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USE OF SEWAGE SLUDGE ON *EUCALYPTUS SALIGNA* INITIAL GROWTH

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ABSTRACT: This study aimed to evaluate the effect of different doses of sewage sludge on *Eucalyptus saligna* initial growth. *E. saligna* seedlings were cultivated for 120 days in pots containing soil (control), soil + 30 Mg ha^{-1} sewage sludge, soil + 60 Mg ha^{-1} sewage sludge and soil + 90 Mg ha^{-1} sewage sludge. The chemical analyses of soils and macro- and micronutrient contents in leaves and biometric, biomass and physiological variables were evaluated. The treatments with sewage sludge were statistically higher than the control treatment for all variables. Treatments of 30 and 60 Mg ha^{-1} sewage sludge were higher than 90 Mg ha^{-1} sewage sludge for plant growth. The liquid photosynthesis (*A*), stomatal conductance (*Gs*) and transpiration (*E*) of *Eucalyptus saligna* in all treatments were higher than the Control treatment. We recommend the application of 30 Mg ha^{-1} sewage sludge for the initial growth of *Eucalyptus saligna* seedlings. Moreover, the use of sewage sludge as substrate conditioner and fertilizer for *Eucalyptus saligna* seedling cultivation is an adequate and sustainable way to manage this urban waste.

Keywords: biosolid, fertilization, seedlings, plant growth.

UTILIZAÇÃO DE LODO DE ESGOTO NO CRESCIMENTO INICIAL DE *EUCALYPTUS SALIGNA*

RESUMO: O estudo teve como objetivo avaliar o efeito de diferentes doses de lodo de esgoto no crescimento inicial de *Eucalyptus saligna*. Mudas de *E. saligna* foram cultivadas por 120 dias em vasos contendo solo (controle), solo + 30 Mg ha^{-1} lodo de esgoto, solo + 60 Mg ha^{-1} lodo de esgoto e solo + 90 Mg ha^{-1} lodo de esgoto. Foram avaliadas as análises químicas dos solos e teores de macro e micronutrientes nas folhas e variáveis biométricas, biomassa e fisiológicas. Os tratamentos com lodo de esgoto foram estatisticamente superiores ao tratamento controle para todas as variáveis.
Os tratamentos de 30 e 60 Mg ha^{-1} de lodo de esgoto foram superiores a 90 Mg ha^{-1} de lodo de esgoto para o crescimento das plantas. A fotossíntese líquida (*A*), condutância estomática (*Gs*) e transpiração (*E*) de *Eucalyptus saligna* em todos os tratamentos foram superiores ao tratamento Controle. Recomendamos a aplicação de 30 Mg ha^{-1} de lodo de esgoto para o crescimento inicial de mudas de *Eucalyptus saligna*. O uso do lodo de esgoto como condicionador de substrato e fertilizante para o cultivo de mudas de *Eucalyptus saligna* é uma forma adequada e sustentável de gerenciar esse resíduo urbano.

Palavras-chave: biossólido, fertilização, mudas, crescimento de plantas.

1 INTRODUCTION

Waste from sewage treatment plants requires safe disposal for environmental reasons. At the same time, agricultural crops require alternative fertilizers in order to reduce production costs (BERTOLAZI et al., 2017). Sewage sludge is a residue that must be used with caution, owing to the probable presence of organic and inorganic contaminants and pathogens, e.g., the use of sewage sludge as fertilizer for crop production, such as

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eucalyptus for cellulose and paper (ABREU-JUNIOR et al., 2017). Sewage sludge was designated a biosolid in order to be reused or recycled in something beneficial, aiming to promote its favorable use in plant cultivation (GUEDES et al., 2006). Sewage sludge incorporated into the soil improves the contact of soil particles increasing CEC, reducing the concentration of exchangeable aluminum, providing a source of macro- (N, P, Ca, Mg and S) and micronutrients, increasing microbial activity in the soil, and improving its physical and biological attributes (PRADO and CUNHA, 2019). The use of sewage sludge in agriculture as fertilizer is regulated by Decree 4.954/2004 and limited by Normative Instruction nº 61/2020 (CONAMA, 2020) which regulate and define criteria for the use of sewage sludge in agricultural areas without undue risk to public health and the environment. The present study aims to investigate the application of sewage sludge to the initial growth of *Eucalyptus saligna* through valuating its effects on plant growth and physiology.

2 MATERIAL AND METHODS

The experiment was conducted in São Paulo City (23°30’S and 46°40’W), São Paulo State, Brazil, located at 770 m above sea level. Six-month-old *Eucalyptus saligna* seedlings (0.40 cm height, 2.73 mm stem diameter, 2.97 g fresh leaf mass, 1.76 g fresh stem mass and 2.09 g fresh root mass, 1.12 g leaf dry mass, 0.65 g stem dry mass and root dry mass 0.57 g) were obtained from cuttings from a commercial producer. The seedlings were planted in 14 L black polyethylene pots filled with Red Latosol soil.

The soil used was collected under the superficial layer (20-40 cm) of Red Latosol (Table 1) from the Atlantic Forest area of the Ipiranga State Park (PEFI) and was sieved in a 2 cm mesh-size. The sewage sludge (Table 2) was generated by the ETA – Sewage Treatment Plant Station of SABESP in Botucatu City, São Paulo State, Brazil. The material was composted during 70 days, reaching temperatures above 50 ºC, allowing its disinfection (CONAMA – Normative Instruction nº 61/2020), and deposited in drying bays where it reached 20% humidity, at FCA/UNESP, Botucatu, São Paulo State, Brazil.

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

pH	**O.M.**	**P_{resine}**	**Al^{3+}**	**H+Al**	**Na**	**K**	**Ca**	**Mg**	**CEC**	**BS**
$CaCl_2$	g dm^{-3}	mg dm^{-3}	--------------------$mmol_c$ dm^{-3} ------------------------							%
3.8	15	2	23	106	-	0.2	4	1	111	5
S	**B**	**Cu**	**Fe**	**Mn**	**Zn**					
---------------------- mg kg^{-1}-----------------------										
105.8	0.5	0.5	43.7	0.2	0.57					

Table 2. Chemical composition of sewage sludge.

N	**P_2O_5**	**K_2O**	**Ca**	**Mg**	**S**	**U-65 °C**	**O.M.**	**C**
---------------------------------------percentage------------------------------								
2.5	3.2	0.1	1.2	0.2	2.4	29	33	18
Na	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**C/N**	**pH**	
---------------------- mg kg^{-1}-------------------------								
568	145	159	33465	315	870	7/1	6.4	

The treatments consisted of soil, soil + 30 t ha^{-1} sewage sludge (dry base), soil + 60 t ha^{-1} sewage sludge (dry base) and soil + 90 t ha^{-1} sewage sludge (dry base). After 150 days of experimentation, three plants of each treatment were randomly selected, and their relative chlorophyll contents were analyzed using SPAD-502® portable equipment (Minolta, Osaka, Japan). For chlorophyll *a*, *b* and carotene contents in leaves, analyses were performed according to Lee et al. (1987). Chlorophyll *a* fluorescence was evaluated on preconditioned leaves in the dark during 30 minutes with an OS5p® Multi-Mode Chlorophyll Fluorometer (Opti-Sciences, Hudson, NH, USA). Gas exchange was evaluated by an infrared gas analyzer (Lcpro + mod., ADC BioScientific Ltd. Global House, Hertfordshire, UK) in an open system between 08:00 and 11:00 hours with irradiance of 1,000 µmol photons m^{-2} s^{-1} in 3 leaves per plant measured in the upper third portion of the leaf.

The seedlings were sectioned into leaves, stems and roots and weighed to obtain fresh mass. The dry mass was obtained after drying in a forced air oven at 65 °C until reaching constant weight. The ratio of root/shoot system dry matter (R/S) was calculated. The chemical analyses of the leaves (MALAVOLTA; VITTI; OLIVEIRA, 2001) and soil (RAIJ et al., 2001) 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. The data were submitted to analysis of variance (ANOVA) and the means compared by Tukey´s test ($p \leq 0.05$), using the SISVAR statistical program.

3 RESULTS AND DISCUSSION

Eucalyptus species cultivated in Brazil are adapted to low levels of soil fertility; tolerating soil acidity from high levels of Al. Dolomitic lime should be used on Eucalyptus crop to supplement the soil with additional amounts of Ca and Mg (GONÇALVES, 1995). Organic matter (O.M.) of the soil increased 66.7% with the application of 30 and 60% Mg ha^{-1} sewage sludge, and 91.2% with the application of 90 Mg ha^{-1} sewage sludge, when compared to the control treatment (Table 3). Sewage sludge is rich in organic matter and other key elements and thus able to act as a substitute for mineral fertilization (KUMMER et al., 2016). Commercial eucalyptus production may deplete nutrients in soil and disrupt soil structure, as well as affect water-holding capacity of the soil and, hence, the long-term productivity of the crop (JONES et al., 1999). In our study, as sewage sludge doses increased, we noticed that cation exchange capacity (CEC) also increased to 87.5%. The increase of CEC is directly correlated with the increase of soil organic C and the nutrients N, P and S uptake by eucalyptus seedlings (GUEDES et al., 2006). The pH of soil decreased from 5.3 (0 Mg ha^{-1}) to 4.0 (90 Mg ha^{-1}). The low pH of sewage sludge and the intermediate products, such as organic acid, may explain the decrease in pH of soils (SHAN et al. 2021). By applying 90 Mg ha^{-1}, P level increased 41-fold, in comparison to control treatment (0 Mg ha^{-1}) (Table 5). Several authors also detected that sewage sludge application could increase extractable P in the soil (GUEDES et al., 2006; PANTANO et al., 2016; SILVA et al., 2012). Phosphorus is a finite resource; thus, phosphorus recovery from sewage sludge should not only be optimized for pollution prevention but should be considered a resultant product and suitable for plant growth and food production (SCHRÖDER et al., 2010). Ca and K contents did not increase, and Mg decreased, as sewage sludge quantities increased; nevertheless, Ca and Mg values were sufficient for the initial development of the culture. The low values for K in sewage sludge may be explained by the high rates of potassium retained in water during the treatment process, owing to its high solubility (LEILA et al., 2017). S concentration in soil with 30, 60 and 90 Mg ha^{-1} was almost 3 times higher compared to the control treatment. All micronutrients and aluminum (Al^{+3} and H+Al) increased as sewage sludge doses increased; however, they were below the levels acceptable for use in forest plantations, thus not reaching levels of soil contamination (CETESB, 2005). B and Zn are important micronutrients for eucalyptus and should be applied in the concentrations of 0.3% B and 0.5% Zn. B application is particularly important, especially in regions where water deficiencies are high, and drought occurs (GONÇALVES, 1995). Through studies, it is possible to observe that some crops, such as corn and beans, when fertilized with sewage sludge presented the same results as those of mineral fertilizer, and in this sense, sewage sludge is shown to be capable of replacing or complementing chemical fertilizer very efficiently (CUNHA MARTINS et al., 2018).

Table 3. Chemical composition of soil treated with sewage sludge (SS) after 150 days of experimentation.

	pH	**O.M.**	$\mathbf{P_{resine}}$	$\mathbf{Al^{3+}}$	**H+Al**	**K**	**Ca**	**Mg**	**BS**	**CEC**
SS (Mg ha^{-1})	$CaCl_2$	g dm^{-3}	mg dm^{-3}	------------mmol$_c$ dm^{-3} ---------						(%)
0	5.3	12	2	0	26	0.5	27	19	47	72
30	4.3	20	25	7	69	0.4	24	14	38	107
60	4.1	20	48	11	89	0.3	31	13	45	134
90	4.0	23	82	14	101	0.3	26	9	35	135

SS (Mg ha^{-1})	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
0	83	0.2	0.6	45	0.2	1.9
30	258	0.4	1.1	91	0.5	6.1
60	280	0.5	1.3	120	0.6	8.8
90	255	0.4	1.7	150	1.1	14.2

N contents in the leaves increased according to the augment of sewage sludge quantities in soil, ranging from 15 to 35 g kg^{-1} (Table 4). N contents in the leaves of the control treatment (0 Mg ha^{-1} sewage sludge) were also within the range of N recommended for adult eucalyptus plants (GONÇALVES, 1995), showing the efficient uptake and use of this nutrient by the species. P contents in leaves with treatments of 30, 60 and 90 Mg ha^{-1} sewage sludge were higher than those considered optimal for eucalyptus in Brazil (0.7 to 1.08 g kg^{-1}; WADT et al., 1998), whereas they were suboptimal for the control treatment (0.2 g kg^{-1}) (Table 4). K, Ca and Mg contents did not increase, and S increased as sewage sludge quantities augmented (Table 4). Sewage sludge is poor in potassium contents, but this element is very soluble in water and remains dissolved in wastewater during the sewage treatment process (MORETTI; BERTONCINI; ABREU JUNIOR, 2015). Leaf chlorosis was observed in older leaves 60 days after transplanting, probably from K deficiency (DELL and ROBINSON, 1993; ZABOTTO et al., 2020). Therefore, a supplementation of potassium is necessary to improve crop production (OLIVEIRA et al., 1995). Ca contents were considered optimal for all treatments (WADT et al., 1998; RAIJ et al., 1997), but Mg was considered lower by Raij et al. (1997). In our study, no visual symptomatology of Mg deficiency was observed, as previously described for *Eucalyptus aculate* (DELL and ROBINSON, 1993). S contents in leaves of plants treated with sewage sludge were considered optimal (RAIJ et al., 1997) and higher than those observed in *Eucalyptus urograndis* adult trees (1.05 mg kg^{-1}; VIERA et al., 2012).

Micronutrient contents in *E. saligna* leaves increased as the quantities of sewage sludge were raised in treatments. Boron contents in leaves of *Eucalyptus saligna* were lower than those recommended for *Eucalyptus* plants (RAIJ et al., 1997) without any visual symptoms. Symptoms of B deficiency in *E. globulus* were rolled and malformed leaves and stem dieback. *E. urophylla*'s leaf margins became chlorotic and then necrotic, while leaves turned brittle and corky, and shoots died back. *E. grandis* leaves turned reddish and gave evidence of stem dieback, and the leaves of *E. tereticornis* developed marginal and interveinal chlorosis and recurved margins (DELL and MALAJCZUK, 1994). Treatment of 30 Mg ha^{-1} sewage sludge exhibited values within the recommended range for eucalyptus crop (150 – 200 mg kg^{-1}; RAIJ et al., 1997); all other treatments had lower values.

Table 4. Chemical analyses of *Eucalyptus saligna* leaves.

Sewage sludge (Mg ha^{-1})	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	---------------g kg^{-1}---------------						------------mg kg^{-1}-------------				
0	15	0.2	5	6	2.4	1.1	10	6	67	207	23
30	30	1.3	6	7	2.7	2.1	21	14	152	397	44
60	30	1.3	7	7	2.9	1.9	23	13	106	503	53
90	31	1.3	8	8	2.7	2.0	27	12	82	727	72

Treatment of 30 Mg ha^{-1} showed the tallest height ($p \leq 0.05$) when compared to the other treatments (0, 60 and 90 Mg ha^{-1}) (Table 5). Treatments of 30 and 60 Mg ha^{-1} showed the highest averages ($p \leq 0.05$) for stem diameter (Table 5). Treatments of 30, 60 and 90 Mg ha^{-1} showed the highest averages ($p \leq 0.05$) for all biomass variables in relation to the control treatment (0 Mg ha^{-1}) (Table 5). The results of biomass, height, and diameter of the stem in *Eucalyptus saligna* are directly related to the 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 in the plants, consequently enhancing plant growth. The plants treated with 30 Mg ha^{-1} sewage sludge showed values of fresh and dry root mass higher than those with treatments of 60 and 90 Mg ha^{-1}, showing that high doses of sewage sludge can inhibit root growth (Table 5). Sewage sludge has high quantities of heavy metals, and the accumulation of these elements in eucalyptus is higher in roots than in the shoot system (MAZEN; FAHEED; AHMED, 2010), which could inhibit root growth. The control treatment (0 Mg ha^{-1} sewage sludge) showed a higher relationship between aerial and root dry mass (Table 5), possibly reflecting a strategy for this plant when the availability of nutrients in the soil is low.

Table 5. Biometry of *Eucalyptus saligna* plants under sewage sludge treatments as plant 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).

Sewage sludge	Height	SD	FLM	FSM	FRM	FTM
(Mg ha^{-1})	(m)	(mm)	---------------------- g -------------------------			
0	0.61 c	7.01 c	21.16 b	17.02 c	33.22 b	71.41 c
30	1.07 a	10.92 a	122.10 a	116.30 a	70.55 a	308.96 a
60	1.00 a	10.30 a	111.94 a	102.88 ab	68.75 a	283.58 ab
90	0.87 b	8.99 b	103.71 a	86.59 b	44.14 b	234.46 b
Sewage sludge	**DLM**	**DSM**	**DRM**	**TDM**	**R/S**	
(Mg ha^{-1})	---------------------- g -------------------------					
	9.37 c	7.29 c	11.75 b	28.42 c	0.80	
	42.68 a	40.73 a	21.23 a	104.55 a	2.02	
	37.43 ab	36.13 ab	18.11 ab	91.68 ab	2.07	
	33.95 b	30.20 b	11.76 b	75.91 b	2.89	

Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$).

The SPAD values for plants treated with 60 Mg ha^{-1} sewage sludge were higher than treatments of 0 and 30 Mg ha^{-1} sewage sludge (Table 6). The leaves of plants fertilized with 30, 60 and 90 Mg ha^{-1} sewage sludge exhibited a more intense green color than control treatment. The Soil-Plant Analysis Development (SPAD) values can be correlated to N levels in leaves, such as those for *E. globulus* (MADEIRA et al., 2009). Treatment of 30 Mg ha^{-1} sewage sludge showed higher averages for chlorophyll *a* ($p \leq 0.05$), carotene ($p \leq 0.05$) and total pigments ($p \leq 0.05$) in relation to the control treatment (0 Mg ha^{-1} sewage sludge) (Table 6). Treatments of 30, 60 and 90 Mg ha^{-1} sewage sludge showed higher averages of chlorophyll *b* ($p \leq 0.05$) in relation to the control treatment (0 Mg ha^{-1} sewage sludge) (Table 6). Plants treated with 30 Mg ha^{-1} sewage sludge showed higher photochemical efficiency (*Fv/Fm*) in relation to the control ($p \leq 0.05$) (Table 6). *Fv/Fm* values between 0.52 and 0.60 are ideal in healthy plants, and these values may vary or be underestimated for each species as a result of climatic conditions, such as low or high temperatures, or cultivation method (CAMPOSTRINI, 2001). Plants treated with 90 Mg ha^{-1} sewage sludge showed higher averages ($p \leq 0.05$) for the variables liquid photosynthesis (*A*) and stomatal conductance (*Gs*) (Table 6). Treatments of 30, 60 and 90 Mg ha^{-1} sewage sludge showed higher averages ($p \leq 0.05$) for transpiration rate *E* (Table 6). Sewage sludge is rich in nutrients, a key factor for eucalyptus crop productivity since they are involved in basic physiological processes related to photosynthesis and energy transfer within leaves (LACLAU *et al.*, 2009). Therefore, use of sewage sludge as fertilizer in the initial growth of *E. saligna* is appropriate and beneficial, as long as it is applied according to appropriate guidelines to minimize environmental and ecological damage and maximize potential benefits for sustained agricultural productivity.

Table 6. *Eucalyptus saligna* physiology under sewage sludge treatments as relative content of chlorophyll (SPAD), chlorophyll *a*, chlorophyll *b*, carotenoids, total pigments, photochemical efficiency (Fv/Fm), liquid photosynthesis (*A*), stomatal conductance (*Gs*) and transpiration (*E*).

Sewage sludge	**SPAD**	**Chlorophyll *a***	**Chlorophyll *b***	**Carotenoids**	**Chlorophyll *a+b***
(Mg ha^{-1})		----------------------------µg cm^{-2}--------------------------------			
0	40.13 c	3.82 b	5.75 b	2.16 b	9.57 b
30	43.33 b	5.85 a	6.97 a	2.59 a	12.82 a
60	45.20 a	4.85 ab	6.68 a	2.37 ab	11.53 a
90	44.82 ab	4.70 ab	6.66 a	2.31 ab	11.36 ab
Sewage sludge	**F*v*/F*m***	***A***	***Gs***	***E***	
(Mg ha^{-1})			(mol m^{-2} s^{-1})		
	0.52 b	4.69 c	0.16 d	2.39 b	
	0.60 a	11.81 b	0.31 c	3.76 a	
	0.57 ab	12.12 b	0.45 b	3.80 a	
	0.58 ab	12.91 a	0.63 a	3.88 a	

Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$).

4 CONCLUSION

The fertilization of soil using sewage sludge resulted in an increase in N, P and O.M. and increase of CEC in soil. The application of sewage sludge showed higher values of biometric variables (growth and development) and positive results in physiological variables in *Eucalyptus saligna*. However, doses above 30 Mg ha^{-1} retracted the root growth in *Eucalyptus saligna*. Thus, 30 Mg ha^{-1} of sewage sludge provides a better result and should be considered a viable technique for fertilization of *Eucalyptus saligna* and to avoid the dispose of the pollutant in the environment.

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