and root system (DMRS), and submitted to drying in an oven with air circulation at 60 °C, until reaching a constant dry mass, which was verified after, approximately, 72 hours. After drying, material was processed in a Willey mill with 1.70 mm mesh. The Dickson Quality Index (DQI) was calculated by the formula  $DQI = (TDM)/((H)/(SBD) + (DMAP)/(DMRS))$ , were: DQI = Dickson Quality Index; TDM = total dry mass (g); H = height (cm); SBD = stem base diameter (mm); DMAP = dry mass of aerial part (g); DMRS = dry mass of root system (g) (DICKSON, LEAF and HOSNER, 1960).

In the soil analysis laboratory of the *Universidade Federal do Rio Grande do Sul*, according to the methodology of Tedesco et al, (1995) and Miyasawa, (1999), the concentrations of macro and micronutrients in the roots and aerial part of the plants were determined.

After data variance analysis, when F test was significant, means were compared by Tukey test at 1% level of error probability, using ESTAT version 2 statistical software (ESTAT, 1994).

## Results

### Combined SS use in different proportions

influenced *P. cattleianum* var. *cattleianum* seedlings growth. SS treatments, especially T3 and T4, were significantly superior when compared to T1 (Table 2).

Among four biometric characteristics analyzed in seedlings (number of leaves, stem base diameter, height and root system length), except for root system, SS addition to substrates provided better results, especially in highest proportions (with 40 and 60% SS added to OC) (Table 2). Seedlings height was higher in T3 and T4 treatments, as demonstrated for number of leaves. In T1 treatment is a lower seedlings height, followed by T2. It was verified that seedlings height in treatments with SS added (T2, T3 and T4) were two to three times higher when compared to T1 (Table 2).

**Table 2** – Number of leaves (NL), aerial part height (H), root system length (RSL), stem base diameter (SBD), height/diameter ratio (H/SBD), Dickson Quality Index (DQI) of *Psidium cattleianum* var. *cattleianum* Sabine seedlings on different substrates.

**Tabela 2** - Número de folhas (NL), altura da parte aérea (H), comprimento do sistema radicular (RSL), diâmetro do colo (SBD), relação altura/diâmetro (H/SBD), Índice de Qualidade de Dickson (DQI) de mudas de *Psidium cattleianum* var. *cattleianum* Sabine cultivadas em diferentes substratos.

Treat.	NF	SBD (mm)	H (cm)	RSL (cm)	H/SBD	DQI
T1	18.50±0.70 b*	1.69±0.13 c	11.54±0.72 c	9.41±0.34 a	6.82±0.81 c	5.90±1.17 c
T2	19.41±0.89 b	3.02±0.18 b	25.61±2.81 b	9.78±1.60 a	8.48±1.03 b	14.06±1.86 b
T3	21.77±1.13 a	3.63±0.06 a	33.51±1.24 a	9.68±1.24 a	9.23±0.44 ab	19.37±0.99 a
T4	21.00±0.50 a	3.55±0.14 a	32.58±1.23 a	9.59±0.22 a	9.17±0.68 a	15.39± 0.80 b
<i>F value</i>	15.19	179.00	167.40	2.80	14.89	122.47
<i>P value</i>	6.0857 x 10 <sup>-5</sup>	1.6182 x 10 <sup>-12</sup>	2.7193 x 10 <sup>-12</sup>	0.0731	6.8159 x 10 <sup>-5</sup>	3.0118 x 10 <sup>-11</sup>
C.V. (%)	4.45	5.16	6.19	3.29	4.86	10.10

Where: Treatments T1 = 50% of commercial substrate Plantmax® + 50% of organic compost from horse bedding (OC), T2 = 20% of sewage sludge (SS) + 80% OC, T3 = 40% SS + 60% OC, and T4 = 60% SS + 40% OC; \*Means ± standard deviation followed by same letters in columns do not differ significantly by Tukey test at 1% level of error probability. C.V. = coefficient of variation.

Stem base diameter was another biometric characteristic that showed same growth behavior, when using 40% and 60% of SS in substrate, diameter values were 3.63 mm and 3.55 mm, respectively; however, 3.02 mm diameter average was also found in T2 (Table

2). Thus, regardless of SS proportion, stem base diameter was a satisfactory result for *P. cattleianum* var. *cattleianum* seedlings quality and, were observed in treatments with organic residue. Relation between height and stem base diameter (H/SBD), which is a characteristic that



seedlings quality expresses at any growth phase, showed values varying from 8.54 to 9.99 in treatments with SS addition (Table 2), when compared to treatment without this organic residue.

Dickson Quality Index (DQI) considers seedlings morphological characteristics and, when showed a higher value (Table 2), seedling quality standard is better. Treatments formulated with SS provided highest averages, especially in T3, with no statistical differences between T2 and T4, with lowest values being evident in treatment without adding this organic residue (T1).

In root system length (Table 2) there were no differences between treatments, however, there were differences of this biomass (Table 3). This can demonstrate that SS did not contribute to

root length growth, but possibly contributed to number of roots increase, with addition of fresh and dry masses of this organ.

Results for number of leaves, stem base diameter, height (Table 2) and biomass (dry mass of root system, dry mass of aerial part and total dry mass) and respective fresh masses of two vegetative structures observed (Table 3), showed that treatments that contained SS positively influenced seedlings growth and biomass production. It was observed that results promoted by seedlings cultivation on substrate formulated with SS were significantly superior when compared to T1 treatment. In these treatments, highest values of height, stem base diameter and seedling biomass are probably related to greater nutrients availability in these substrates (Table 1).

**Table 3** - Biomass of *Psidium cattleianum* var. *cattleianum* Sabine seedlings planting on different substrates.

**Tabela 3** - Biomassa de mudas de *Psidium cattleianum* var. *cattleianum* Sabine cultivadas em diferentes substratos.

Treat.	FMAP (g)	FMRS (g)	TFM (g)	DMAP (g)	DMRS (g)	TDM (g)
T1	47.22±5.08 d*	38.73±5.86 c	85.96±10.49 c	26.17±2.51 d	21.32±2.87 c	47.49±5.31 d
T2	160.89±11.38 c	110.93±6.66 b	267.82±6.90 b	83.97±6.17 c	56.25±6.39 b	140.23±11.50 c
T3	249.09±5.91 a	150.36±2.86 a	403.46±13.80 a	130.10±5.51 a	80.01±4.42 a	210.18±2.25 a
T4	220.71±7.66 b	107.46±7.55 b	290.00±15.48 b	114.75±4.08 b	57.18±2.82 b	171.93±4.10 b
<i>F</i> value	641.85	99.4419	35.0448	460.92	152.68	528.65
<i>P</i> value	7.0741 x 10 <sup>-17</sup>	1.4703 x 10 <sup>-10</sup>	2.9055 x 10 <sup>-7</sup>	9.7342 x 10 <sup>-16</sup>	5.5370 x 10 <sup>-12</sup>	3.2912 x 10 <sup>-16</sup>
C.V. (%)	17.58	10.50	14.09	6.19	8.83	5.87

Where: Treatments T1 = 50% of commercial substrate Plantmax® + 50% of organic compost from horse bedding (OC), T2 = 20% of sewage sludge (SS) + 80% OC, T3 = 40% SS + 60% OC, and T4 = 60% SS + 40% OC; \*Means ± standard deviation followed by same letters in columns do not differ significantly by Tukey test at 1% level of error probability. C.V. = coefficient of variation; FMAP = fresh mass of aerial part; FMRS = fresh mass of root system; TFM = total fresh mass; DMAP = dry mass of aerial part; DMRS = dry mass of root system; TDM = total dry mass.

Regarding dry biomass of aerial part, dry biomass of root system and total dry mass (Table 3), it was found that *P. cattleianum* var. *cattleianum* seedlings produced with treatments with SS application, differed significantly from treatment without this organic residue. This result is a consequence of higher values in number of leaves, height and stem base diameter of seedlings in treatments with SS.

Seedlings that showed adequate parameters of number of leaves, height, stem base diameter and biomass were grown in treatments with SS, which have the highest nutrients concentration in substrate (Table 1) and, consequently, there was a higher macro and micronutrients concentration in seedlings vegetative parts (Table 4).

Highest N, Ca and S concentrations in

leaves, stem and root of seedlings were observed in treatments with SS application and, for Mg and K in three vegetative structures, there was a nutrients concentration reduction in treatment with SS addition, when compared to T1 (OC) (Table 4). As for P, it was observed in Table 4 that concentration, in three vegetative structures, were not influenced by SS

increasing addition, with only a small increase in root.

Mg concentration was increase as a function of SS doses, which occurred within a short range, which was also verified for S (Table 4). This tendency was not observed for K, with a decrease in total concentration when different SS doses were added (Table 4).

**Table 4** - Macronutrients concentrations in leaves, stem and roots of *Psidium cattleianum* var. *cattleianum* Sabine seedlings planting on different substrates.

**Tabela 4** - Concentração de macronutrientes nas folhas, caule e raízes de mudas de *Psidium cattleianum* var. *cattleianum* Sabine cultivadas em diferentes substratos.

		N	P	K	Ca	Mg	S
		<i>g kg<sup>-1</sup></i>					
Leaves	T1	8.1 ± 0.01cB*	2.7 ± 0.10abA	13.0 ± 0.02aA	9.2 ± 0.06bA	2.3 ± 0.05bC	0.9 ± 0.01bB
	T2	8.5 ± 0.11cB	3.4 ± 0.08aA	7.9 ± 0.10bA	13.0 ± 0.03aA	3.4 ± 0.08aB	1.1 ± 0.07bB
	T3	11.0 ± 0.00bA	2.5 ± 0.12abAB	4.7 ± 0.11cA	13.5 ± 0.10aA	3.2 ± 0.01abB	1.3 ± 0.03abB
	T4	14.0 ± 0.08aA	2.1 ± 0.05bB	4.3 ± 0.07cA	14.0 ± 0.11aA	3.2 ± 0.00abB	1.6 ± 0.00aB
Stem	T1	5.0 ± 0.12bC	1.9 ± 0.08aB	9.2 ± 0.00aB	8.5 ± 0.11bB	3.1 ± 0.10aB	0.7 ± 0.01aB
	T2	5.1 ± 0.18bC	2.3 ± 0.01aB	7.1 ± 0.03bB	11.0 ± 0.10aB	3.7 ± 0.11aB	0.7 ± 0.00aB
	T3	5.9 ± 0.08bB	2.0 ± 0.07aB	3.7 ± 0.01cB	11.0 ± 0.08aB	3.4 ± 0.08aB	0.8 ± 0.02aB
	T4	8.8 ± 0.11aB	2.2 ± 0.06aB	3.5 ± 0.05cAB	12.0 ± 0.10aB	3.3 ± 0.21aB	1.0 ± 0.01aB
Roots	T1	8.8 ± 0.04cA	2.9 ± 0.01bcA	8.8 ± 0.10aB	5.4 ± 0.06bC	7.6 ± 0.08aA	1.7 ± 0.00bA
	T2	9.9 ± 0.01bcA	2.6 ± 0.06cB	3.1 ± 0.13bC	5.4 ± 0.02bC	6.3 ± 0.07bA	1.9 ± 0.01bA
	T3	11.0 ± 0.05abA	3.3 ± 0.02abA	3.0 ± 0.11bB	6.7 ± 0.01aC	6.7 ± 0.10bA	2.6 ± 0.01aA
	T4	15.0 ± 0.08aA	3.9 ± 0.00aA	3.0 ± 0.10bB	7.1 ± 0.01aC	5.4 ± 0.06cA	2.8 ± 0.03aA

Where: Treatments T1 = 50% of commercial substrate Plantmax® + 50% of organic compost from horse bedding (OC), T2 = 20% of sewage sludge (SS) + 80% OC, T3 = 40% SS + 60% OC, and T4 = 60% SS + 40% OC; \*Means ± standard deviation followed by same lowercase letters in columns within treatments and uppercase letters for organ between treatments do not differ significantly by Tukey test at 1% level of error probability.

Macronutrients concentration in leaves followed the decreasing order: N > Ca > K > Mg > P > S; in stem: Ca > N > K > Mg > P > S and root system: N > Ca > Mg > P > K > S, being that, in three vegetative structures S was less concentrated and, N was highest content in leaves and root system, (Table 4). Thus, it was found that SS addition to substrate, in different proportions, significantly influenced nutrients

accumulation in three vegetative structures, except for P in leaves, K in three vegetative structures and, Mg in root. Considering three vegetative structures, there was a higher concentration of Mg and S in root system, K and Ca in leaves and N and P in leaves and roots (Table 4).

Micronutrient concentrations differed between vegetative parts, except for Mo. With



different concentrations of SS addition to substrate, B concentration differed between the three vegetative structures, with reduction of B concentration in treatments with SS, when

compared to T1 and, in treatments with SS addition; higher concentration of B was found in T2, regardless of vegetative structure (Table 5).

**Table 5** - Micronutrients concentrations in leaves, stem and roots of *Psidium cattleianum* var. *cattleianum* Sabine seedlings planting on different substrates.

**Tabela 5** - Teores de micronutrientes nas folhas, caule e raízes de mudas de *Psidium cattleianum* var. *cattleianum* Sabine cultivadas em diferentes substratos.

		Cu	Zn	Fe	Mn	Mo	B
		mg kg <sup>-1</sup>					
Leaves	T1	4 ± 0.01cC	27 ± 0,11dC	246 ± 0.12aB	20 ± 0.08cB	2 ± 0.02bA	43 ± 0.06aA
	T2	6 ± 0.08bB	34 ± 0,10cB	113 ± 0.10bC	17 ± 0.10dB	3 ± 0.01abA	35 ± 0.05bA
	T3	7 ± 0.06abB	43 ± 0,10bC	89 ± 0.11cC	23 ± 0.08bB	4 ± 0.00aA	24 ± 0.01cA
	T4	8 ± 0.06aB	107 ± 0,12aC	58 ± 0.10dC	36 ± 0.10aB	3 ± 0.01abA	21 ± 0.00dA
Stem	T1	8 ± 0.01aB	54 ± 0.11bB	830 ± 0.10aA	17 ± 0.08aC	2 ± 0.00aA	21 ± 0.10aB
	T2	7 ± 0.03aB	34 ± 0.08cB	133 ± 0.08bB	8 ± 0.10bC	2 ± 0.01aA	17 ± 0.11bB
	T3	7 ± 0.00aB	57 ± 0.10bB	99 ± 0.11cB	8 ± 0.06bC	2 ± 0.00aB	12 ± 0.13cB
	T4	7 ± 0.01aB	135 ± 0.10aA	65 ± 0.10dB	8 ± 0.08bC	2 ± 0.01aA	12 ± 0.09cB
Roots	T1	16 ± 0.12dA	219 ± 0.11cA	200 ± 0.12dA	135 ± 0.12cA	2 ± 0.00aA	11 ± 0.01aC
	T2	35 ± 0.10cA	446 ± 0.10aA	330 ± 0.10cA	117 ± 0.10dA	2 ± 0.01aA	10 ± 0.00aC
	T3	50 ± 0.08bA	397 ± 0.03bA	630 ± 0.10bA	167 ± 0.10bA	2 ± 0.01aB	8 ± 0.02bC
	T4	86 ± 0.10aA	120 ± 0.05dB	1200 ± 0.10aA	189 ± 0.10aA	2 ± 0.01aA	8 ± 0.01bC

Where: Treatments T1 = 50% of commercial substrate Plantmax® + 50% of organic compost from horse bedding (OC), T2 = 20% of sewage sludge (SS) + 80% OC, T3 = 40% SS + 60% OC, and T4 = 60% SS + 40% OC; \*Means ± standard deviation followed by same lowercase letters in columns within treatments and uppercase letters for organ between treatments do not differ significantly by Tukey test at 1% level of error probability.

Contrary to what was observed for B, Cu and Mn concentrations differed between leaves and roots, when compared to stem, and with SS addition there was an increase in these micronutrients in biomass, except in stem. When SS was added to substrate, Fe concentration in roots increased, with micronutrient higher concentrations found in vegetative structures (Table 5).

In Table 5, higher Zn concentration can be seen in stem and leaves of *P. cattleianum* var. *cattleianum* seedlings as there is an increase in proportions of SS to substrate, whereas in roots this behavior was not observed. Zn

concentration in substrates was higher in treatments that SS contained and there was a gradual increase in Zn concentration in three different dosages. In roots there were highest micronutrient concentrations, except for B, which was more concentrated in leaves (Table 5).

Micronutrients concentration in leaves and stem followed the decreasing order: Fe > Zn > B > Mn > Cu > Mo and in root system: Fe > Zn > Mn > Cu > B > Mo. It was noted that in three vegetative structures there were high Fe and Zn and, lowest Mo concentration (Table 5).

## Discussion

Biometric parameters of number of leaves, stem base diameter, height, except for root length, evidenced the effect of SS addition on seedling growth, providing rusticity and good quality for seeding. SS is organic residue and is an alternative to reduce costs in seedlings production, with partial replacement of commercial substrate and chemical fertilization reduction. This sustainable practice aim minimizes the environmental impact that would be caused by residue disposal, often from basic sanitation, inappropriately in nature, causing environmental pollution.

Kerbaux (2008), Araújo and Sobrinho (2011) and, Taiz and Zeiger (2017), emphasize that number of leaves is a factor entirely linked to plant development, since they are responsible organ for photosynthesis. Authors also relate out that leaves are reserve centers, auxin resource and rooting cofactors that are base translocate, thus contributing to new vegetative and reproductive organs formation. Such information agrees with present study, indicating higher values of height, stem base diameter and number of leaves of *P. cattleianum* seedlings with SS addition to substrate.

Seedling height is a parameter that provides an excellent estimate of initial growth prediction after seeding in field, and is technically accepted as a good measure of seedling development potential (CAMPOS and ALVES, 2008; CALDEIRA et al., 2014). Is related by Gonçalves et al. (2014) that, to quality of forest species seedlings, are recommended height limits between 20 and 35 cm. Considering seedlings height, T2, T3 and T4 treatments are recommended for cultivation, as they were within that height range that was considered as a quality standard.

When SS was added to substrate for *Ateleia glazioveana* Baill cultivation at 90 days, aerial part height varied between 11.09 and 30.32 cm (CALDEIRA et al., 2012). For *Parapiptadenia rigida* (Benth.) Brenan cultivated in substrate

that SS contained same formulations of this study, there was a variation in aerial part height from 17.76 to 24.42 cm (SANTOS et al., 2019). Seedlings height observed in these studies is lower when compared to presented results here, however with *P. cattleianum* var. *cattleianum* seedlings height was 33.51 cm, after 180 days of cultivation (Table 2).

In addition to seedling height and number of leaves, SS provided satisfactory results for stem base diameter. This variable being considered as one of the most important characteristics for estimating the forest species seedlings survival in field, which can be considered separately or combined with height (GOMES and PAIVA, 2004). Thus, it was found that biometric variables values in this study are consistent with what was observed in other studies (ARAÚJO and SOBRINHO, 2011; CALDEIRA et al., 2012; SANTOS, KUNZ and CALDEIRA, 2013; GONÇALVES et al., 2014; SANTOS et al., 2019). Probably, satisfactory biometric parameters of seedlings (number of leaves, stem base diameter and height) obtained in treatments with SS addition are related to greater nutrients supplied availability to these substrates (Table 1).

Studies demonstrate that H/SBD ratio and DQI are variable characteristics (GOMES and PAIVA, 2004; CAMPOS and ALVES, 2008; CALDEIRA et al., 2012; TRAZZI, DELARMELINA and CALDEIRA, 2014). These parameters may vary depending on species, seedlings management in nursery, type and substrate formulation, container volume and, mainly, according to seedling age was evaluated (CALDEIRA et al., 2012). As it is a very variable characteristic, comparison between species and substrate formulations is not viable. For H/SBD it was found that values from 5.4 to 8.1 are in above-ideal range, according to Carneiro (1995).

Among biometric characteristics evaluated, root system length did not respond positively to SS added in OC and treatments did not differ. In studies that evaluate biometric characteristics as a quality parameter of plant species, it is emphasized that cultivation container is one



factor that influences growth of root (TRAZZI, DELARMELINA and CALDEIRA, 2014). However, although difference in root length was not observed, there was variation in root biomass (Table 3). Possibly, SS presence in treatments contributed to number of roots increase, which was corroborated by fresh and dry biomass increase of this organ, being an important characteristic for seedlings rooting after planting in field. This result was also observed in *Parapiptadenia rigida* seedlings, when using same SS proportions as in present study (SANTOS et al., 2019).

The SS addition to substrate influenced dry, root and total biomass production (Table 3) of *P. cattleianum* var. *cattleianum* seedlings. This result is a consequence of higher values of number of leaves, height and stem base diameter of seedlings grown on substrate where SS was added. Cruz et al. (2011) reported when was a higher value of total dry mass (TDM), better is seedling quality. Corroborating this information, Gomes and Paiva (2004) state that it is important to evaluate this characteristic, as it seedling rusticity indicates. That is, when TDM value is higher, seedlings are more rusted, which must be hardened at planting, that is, with greater biomass.

Additionally, studies have evaluated seedlings quality of forest species, where SS use, added in different proportions to substrates, resulted in better quality seedlings. The SS use can become an alternative as a substrate component in seedlings production, reducing fertilizers use and providing environmental benefit, due to residue reuse (CALDEIRA et al., 2014; TRAZZI, DELARMELINA and CALDEIRA, 2014). It should be noted that the seedlings with better results in height, stem base diameter and biomass were grown in treatments with SS addition. This can be explained by the fact that SS provides higher nutrients contents in substrate (Table 1) and, consequently, seedlings responded with greater nutrients accumulation, both in root and aerial part (Tables 4 and 5).

According to Schumacher et al. (2001), macronutrient concentration can be an nutrition

indication, due to different type of nutrients resources. This is evidenced in present study, where SS addition, in three different proportions to OC, provided an increase in N, Ca and S and a decrease in Mg and K concentration in three vegetative structures (Table 4). Caldeira et al. (2014) and Antunes et al. (2016) found a variation in nutritional efficiency of P, K, Mg and N in *Acacia mearnsii* De Wild., grown on different substrates with bovine and sheep manure addition, where macronutrient concentration, in general, were similar, or compared, to present study. Aforementioned authors emphasized that age and species factors influence the nutrients concentration in biomass, which, in a way, makes it difficult to results compare.

Camargo et al. (2013) analyzed macronutrient concentration in leaves of *Jatropha curcas* L. at 60 days, and found that N and S were in higher and lower concentrations, respectively, according to order  $N > K > Mg > Ca > P > S$ . Laviola and Dias (2008), evaluating the same species, observed that macronutrient concentration in leaves was similar to present study, with order  $N > Ca > K > Mg > P > S$ .

Fernández et al. (1994) stated that N supply increase in substrate stimulates seedling growth, delays senescence and changes plants morphology. In addition, higher levels of nitrogen fertilization cause a significant increase in chlorophyll concentration of leaves. According to Kerbauy (2008), N demand varies according to species and nutritional status and, for an adequate growth; N concentration is in range of 20 to 50 g kg<sup>-1</sup> of plant dry matter. In present study, for both leaves and root system, N was the nutrient in highest concentration (Table 4) and was also found in high concentrations in substrates with SS addition (Table 1).

The SS addition in different proportions to substrate significantly influenced the nutrients accumulation in three vegetative structures, except for P in leaves, K in total biomass and Mg in root. According to Kerbauy (2008), Ca demand by plants, for optimal growth, corresponds to a range of 10 to 50 g kg<sup>-1</sup>,

contents below the accumulated in seedlings biomass in all treatments (Table 4).

Except for B, all micronutrients were more concentrated in root, and Fe was more concentrated in biomass, in all treatments. According to Antunes et al. (2016), higher concentration of some micronutrients in vegetative parts, when adding biological sludge to substrate, may be due to availability of these nutrients, as these treatments showed high concentration in substrates (Table 1).

The B concentration decreased gradually with SS addition in substrate (Table 5), with T2 presenting the highest levels of this micronutrient. This result was not observed by Camargo et al. (2013), when SS using, as there was a gradual B concentration increase with SS proportion in substrate increase. However, in present study, when used 20% of SS ( $35 \text{ mg kg}^{-1}$ ), results differed from what was found by Camargo et al. (2013), where levels were  $28.33 \text{ mg kg}^{-1}$ , and by Laviola and Dias (2008), with levels of  $29.2 \text{ mg kg}^{-1}$ .

Additional effects produced by organic matter are due to solubility of formed organic complexes increased, especially with micronutrients (KERBAUY, 2008). B is essential for pollen grains germination, pollen tube growth, seeds and proteins formation. Considering the pH range of most soils cultivated with forest species, B occurs in soil solution in boric acid form ( $\text{H}_3\text{BO}_3$ ), therefore, the only nutrient that occurs in neutral form.

Literature evidence conflicting results regarding the dynamics of nutrient accumulation in plant tissues due to SS application. However, one must consider the possibility of many factors effect, such as SS chemical composition, period between application to the soil and tissues collection for analysis, species characteristics and possible interactions with other factors.

Beneficial effects of organic residue adding varying from better biological activity, soil conditioning, physical stability, lower mineralization rate, among others. Antunes et al. (2016) stated that organic fertilization is shown as a more interesting alternative to soil

and environment. The residue reuse has an environmentally correct aspect, because if improperly disposed they become an environmental problem. Thus, only fact that a polluting residue is removed from environment and transformed into an input for *P.cattleianum* var. *cattleianum* seedlings production is an adequate solution.

## Conclusions

Sewage sludge addition to substrate provided better conditions for growth and development of *P. cattleianum* var. *cattleianum* seedlings, evidenced by height, stem base diameter and seedlings biomass. Thus, for specie seeding it is recommended to add 40% of sewage sludge + 60% organic compost and/or 60% of sewage sludge + 40% organic compost to substrate. The sewage sludge increased the nutrients contents in leaves, stem and root, which are essential for *P. cattleianum* var. *cattleianum* seedlings quality.

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