

RESEARCH ARTICLE

Nutrition and physiology of hybrid *Eucalyptus urograndis* in soil fertilized with sewage sludge

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ABSTRACT

Sewage sludge is a residual pollutant product from the treatment of urban effluent and must be adequately processed before final disposal in order to avoid environmental contamination. The use of sewage sludge in agricultural and forestry areas can improve the physical, chemical and biological properties of soil; it can also be a source of nutrients, increasing crop productivity. Accordingly, this study aimed to evaluate the growth and development of hybrid *Eucalyptus urograndis* (*Eucalyptus grandis* x *E. urophylla*) seedlings cultivated in soil with different doses of sewage sludge. To accomplish this, hybrid *E. urograndis* seedlings were cultivated for 120 days in pots containing soil (dystrophic Red-Yellow Latosol -LVA) with different doses of sewage sludge (0, 30, 60 or 90 Mg ha⁻¹). The experiment was carried out in a randomized complete block design consisting of four treatments, three replications with four plants per replication, totaling 48 plants. Biometric, biomass and physiological variables (chlorophyll, gaseous exchange, stomatal conductance and transpiration); in addition to macro- and micronutrient contents in leaves and soils of each treatment were evaluated. The treatments with sewage sludge were statistically superior to control treatment for all variables. Therefore, we recommend the application of 60 Mg ha⁻¹ sewage sludge for the growth of hybrid *Eucalyptus urograndis* seedlings. Conversely, 90 Mg ha⁻¹ sewage sludge inhibited hybrid *E. urograndis* root growth. Moreover, the use of sewage sludge as organic fertilizer in tropical soils is a viable and sustainable alternative for *Eucalyptus* cultivation.

Keywords: Biosolid; *Eucalyptus*; Fertilization; Organic nutrition

INTRODUCTION

The most important destination for sewage sludge is in agriculture since it is rich in nutrients and, consequently, an excellent organic fertilizer (Contin et al., 2012). Sewage sludge has high contents of organic matter and nitrogen, as well as other minerals of agronomic interest (Lima et al., 2011). The use of sewage sludge and its potential for use as fertilizer and soil conditioner represent the possibility of reducing the use of mineral fertilizers and increasing crop productivity (Guedes et al., 2006), as long as it is used according to appropriate procedures to minimize environmental and ecological impact (Usman et al., 2012). Sewage sludge improves soil fertility by increasing cation exchange capacity (CEC) when incorporated into soil, reducing concentrations of exchangeable aluminum and increasing soil microbial activity, as well as improving its physical and biological attributes. It is also a source of macro- and micronutrients for plants (Prado and

Cunha, 2011). In contrast, sewage sludge contains high amounts of heavy metals that can cause environmental damage, polluting the soil and groundwater and presenting toxicity to plants (Bettioli and Ghini, 2011). Use of the parameters and procedures defined in Resolution 375/2006 of CONAMA - National Environment Council (CONAMA, 2006) for sewage sludge should be considered.

Eucalyptus forest plantations are economically and ecologically important in Brazil, producing several products and raw materials, mostly for the timber and cellulose industry, and contributing to deforestation reduction owing to the replacement of wood from native forests for those of cultivated forests (Moreira et al., 2017). The production cycle of the crop is long, requiring the application of slow release fertilizers because the soils used for forest plantations are usually poor in nutrients (Ibrahim et al., 2019). Several studies have been carried out with the use of sewage sludge in forest plantations;

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however, further studies are needed to understand its effects on soil and plants (Zabotto et al., 2018).

The objective of our study was to gain insight into the effects of the application of sewage sludge on the growth, physiology and nutrition of hybrid *Eucalyptus urograndis* (*Eucalyptus grandis* x *E. urophylla*) seedlings submitted to different doses of sewage sludge in soil.

MATERIAL AND METHODS

The experiment was conducted in São Paulo (23°30'S and 46°40'W), State of São Paulo, Brazil, located at 770 m above sea level. The experiment was carried out in a greenhouse and it was diary watered. The analysis of irrigation water showed pH - 7.7; K - 0.07 mM; Ca - 0.320 mM; Mg - 0.060 mM; Cl - 0.960 mM; Na - 0.100 mM; CO₃ - 0.000 mM; HCO₃⁻ - 0.420 mM; SAR (Sodium Absorption Ratio) - 0.23 and EC - 0.080 dS m⁻¹.

The hybrid *Eucalyptus urograndis* (*Eucalyptus grandis* x *E. urophylla*) seedlings were obtained from cuttings of a commercial producer. Six-month-old plants (0.41 cm height, 3.01 mm stem diameter, 3.61 g fresh leaf mass, 2.18 g fresh stem mass and 2.26 g fresh root mass, 1.24 g leaf dry mass, 0.80 g stem dry mass and root dry mass 0.77 g) were transplanted into 14 L polyethylene pots.

The sewage sludge (Table 1) was obtained from a sewage treatment plant (ETE) located in Botucatu, São Paulo State, Brazil, where it underwent the composting process. The sewage sludge remained in the drying bay for about 70 days, presenting temperatures above 55 °C, which permitted the disinfection (according to CONAMA Resolution No. 375/2006) and drying of the material (20% humidity). The soil used was dystrophic Red-Yellow Latosol (LVA) soil from the forest area (Table 2). It was collected at depths of 20 cm and sieved in a 2 cm mesh. The soil was incorporated with 2 Mg ha⁻¹ of agricultural limestone (PRNT 92%) for pH correction (Rajj et al. 1996).

The treatments were Soil (dystrophic Red-Yellow Latosol (LVA) soil) - Control; 30 Mg ha⁻¹ sewage sludge + Soil;

60 Mg ha⁻¹ sewage sludge + Soil or 90 Mg ha⁻¹ sewage sludge + Soil. After 150 days of experimentation (Fig. 1), three seedlings from each treatment were randomly selected and analyzed for relative chlorophyll content using SPAD-502® portable equipment (Minolta, Osaka, Japan). The analysis of chlorophyll *a* and *b*, in addition to carotenoid contents in leaves, was performed according to Lee et al. (1987). Chlorophyll *a* fluorescence (Multi-Mode Chlorophyll Fluorometer OS5p®, Opti-Sciences, Hudson, USA) and gas exchange (Infrared Gas Analyzer LCpro+, ADC BioScientific Ltd. Global House, Hertfordshire, UK) were evaluated as described by Silva et al. (2017). The plants were sectioned into leaves, stems and roots and weighed to obtain fresh mass. Dry mass was obtained after drying in a forced air oven at 65 °C until constant weight. The ratio of root and shoot dry matter (R/S) was calculated. Chemical analyses of the leaves and soil were carried out in the Laboratory of Mineral Nutrition in Plants at UNESP, Botucatu, São Paulo State, Brazil.

The experimental design was a randomized block design with four treatments and three blocks with four plants per replication. Data were analyzed by variance analysis (ANOVA) and the means compared by Tukey's test ($p \leq 0.05$), using the Sisvar 5.3 Statistical Software.



Fig 1. Hybrid *Eucalyptus urograndis* after 150 days of experimentation submitted to treatments Soil (dystrophic Red-Yellow Latosol - LVA) - Control; 30 Mg ha⁻¹ sewage sludge (SS) + Soil; 60 Mg ha⁻¹ sewage sludge (SS) + Soil or 90 Mg ha⁻¹ sewage sludge (SS) + Soil.

Table 1: Chemical composition of dystrophic Red-Yellow Latosol (LVA) soil

pH	O.M.	P _{resine}	Al ³⁺	H+Al	Na	K	Ca	Mg	CEC	BS	S	B	Cu	Fe	Mn	Zn
CaCl ₂	g dm ⁻³	mg dm ⁻³	mmol _c dm ⁻³			%		mg dm ⁻³								
3.8	15	2	23	106	-	0.2	4	1	111	5	105.8	0.5	0.5	43.7	0.2	0.57

Table 2: Chemical composition of sewage sludge

N	P ₂ O ₅	K ₂ O	Ca	Mg	S	U-65 °C	O.M.	C	Na	B	Cu	Fe	Mn	Zn	C/N	pH
Percentage									mg kg ⁻¹							
2.5	3.2	0.1	1.2	0.2	2.4	29	33	18	568	145	159	33465	315	870	7/1	6.4

RESULTS AND DISCUSSION

Organic matter (O.M.) of the soil increased 59% with the application of 30, 60 or 90 Mg ha⁻¹ of sewage sludge when compared to the control treatment. Sewage sludge is rich in organic matter and a source of N, P and S, and it is fundamental to the availability of plant nutrients (Cunha et al., 2016). O.M. in soil can also form aggregates, increasing its stability and reducing erosive potential (Galdos et al., 2009). The capacity of cation exchange (CEC) increased as the doses of sewage sludge increased in treatments. Soils with higher CEC can maintain adequate quantities of Ca²⁺, Mg²⁺ and K⁺ than soils with low CEC; however this soils can retain acid cations such as hydrogen (H⁺) and aluminum (Al³⁺) (Ross and Ketterings, 2011).

The application of sewage sludge did not alter the pH of the soils, allowing them to remain in the range suitable for the growth of the species, which is usually adapted to and cultivated in acidic soils. The increase in pH in degraded areas after application of sewage sludge results from the lime (CaO) used to treat the residue (Ricci et al., 2010). The application of 30, 60 and 90 Mg ha⁻¹ sewage sludge added 750, 1,500 and 2,250 kg ha⁻¹ N to the soil, respectively. The treatments with sewage sludge also increased P contents in soil (Table 3). Phosphorus has been a finite resource and extremely important for the maintenance of agricultural productivity (Pantano et al., 2016); consequently, the use of sewage sludge is a sustainable way to provide this element to plants, allowing its recycling and minimizing the need to exploit this resource (Guimarães et al., 2018). Sewage sludge can partially replace phosphate mineral fertilizers in corn crops, obtaining results equivalent to those reached by traditional inorganic fertilizers (Bártolo et al., 2017). Ca and Mg contents were not modified by treatments; however, the values were shown to be sufficient for the initial development of the culture, maintaining BS (Base Sum) close to 25%, which is suggested by several

authors as ideal for the development of eucalyptus species. The micronutrients B, Cu, Fe, Mn and Zn were below the content levels acceptable for use in forest plantations; thus, not reaching levels of soil contamination (CETESB, 2005).

The results of foliar analyses (Table 4) show a deficiency of K in all treatments (Gonçalves et al., 1997) with marginal chlorosis in older leaves after 60 days of planting, a symptom of deficient K in *Eucalyptus* (Silveira et al., 2005). Sewage sludge is nutritionally unbalanced, as for example K, which is trapped in water during the process of sewage sludge separation at the sewage treatment plant (Oliveira et al., 2010). N contents in the leaves increased with higher sewage sludge doses, ranging from 14 to 35 g kg⁻¹. N concentrations in the leaves of control treatment were also within the range of recommended N for adult *Eucalyptus* plants (Gonçalves et al., 1997), showing the efficient uptake and use of this nutrient in this species. P concentrations in leaves with treatments of 30, 60 and 90 Mg ha⁻¹ sewage sludge were adequate (1.0 to 1.3 mg kg⁻¹) for *Eucalyptus* (Gonçalves, 2015), while those of control treatment were lower. Ca and Mg contents were comparable to the contents in leaves of *Eucalyptus urophylla* x *Eucalyptus globulus* adult plants (Vieira et al., 2013), but lower than those in leaves of hybrid *Eucalyptus urograndis* seedlings (10.1 to 12.7 g kg⁻¹ Ca, 3.1 to 3.9 g kg⁻¹ Mg) (Melo Silva et al., 2018). S contents in leaves of plants treated with sewage sludge were higher than those reported by Viera et al. (2012) in hybrid *Eucalyptus urograndis* adult trees (1.05 mg kg⁻¹). Leaf contents of B were lower than those recommended for *Eucalyptus* plants (Gonçalves et al., 1997). B can limit *Eucalyptus* productivity (Gonçalves et al., 2015), and its deficiency can be visually distinguished by the drought of the apical meristem, followed by loss of dominance (Ferreto et al., 2016), which was not observed in this experiment. The control treatment had lower Fe contents than the recommended levels (Gonçalves et al., 1997). Treatments of 60 and 90 Mg ha⁻¹ sewage sludge exhibited

Table 3: Chemical composition of soil treated with sewage sludge after 150 days of experimentation

Sewage sludge (Mg ha ⁻¹)	pH	OM	P _{resine}	Al ³⁺	H+Al	K	Ca	Mg	BS	CEC	S	B	Cu	Fe	Mn	Zn
	CaCl ₂	g dm ⁻³	mg dm ⁻³	mmol _c dm ⁻³ (%)							mg dm ⁻³					
0	4.2	12	2	6	54	0.4	10	7	17	71	120.7	0.3	0.7	59.9	0.2	2.3
30	4.1	19	24	11	66	0.5	17	7	25	91	166.5	0.3	1.1	86.4	0.4	5.6
60	3.9	19	46	15	85	0.2	17	7	25	110	270.1	0.4	1.3	116.4	1.2	9.7
90	4.1	19	76	12	87	0.3	37	13	51	138	284.1	0.4	1.6	135.5	1.4	14.2

Table 4: Chemical analyses of hybrid *Eucalyptus urograndis* leaves

Sewage sludge (Mg ha ⁻¹)	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	g kg ⁻¹						mg kg ⁻¹				
0	14	0.3	4	7	2.6	1.4	23	4	57	199	30
30	31	1.3	6	6	2.8	2.9	25	13	370	477	60
60	32	1.2	7	6	2.9	2.4	22	13	111	852	71
90	35	1.4	6	6	2.8	2.8	28	13	129	822	85

values within the recommended range for *Eucalyptus* crop (150 - 200 mg kg⁻¹), while plants treated with 30 Mg ha⁻¹ sewage sludge presented higher Fe contents.

Sewage sludge application increased plant height (Table 5) when compared to the control treatment ($p \leq 0.05$). Treatment with 30 Mg ha⁻¹ sewage sludge resulted in plants with larger diameter of the stem than other treatments ($p \leq 0.05$). Both fresh and dry mass of leaves, stem and total (roots + stem + leaves) of plants in treatments of 30, 60 and 90 Mg ha⁻¹ sewage sludge were higher ($p \leq 0.05$) compared to control (0 Mg ha⁻¹ sewage sludge). Plants fertilized with 30 Mg ha⁻¹ sewage sludge presented higher fresh root mass than control treatment, and treatments of 60 and 90 Mg ha⁻¹ sewage sludge resulted in greater root dry mass than control, and 90 Mg ha⁻¹ sewage sludge treatments. Both fresh and dry mass of plants treated with 30, 60, and 90 Mg ha⁻¹ sewage sludge were higher than the control. Plants treated with 30 Mg ha⁻¹ sewage sludge showed 270% higher yield for fresh mass and 231% for dry mass in relation to control (0 Mg ha⁻¹ sewage sludge). The results of biomass, height and diameter of the stem of hybrid *Eucalyptus urograndis* are directly related to chemical analysis of soil and leaves that showed the nutritional effect of sewage sludge with increases of macro and micronutrient contents in the soil and, hence, plant uptake. *E. camaldulensis* was 20% taller and had 40% more leaves than control, showing the excellent fertilization properties of soil/sludge (40/60) mixture (Leila et al., 2017). Plants treated with 30 Mg ha⁻¹ sewage sludge showed fresh and dry root mass greater than treatments of 60 and 90 Mg ha⁻¹ sewage sludge, demonstrating that high doses of sewage sludge can inhibit root growth. This effect may be related to the high levels of heavy metals in sewage sludge,

e.g., 12 mg dm⁻³ Fe, 1.2 mg dm⁻³ Zn and 10 mg dm⁻³ P, all considered high for this crop (Raij et al., 1996). *Eucalyptus urophylla* seedlings showed inhibition of root and shoot growth with increasing doses of Zn in nutrient solution (0, 2, 50, 150, 300 or 450 $\mu\text{mol L}^{-1}$ as ZnSO₄·7H₂O) (Ramos et al., 2009). The control treatment (0 Mg ha⁻¹ sewage sludge) presented a higher relationship between shoot and root dry mass, possibly reflecting a strategy for this plant when availability of nutrients in the soil is low; in this case, the greatest root expansion occurs in order to uptake the maximum nutrients in this condition (Edwards, 2004).

Soil-plant analysis development (SPAD) values for plants treated with 60 and 90 Mg ha⁻¹ sewage sludge were higher than control treatments (0) and 30 Mg ha⁻¹ sewage sludge (Table 6). Visually, the leaves of plants on sewage sludge treatments showed intense green coloration, while plants of control treatment (0 Mg ha⁻¹ sewage sludge) showed reddish leaves, confirming the SPAD readings. N contents of leaves and SPAD values for *Eucalyptus globulus* are directly related, making the technique helpful for management of N in *Eucalyptus* plants up to two years old (Madeira et al., 2009). The noncorrelation between SPAD readings and chlorophyll content in our experiment could be explained by the fact that N in leaves is not associated with chlorophyll molecules (Duru, 2002). Since SPAD meters cannot identify N not incorporated into chlorophyll molecules (luxury consumption), no change in the intensity of green coloration will be reflected (Mascarello et al., 2016).

Plants treated with 60 Mg ha⁻¹ sewage sludge presented higher photochemical efficiency (Fv/Fm) in relation to control ($p \leq 0.05$). Fv/Fm values between 0.75 and 0.85 are ideal in healthy plants; however, it is important to

Table 5: Height, stem diameter (SD), fresh leaf mass (FLM), fresh stem mass (FSM), fresh root mass (FRM), total fresh mass (TFM), dry leaf mass (DLM), dry stem mass (DSM), dry root mass (DRM), total dry mass (TDM) and root dry mass/shoot dry mass ratio (R/S) of hybrid *Eucalyptus urograndis* plants under sewage sludge treatments. Means followed by the same letter do not differ by Tukey's test ($p \geq 0.05$)

Sewage sludge (Mg ha ⁻¹)	Height (m)	SD (mm)	FLM	FSM	FRM	TFM	DLM	DSM	DRM	TDM	R/S
0	0.74 ^B	8.50 ^C	39.12 ^B	37.18 ^B	37.73 ^C	114.04 ^B	14.69 ^B	14.63 ^B	14.36 ^B	43.70 ^B	0.49
30	1.21 ^A	11.55 ^A	119.81 ^A	126.32 ^A	62.41 ^A	308.55 ^A	38.42 ^A	42.60 ^A	20.01 ^A	101.04 ^A	0.25
60	1.15 ^A	10.22 ^B	123.40 ^A	120.00 ^A	54.59 ^{BC}	298.00 ^A	37.55 ^A	40.61 ^A	17.23 ^{AB}	95.40 ^A	0.22
90	1.19 ^A	10.19 ^B	121.96 ^A	117.62 ^A	46.39 ^B	285.97 ^A	38.03 ^A	39.46 ^A	13.39 ^B	90.89 ^A	0.17

Table 6: Relative content of chlorophyll (SPAD), chlorophyll a, chlorophyll b, carotenoids, total chlorophyll, photochemical efficiency (Fv/Fm), liquid photosynthesis (A), stomatal conductance (G_s) and transpiration (E) of hybrid *Eucalyptus urograndis* under sewage sludge treatments. Means followed by the same letter do not differ by Tukey's test ($p > 0.05$)

Sewage sludge (Mg ha ⁻¹)	SPAD	mg cm ⁻²				Fv/Fm	$A \mu\text{mol m}^{-2} \text{s}^{-1}$	$G_s \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$	$E \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$
		Chl a	Chl b	Carot	Total chl				
0	34.09 ^C	2.99 ^B	5.20 ^A	0.68 ^B	8.20 ^A	0.49 ^B	5.68 ^C	0.38 ^C	2.89 ^B
30	41.24 ^B	3.93 ^A	5.25 ^A	1.05 ^A	9.19 ^A	0.57 ^{AB}	12.70 ^B	0.69 ^B	4.18 ^A
60	44.24 ^A	3.73 ^{AB}	6.46 ^A	0.86 ^{AB}	10.20 ^A	0.58 ^A	14.67 ^A	0.92 ^A	4.19 ^A
90	44.90 ^A	3.20 ^{AB}	5.70 ^A	0.72 ^B	8.91 ^A	0.55 ^{AB}	14.92 ^A	0.98 ^A	4.21 ^A

note that these values may vary, or be underestimated, for each species owing to climatic conditions, such as low or high temperatures, or cultivation method (Björkman and Demmig, 1987). Nutrient supply to plants increases the photochemical efficiency in the reaction centers when exposed to light. For example, *Pinus radiata* showed increasing Fv/Fm values with the elevation of N fertilization (Bown et al., 2009). On the other hand, *Ochroma pyramidale* showed a considerable reduction in photochemical efficiency in the absence of P (Cunha et al., 2016).

Plants treated with 60 and 90 Mg ha⁻¹ sewage sludge showed higher photosynthetic rates (A) with 14.67 and 14.92 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and higher stomatal conductance (G_s) with 0.92 and 0.98 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, respectively. Meanwhile, control had the lowest values (5.68 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and 0.38 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Photosynthetic rate is one of the factors responsible for the higher productivity of *Eucalyptus* with a linear relationship between maximum photosynthesis and productivity (Otto et al., 2013). Values are equivalent to the photosynthetic rates of *Eucalyptus* plants submitted to water deficit (1.2 to 3.4 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and water availability (12.5 to 16.4 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Otto et al., 2013), showing that nutritional deficiency in *Eucalyptus* can be as limiting to photosynthesis and, consequently, to productivity as water deficiency. The transpiration rate (E) was higher in plants fertilized with 30, 60 or 90 Mg ha⁻¹ sewage sludge.

CONCLUSION

Our results indicated that sewage sludge increase soil fertility by augment of N, P and O.M. contents and CEC in soil. The application of 60 Mg ha⁻¹ sewage sludge in the soil provided higher values of growth and development variables and positively impacted the physiological variables studied in hybrid *E. urograndis*. However, 90 Mg ha⁻¹ sewage sludge inhibited hybrid *E. urograndis* root growth.

Author's contributions

All authors contributed equally to the production of the manuscript.

Declaration of conflicting interests

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