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Application of biosolid and wastewater as nutritional sources for Eucalyptus clones development

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Abstract: The use of sewage sludge (Biosolid) and wastewater as sources of nutrients for the development of eucalyptus plants brings the possibility of maintaining or increasing the productivity of this species. In addition, it can provide a more suitable purpose for urban waste by promoting the recycling of nutrients. The present work aimed to evaluate the nutritional potential of biosolids (sludge) and wastewater in the initial development of *Eucalyptus urograndis* plants. The experimental design used was in randomized blocks, consisting of 5 blocks and 4 treatments: Conventional fertilization or control (NPK); irrigation with wastewater (WW); Incorporation of sludge with 25% of the soil volume (B25); Incorporation of sludge with 50% of the soil volume (B50). The experiment lasted 120 days. Biometric analyzes and macro and micronutrient contents were performed in the leaves, stem and roots. The application of sludge and wastewater as nutritional sources showed promising results for the initial development of eucalyptus. Among the treatments, the incorporation of sludge was highlighted, and the application of 25% of the biosolid was efficient to supply the nutritional demand of plants.

Keywords: hydric resource, organic nutrition, plant growth, sewage sludge, silviculture.

Introduction

The rational use of any type of waste provides the benefit of reducing its disposal in the environment. Thus, the challenge of using the most diverse waste generated by industries and urban centers arises, inserting them again in the productive means and adapting them to the most technically, economically, and environmentally viable uses. In this way, the application of urban waste as a nutritional source in the eucalyptus crop can bring the benefits of nutrient recycling in addition to increasing wood productivity.

Wastewater and/or sludge from STP (Sewage Treatment Plant) are rich in macro and micronutrients (Gouqing et al., 2019; Minhas et al., 2015; Sharma et al., 2017) essential to plants, presenting potential to contribute to plant production. Studies have shown interesting results regarding the accumulation of biomass in forest species treated with domestic effluents (Leila et al., 2017; Minhas et al., 2015). Important economic advantages were also reported, since materials from STP are less expensive when compared to commercial fertilizers. The application of sewage sludge has increased significantly in recent years (Abreu-Junior et al., 2019), as it provides positive effects on plant growth. After treatment and stabilization this residue is called biosolid.

This material can act as a slowly mineralizing fertilizer and soil conditioner (Lira et al., 2008), as it contains considerable amounts of macro and micronutrients (mainly N and P) in addition to organic matter that provides soils with greater water retention capacity and greater erosion tolerance (Dores-Silva et al., 2011).

With regard to wastewater, in addition to providing nutrients to plants, it represents an alternative as a water source. Fertigation is a technique that combines fertilization and irrigation, this combination enhances the growth of forest crops, as this technique increases the conduction of nutrients to the roots (Pereira et al., 2019). Fertigation of forest species is beneficial for the recycling and use of reused water by converting energy from nutrients into biomass, thus increasing wood production and eco- restoration (Minhas et al., 2015).

Given this perspective, the application of biosolids and wastewater in forest plantations can favor the consolidation of more sustainable practices, since these residues demonstrate high potential for plant nutrition.

Holder of a vast area of native forests and highly productive planted forests, Brazil stands out on the world stage with 7.8 million hectares of reforestation in 2015. Eucalyptus plantations are responsible for approximately 72% of this total (IBÁ, 2019). Among the main Brazilian industrial segments responsible for eucalyptus planting are pulp and paper (61%), industrialized wood panels (25%), and solid wood products (8%) (IBÁ, 2019).

Fertilization in eucalyptus plantations can bring about changes even at anatomical levels, which can be reflected in the density and productivity of the wood (Barreiros et al., 2007). It is estimated that fertilization is responsible for increasing wood production by 30 to 50% (Bazani et al., 2016).

Fertilizers can be of mineral or organic origin, both of which are efficient for planting species of the genus Eucalyptus (Barreiros et al., 2007). The application of mineral fertilizers accounts for approximately 25% of the cost of eucalyptus plantations (Bazani et al., 2016). Therefore, the application of alternative sources of fertilizers is being evaluated, such as sewage sludge, which has been shown to be a viable organic fertilizer alternative (Barreiros et al., 2007). In addition to plant productivity, fertilization must be adjusted to the lowest cost without negatively affecting the environment (Silva et al., 2013).

The irrigation of forest species is beneficial for the recycling and reuse of water, as it conserves nutrient energy in biomass, increases wood production, and promotes environmental sanitation and eco-restoration (Minhas et al., 2015). Sousa et al. (2011) demonstrated that the productivity and oil content of *Jatropha curcas* L. seeds were positively and significantly affected by the application of wastewater. In the cultivation of eucalyptus species, the wood production in plants that received reused water was higher compared to plants that did not receive this type of water (Marinho et al., 2014; Minhas et al., 2015). In addition, they enable savings with inputs and favor a more adequate destination of urban effluents. The aim of the assay was to evaluate the potential use of biosolids and wastewater as a source of nutrients for plants, in partial replacement of inorganic fertilizers in the development of *Eucalyptus urograndis* plants.

Results and Discussion

Growth analysis

The application of biosolids promoted greater plant height in relation to the control treatment (NPK), and the B50 treatment was more effective for this parameter (plants grew on average 316.2 cm). The highest averages for stem diameter were recorded in plants of treatments B25 and B50 reaching 20.0 and 20.4 mm, respectively (Table 4). In the study by Soudani et al. (2017), the greater height of *Eucalyptus camaldulensis* plants was also a result of the application of biosolid doses in the proportion of 60% of the substrate volume. For the present assay, higher growth rates can be associated with the process mineralization graduation of organic matter present in treatments B25 and B50, which leads to constant availability of nutrients throughout the test. Andrade et al. (2013) reported higher mineralization velocity in soils with higher doses of biosolid from station treatment urban.

The height of the plants did not show a statistical difference between the WW and NPK treatments. However, the plants in the WW treatment reached a height of 236.3 cm, while the NPK treatment had an average height of 219.8 cm. Shah et al. (2010) evaluated irrigation with urban WW in Eucalyptus Camaldulensis and observed a greater plant height for the WW treatments compared to plants irrigated with pure water. It is possible that the constant application of reuse water for a longer period, thus generating an accumulation of nutrients, promotes greater plant growth.

Regarding leaf area, treatment B50 showed a higher average (21316.7 cm^2), differing from treatments WW and NPK. Thus, there was greater production of dry biomass of leaf, stem, root and total (Table 4) of plants treated with biosolid.

Plants from treatments B25 and B50 produced more (117.6 and 127.6 g, respectively) in relation to plants from the NPK treatment (45.6 g). The increase in stem dry mass was even more pronounced in treatments with biosolid application. Plants from the B50 treatment (310.8 g) presented an average approximately four times higher compared to the plants from the NPK control treatment (74.8 g), evidencing excellent fertilization properties of BS.

The results of height, stem diameter, leaf area and dry biomass of the plants were similar with the result levels of macro and micronutrients in the soils enriched with BS, confirmed by chemical analyzes (Table 2), indicating that there was greater nutritional availability to the plants.

Nutritional parameters

There was variation in relation to the accumulation of nutrients in the leaves, stems and roots of *Eucalyptus urograndis* as a function of the treatments applied (Tables 5, 6 and 7). Plants that received biosolids (B25 and B50) had higher means of N content in leaf tissues (Table 6) with 27.4 g kg^{-1} and 28.9 g kg^{-1}, respectively. These values did not differ from the N concentration of plants from the WW treatment (21.4 g kg^{-1}). The plants of the NPK treatment showed the lowest average of the assay (13.8 g kg^{-1}) for this nutrient. The N concentrations observed in the leaves of all treatments were above the minimum value (13.0 g kg^{-1}) recommended by Raij et al. (1997), for the cultivation of eucalyptus. The use of BS and WW showed greater potential to supply the N demand in the *E. urograndis* hybrid compared to plants fertilized with NPK. However, the N content in the leaves of plants fertilized with WW did not reflect in higher growth rates (Table 4).

For P contents, the highest averages were found in plants from treatments B25 and B50 (1.6 g kg^{-1} in both). Responses to P application have been more frequent and to a greater extent for eucalyptus, since Brazilian soils generally have low availability of the macroelement and high adsorption capacity (Bassaco et al., 2018). Furthermore, eucalyptus is a very demanding P crop in the initial stage of development in the field. The higher concentrations of P found in leaves, stems and roots (Tables 5, 6 and 7) of plants fertilized with BS might be one of the main reasons for the higher plant growth performance in these treatments. Thus, the application of BS in eucalyptus plants demonstrated, in this study, a high potential for an alternative source of P, capable of supplying the plants' demand for this nutrient.

The plants in the WW treatment had lower average phosphorus levels in the leaves (0.7 g kg^{-1}), which is below the recommended range of 0.9 to 1.3 g kg^{-1} according to Raij et al. (1997). According to Graciano et al. (2006), N and P are the nutrients that most limit tree growth. The authors indicate that, in *E. grandis* plants, P fertilization favored higher growth rates than N fertilization. *E. urograndis* plants grown in southern Brazil were more responsive to P fertilization, but no to the addition of N (Bassaco et al., 2018). Thus, once the N concentration in the leaves of the WW treatment plants was within the range recommended by RAIJ et al. (1997), it is suggested that the most limiting nutrient for growth was P.

NPK fertilization provided higher K content in leaves (11.7g kg^{-1}). A higher concentration of K was expected in the leaves of plants treated with WW, since this nutrient is highly soluble in water. However, its concentration in the leaves for this treatment was 6.7 g kg^{-1}, which is lower than that recommended by Raij et al. (1997). A partial replacement of cations K^+ by Na^+ in the physiology of the trees seems to have been the mechanism that can explain this behavior, since in the chemical analysis of the reuse water (Table 3), it was found that there was a three-fold higher concentration of Na (62, 4 mg L^{-1}) when compared to the K concentration (19.0 mg L^{-1}). Almeida et al. (2010) observed that Na was considered a potential fertilizer in K-deficient soils, because the growth of *E. grandis* showed a positive response to NaCl applications in partial replacement of KCl. This substitution in some plants is possible when Na^+ is used for nonspecific functions performed by K^+ in the plant cell, such as maintaining the osmotic potential in vacuoles, which can be affected by other cations such as Na^+ (Wakeel et al., 2011).

Regarding the concentrations of Ca and Mg in the leaves, the highest averages were found for plants treated with NPK (14.3 g kg^{-1} and 3.4 g kg^{-1}, respectively). The calcium concentration in the leaves of all four treatments fell within the recommended range of 6 to 10 g/kg for eucalyptus, as suggested by Raijj et al. (1997). These values were higher than those reported by Zabotto et al. (2020) for *E. urograndis* fertilized with increasing doses of biosolids.

The Mg concentration was below the recommended (Raij et al., 1997) in all treatments. However, in treatments B25 and B50, these concentrations (2.5 g kg^{-1} and 2.7 g kg^{-1}, respectively) were above the observed values (1.05 g kg^{-1} and 1.17 g kg^{-1}, respectively) by Bertolazi et al. (2017), working with the same hybrid at the same BS concentrations.

The B25 and B50 treatments resulted in the highest sulfur levels (2.0 g kg-1 and 2.2 g kg-1), which were higher than those reported by Bertolazi et al. (2017). The initial growth of *E. urograndis* was lower when treated with biosolids from a domestic sewage treatment plant compared to the findings of Zabotto et al. (2020) in *E. urograndis* fertilized with increasing doses of BS.

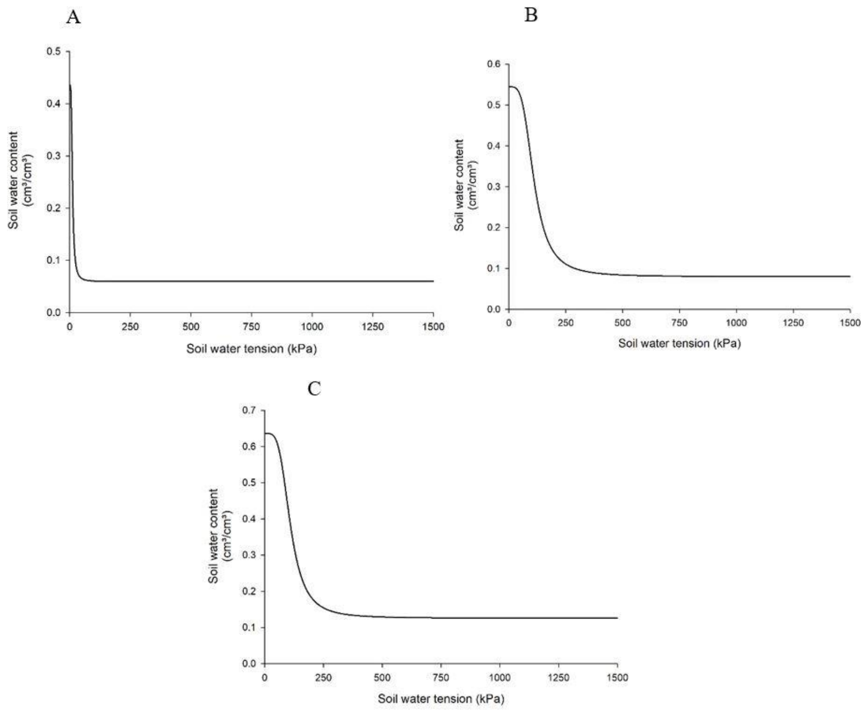

Figure 1. Water retention curves in the natural soil (A), soil + 25% biosolids (B), and soil + 50% biosolids (C).

Regarding the concentration of micronutrients in the leaves, there was no statistical difference between treatments for Cu and Fe, which showed averages above the minimum recommended value (Raij et al., 1997). For WW treatment plants, there was a higher mean for B content (26.5 mg kg^{-1}), which may be related to the fact that B is easily leached and soluble in water (Chen et al., 2017).

However, the average concentrations for this nutrient were below the recommended minimum, which is 30 mg kg^{-1} of leaf biomass (Raij et al., 1997). Still, no symptoms of B deficiency were observed in the plants.

The highest averages for Mn contents in the leaf were found in plants from treatments B25 and B50, and B25 differed from treatments NPK and WW. Mn levels in all treatments were above the recommended range (Raij et al., 1997), however there were no visual symptoms of toxicity in the plants. It was verified that the foliar contents of Zn were higher in the treatments with BS however in the plants of the WW treatment the content was lower than recommended by Raij et al., 1997. In this way, the BS treatment showed to be more promising than the WW in the supply of this micronutrient. The levels of P, K, Mg, S, B, and Zn for the plants in the WW treatment were below the recommended averages by Raij et al. (1997) compared to the other treatments, demonstrating a greater nutritional imbalance, resulting in less growth compared to the plants treated with biosolids (Table 5).

As for the leaves (Table 5), the N concentration in the stem (Table 6) was positively affected by treatments B25 and B50 (9.5 g kg^{-1} and 9.9 g kg^{-1}), differing from the NPK treatment (6.8 g kg^{-1}). The content of P in the stem tissue was statistically higher in plants treated with B50 (1.1 g kg^{-1}). Similar as observed in the leaf tissue, the P content in the stem of the plants treated with WW was lower (0.3 g kg^{-1}), demonstrating the low availability of this nutrient and corroborating the hypothesis that P was a limiting factor for plant growth in this treatment.

For the contents of K, the highest average was observed in the plants of the NPK treatment (8.2 g kg^{-1}) and the lowest averages in the plants in B25 and B50 (2.8 g kg^{-1} and 3.3 g kg^{-1}, respectively). As this behavior also occurred in the leaves, the evidence that the high concentration of Na verified in the WW may have partially replaced the absorption of K is reiterated.

The concentrations of Ca and Mg in the stem (8.6 g kg^{-1} and 1.3 g kg^{-1}, respectively) were significantly influenced by the application of WW. Ca, after Na and N, was the nutrient present in the highest concentration (23.00 mg L^{-1}) in the chemical

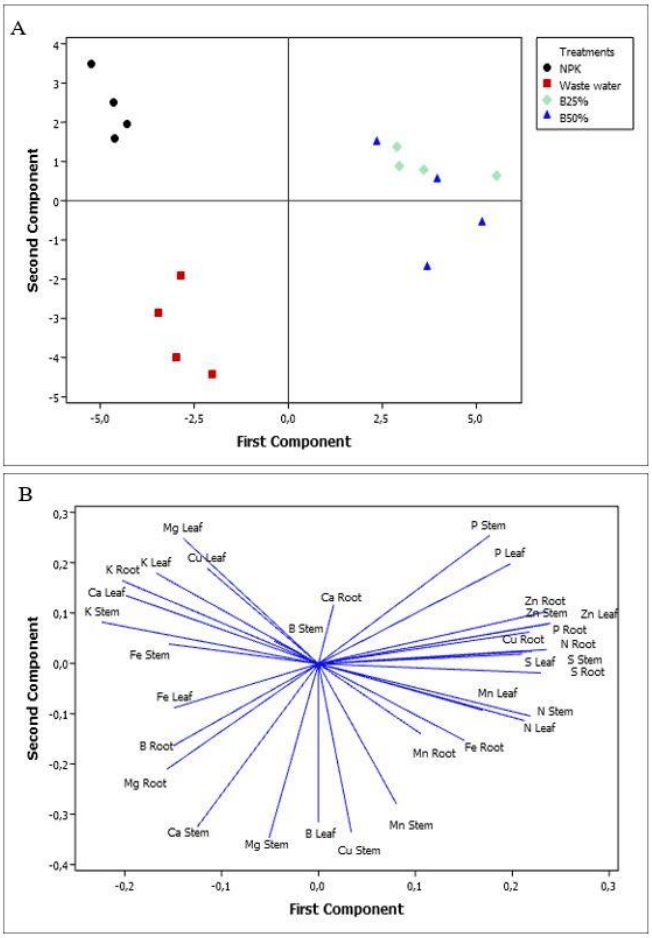

Figure 2. Dispersion of the treatments considered (NPK, WW, B25 and B50) by the analysis of the main components according to the concentrations of macro and micronutrients in the leaf tissue, stem and root of E. urograndis plants.

analysis of WW (Table 3). This factor may justify the Ca levels found both in the stem (Table 6), as well as in the leaves and roots (Tables 5 and 7) of the plants of this treatment. For S, the highest averages were found in plants of treatments B25 and B50 (7.0 g kg^{-1}), not differing from the WW treatment.

Table 1. Chemical characterization of the integral biosolid after disinfection.

N	P_2O_5	K_2O	Ca	Mg	S	U-65°C	O.M.	C	Na	B	Cu	Fe	Mn	Zn	C/N	pH
---------- % ----------									---------- 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

Table 2. Chemical characteristics of natural soil and soil with addition of biosolid to the volume of the pot.

	pH	O.M.	P resin	S	H+Al	K	Ca	Mg	SB	CCC	V%	B	Cu	Fe	Mn	Zn
	$CaCl_2$	g dm^3	----mg dm^3---		---------- mmolc dm^3 ----------							---------mg dm^3 ---------				
Soil	4.4	7	3	16	23	0.34	6	3	10	33	30	0.20	0.6	6	1.6	0.2
B25	5.1	17	1327	1175	22	2.78	86	18	107	145	73	0.69	1.2	34	13.8	5.5
B50	6	40	2029	2295	21	5.41	175	24	204	225	91	0.76	1.9	73	21.6	6.0

B25, B50, Pots with 25% or 50% of biossolid mixed to the natural soil; O.M, organic matter; SB, sum of bases; CCC, Cation change capacity.

Table 3. Chemical, physical, and microbiological analysis of wastewater after decontamination treatment (filtration and ozonation).

Parameters	value	Parameters	value
N	51.80 mg L^{-1}	Color	20
P	0.93 mg L^{-1}	Odor	-
K	19.0 mg L^{-1}	Turbidity	5.2 NTU
Ca	23.00 mg L^{-1}	Total iron	0.021 mg L^{-1}
Mg	3.00 mg L^{-1}	Total hardness	60 $CaCO_3$ mg L^{-1}
S	10.36 mg L^{-1}	Calcium hardness	50 $CaCO_3$ mg L^{-1}
Na	62.4 mg L^{-1}	Magnesium hardness	8.4 $CaCO_3$ mg L^{-1}
B	0.18 mg L^{-1}	Chlorides	58.436
Cu	0.00 mg L^{-1}	Total solids	0.02 mg L^{-1}
Fe	0.04 mg L^{-1}	Total solids/suspension	0.7 g L^{-1}
Mn	0.00 mg L^{-1}	Fluoride	0.025 mg L^{-1}
Zn	0.01 mg L^{-1}	TCC	30.6 mg L^{-1}
pH	6.25	Free residual Chlor	2.70 mg L^{-1}
E.C.	0.488 mS cm	Total residual Chlor	2.27 mg L^{-1}
$BOD_{5,20}$	5.00 mg L^{-1}	Total coliforms	5.1 MLN 100 mg^{-1}
Aspect	turbid	Thermotolerant coliforms	5.1 MLN 100 mg^{-1}

E.C., Electric Conductivity; BOD, Biochemical Oxygen Demand; TCC, Total Carbon Content; MLN, Most Likely Number; NTU, Nephelometric Turbidity Unit.

Regarding the contents of B, Fe and Mn, no differences were observed with the application of any of the treatments. A higher average for Cu, could be observed in the plants of the WW treatment (11.2 mg g^{-1}), not differing from the treatments with B25 and B50 (8.0 mg kg^{-1} and 8.8 mg kg^{-1} respectively).

The Mn contents in leaves and stems in all treatments were higher than in roots. Similar behavior was described by Dick et al. (2017), when evaluating micronutrient stocks in a 60-month-old *E. dunnii* stand. Treatments B25 and B50 promoted higher levels of Zn (149.2 mg kg^{-1} and 157.5 mg kg^{-1}, respectively).

As for the concentration of nutrients in the roots (Table 7), the treatments with B25 and B50 had the highest means of N (15.2 g kg^{-1} and 16.2 g kg^{-1}, respectively), P (2.8 g kg^{-1} and 3.5 g kg^{-1}, respectively) and S (2.6 g kg^{-1} and 2.5 g kg^{-1}, respectively). The application of biosolids in eucalyptus increases the availability mainly of N and P in the soil (Gouqing, et al., 2019), justifying the higher levels of N and P in leaves, stems and roots verified in this assay. Thus, the use of BS represented an excellent alternative to the use of mineral N and P fertilizers. For K, as occurred in leaves and stems, the highest average was observed in plants treated with NPK (6.1 g kg^{-1}). In poplar plants treated with BS, it was found that K content in the roots decreased with increasing sludge doses, the authors attributed this effect to the inhibition of K by heavy metals (Gouqing et al., 2019).

For the Ca and Mn contents in the root, no differences were observed with the application of any treatment. The plants of the WW treatment showed the highest mean in relation to the Mg concentration (1.7 g kg^{-1}), not differing from the NPK treatment (1.5 g kg^{-1}).

For B concentration, the highest mean was also observed in the WW treatment (21.4 g kg^{-1}), differing from the B25 treatment. As mentioned before, B is highly soluble, so its availability is greater in the liquid phase (WW) than in the solid phase (sludge) of sewage. The highest average concentration of Cu in the root was measured in plants of treatment B50 (44.3 mg kg^{-1}), not differing from treatment B25.

A higher average Fe content was observed in the roots of the plants of the B25 treatment (9053.5 mg g^{-1}), compared to the NPK treatment (Table 7). Fe is an essential micronutrient for the biosynthesis of constitutive proteins of chloroplasts, mitochondria and other organelles (Lima et al., 2018.It is important to note that regardless of the treatment, iron retention was significantly higher in the roots, as observed in *Eucalyptus dunnii* by Dick et al. (2017). This may be due to the low translocation of the nutrient to the aboveground parts and/or the adsorption of oxide particles on the root surface, which are difficult to remove during sampling (Mavolta et al., 1997).

Plants treated with B25 and B50 had the highest average Zn content at 378.2 mg kg^{-1} and 368.5 mg kg^{-1}, respectively, significantly higher than plants from NPK and WW treatments (60.5 mg kg^{-1} and 24.6 mg kg^{-1}). Excessive Zn in the soil can harm plant development, but the doses of BS used in this study did not cause toxicity in the plants, as evidenced by the absence of symptoms and the favorable growth of plants in the B25 and B50 treatments. The concentration of Zn in the root of the NPK treatment plants was approximately 2.5 times higher than the concentration in the WW treatment, which differs from the results verified by Rasheed et al. (2020), in which the concentration of Zn in the roots of *Conocarpus lancifolius* fertilized with WW was significantly higher in relation to the treatment of the plants of the control treatment.

Sewage sludge usually contains non-toxic organic compounds (organic matter), inorganic compounds and a relatively small amount of heavy metals such as Cd, Pb, Zn, Cu, Mn and Fe (Nissim et al., 2018; Simonette and Kiehl, 2002). Therefore, the highest concentrations of Fe and Zn in the root system of fertilized plants B25 and B50 may be related to the high amount of these metals verified in the chemical analysis of pure sewage sludge (Table 7).

The statistical treatment for principal components (PCA) in relation to nutrients (Figure 2A), considered the first and second principal components (x and y axes) with 65.9% of the sample variance. Thus, there was the formation of three distinct response groups as the NPK treatment group, WW treatment

Table 4. Height (H), stem diameter (SD), leaf area (LA), leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM), total dry mass (TDM) of *Eucalyptus urograndis* plants after 120 days of planting, submitted to different nutritional sources.

Treatments	H (cm)	SD (mm)	LA (cm²)	LDM (g)	SDM (g)	RDM (g)	TDM(g)
NPK	219.8 C	13.7 B	7010.0 B	45.6 B	74.8 B	52.4 B	172.8 B
WW	236.6 BC	13.2 B	11033.4 B	57.6 B	72.0 B	53.6 B	183.2 B
B25	291.0 AB	20.0 A	15728.8 AB	117.6 A	271.6 A	130.4 A	519.6 A
B50	316.2 A	20.4 A	21316.7 A	127.6 A	310.8 A	139.2 A	577.6 A

Means followed by the same letters in the column do not differ significantly by Tukey's test ($p \leq .05$). NPK – control treatment; B25 - biosolid with addition of 25% of the vessel volume; B50 - biosolid with addition of 50% of the vessel volume; WW – Wastewater.

Table 5. Concentration of macro and micronutrients in *E. urograndis* leaves after 120 days of planting, subjected to different nutritional sources.

	Treatments			
	NPK	**WW**	**B25**	**B50**
	----------g kg⁻¹----------			
N	13.8 b	21.4 ab	27.4 a	28.9 a
P	1.1 b	0.7 c	1.6 a	1.6 a
K	11.7 a	6.7 b	5.1 b	5.8 b
Ca	14.3 a	12.1 ab	9.7 b	10.8 b
Mg	3.4 a	2.6 b	2.5 b	2.7 b
S	1.0 c	1.4 b	2.0 a	2.2 a
	----------mg kg⁻¹----------			
B	18.9 b	26.5 a	20.5 b	22.9 ab
Cu	44.5 a	32.3 a	23.7 a	34.5 a
Fe	305.8 a	313.5 a	217.3 a	224.0 a
Mn	650.8 b	862.7 b	1523.0 a	1083.0 ab
Zn	39.2 b	33.3 b	156.5 a	160.0 a

Means followed by the same letters on the line did not differ significantly by Tukey's test ($p \leq .05$). NPK – control treatment; B25 - biosolid with addition of 25% of the vessel volume; B50 - biosolid with addition of 50% of the vessel volume; WW – Wastewater.

Table 6. Macro and micronutrients in the stem tissue of *E. urograndis* after 120 days of planting, submitted to different nutritional sources.

	Treatments			
	NPK	**WW**	**B25**	**B50**
	----------g kg⁻¹----------			
N	6.8 b	8.2 ab	9.5 a	9.9 a
P	0.8 b	0.3 c	1.0 ab	1.1 a
K	8.2 a	5.7 b	2.8 c	3.3 c
Ca	6.8 b	8.6 a	6.4 b	5.7 b
Mg	1.0 b	1.3 a	1.0 b	0.9 b
S	0.6 b	0.6 ab	0.7 a	0.7 a
	----------mg kg⁻¹----------			
B	15.2 a	13.7 a	14.0 a	12.7 a
Cu	5.5 b	11.2 a	8.0 ab	8.8 ab
Fe	81.5 a	58.3 a	28.5 a	34.7 a
Mn	205.5 a	443.7 a	456.0 a	302.0 a
Zn	24.7 b	14.7 b	149.2 a	157.5 a

Means followed by the same letters on the line, do not differ significantly by Tukey's test ($p \leq 0.05$). NPK – control treatment; B25 - biosolid with addition of 25% of the vessel volume; B50 - biosolid with addition of 50% of the vessel volume; WW – Wastewater.

and sewage sludge treatments (B25 and B50). There was greater similarity between B25 and B50, probably because they are from the same nutritional source.

For plants fertilized with NPK, there was greater representation of K content in leaves, stem and root, while Ca and Mg were more representative for leaf tissue.

In plants that received WW, there was greater representation for the levels of Ca in the stem, B in the root and Mg in the stem and root. Regarding the leaf tissue for this treatment, only the Fe content was representative.

P contents in stem, leaves and root showed greater variability, being more representative in treatments B50 and B25 and less in plants of WW treatment. Treatments with sewage sludge were also more representative for the contents of N, S and Zn in leaves, stem and root. With the exception of B, the root contents of the other analyzed micronutrients (Cu, Fe, Zn and Mn) were more representative in treatments B25 and B50.

The greater representation of nutrients in the root, especially metals, in treatments with sludge is due to the presence of these elements in the sludge and demonstrates the ability of *Eucalyptus urophylla* to retain high levels of these elements in the roots. Forest species play an important role in protecting the environment by absorbing harmful heavy metals from the soil through the development of extensive root systems (Bhati and Singh, 2003). Several studies corroborate the potential for eucalyptus species to accumulate large amounts of these metals (Luo et al., 2019; Nissim et al., 2018; Peng et al., 2012; Simonette and Kiehl, 2002).

The fact that B in the root presents a negative correlation with the treatments with B25 and B50 and a positive correlation with the WW treatment is due to the high solubility of this nutrient, which is concentrated in the wastewater when it is separated from the sludge.

Material and Methods

Characterization of the local

The study was carried out at the Department of Chemical and

Table 7. Macro and micronutrients in *E. urograndis* roots after 120 days of planting, submitted to different nutritional sources.

	Treatments			
	NPK	**WW**	**B25**	**B50**
	----------------------	---------------- $g\ kg^{-1}$	----------------	----------------------
N	7.4 b	8.5 b	15.2 a	16.2 a
P	0.3 b	0.3 b	2.8 a	3.5 a
K	6.1 a	3.8 b	2.9 b	2.8 b
Ca	5.6 a	5.3 a	5.7 a	5.5 a
Mg	1.5 ab	1.7 a	1.3 b	1.1 b
S	0.8 b	1.0 b	2.6 a	2.5 a
	----------------------	---------------- $mg\ kg^{-1}$	----------------	----------------------
B	19.8 ab	21.4 a	15.7 b	17.3 ab
Cu	8.2 b	13.0 b	34.3 ab	44.3 a
Fe	5221.3 b	7828.2 ab	9053.5 a	8295.0 ab
Mn	123.5 a	150.3 a	190.0 a	140.5 a
Zn	60.5 b	24.3 b	378.2 a	368.5 a

Means followed by the same letters on the line do not differ significantly by Tukey's test ($p \leq 0.05$). NPK – control treatment; B25 - biosolid with addition of 25% of the vessel volume; B50 - biosolid with addition of 50% of the vessel volume; WW – Wastewater.

Biological Sciences - IBB/São Paulo State University, Botucatu Campus, SP Brazil, located at the geographic coordinates 22°53'42.4" South latitude and 48°29'36.6" West longitude and approximately 840 m altitude, in a greenhouse condition, from February to June (spring-summer). The climate, according to the Köppen classification, is characterized as hot and humid temperate, with rainy summers, dry winters and an average annual rainfall of 1,428 mm. The average annual temperature is 20.3 °C, the highest rainfall occurs between December and February and the dry season between June and August (Cunha; Martins, 2009).

Plant material

The genetic material chosen was the clone named I 144 (*Eucalyptus urograndis*), from the seedling nursery in the municipality of Avaré, São Paulo - Brazil. The seedlings were transplanted at 80 days into 22 L polyethylene pots. The trial was carried out for 120 days, a time that characterizes the initial development of the eucalyptus crop.

Biosolid and wastewater

The biosolid used (Table 1) came from the Sewage Treatment Station, located on the Campus of Experimental Lageado Farm, municipality of Botucatu, SP. Leaving the settling pond, the biosolid was transferred to the drying bay where it spent about 70 days and reached a temperature above 55 °C, allowing its disinfection in accordance with the processes of significant reduction of pathogens (Brazilian National Environment Council – CONAMA, Resolution nº 375/2006).

The soil used was the Dystrophic Red Latosol, with a sandy loam texture. The total amount of soil in the pots varied according to the treatment applied. After chemical analysis (Table 2), 1.6 t ha^{-1} of agricultural limestone (TNRP 70%) was incorporated into the soil to supply Ca and Mg and pH correction (Gonçalves, 1995) for the treatments with NPK and treated wastewater. The treatments with biosolids did not receive limestone since the incorporation of the material was enough to raise the pH of the soil (Table 1). For fertilization of the control treatment (NPK), urea, simple superphosphate, potassium chloride, borax and zinc sulfate (60 kg ha^{-1} of N, 100 kg ha^{-1} of P_2O_5, 80 kg ha^{-1} of K_2O, 1 kg ha^{-1} of B and 1.5 kg ha^{-1} of Zn) were incorporated to the soil, according to Gonçalves (1995).

The wastewater, used (Table 3) was generated at the Sewage Treatment Station (ETE - Sabesp) that treats urban sewage, located on the campus of Fazenda Experimental Lageado, municipality of Botucatu, SP. To meet the class 2 standard of the National Water Resources Council (CNRH, 2005) for reuse in tree crops, it went through the primary treatment system at the ETE and was transported to the experimental area. Before being applied to plants, this water passed through sand filtration and ozonation to eliminate the microbial load. The wastewater underwent filtration and ozonation. The filter, which had a diameter of 0.20 m and a height of 0.70 m, contained sterilized sand with coarse and fine granulometry of 0.06 m, a layer of 0.01 m of spongy material, and a layer of 0.09 m of gravel No. 0, as proposed by the methodology of Gomes (2016). After passing through the filter, the wastewater was transferred via plastic pipe to the ozone reactor to eliminate the microbial load from the water. The water, suitable for reuse in plants, was stored in a one-thousand-liter tank.

Treatments

The treatments consisted of different sources of nutrients for the plants, as follows: Conventional fertilization with NPK (NPK); Wastewater irrigation (WW); Incorporation of sewage sludge (biosolid) into the soil in the range of 25% of the vessel volume (B25);Incorporation of sewage sludge (biosolid) into the soil in the range of 50% of the vessel volume (B50). The treatment defined as ''conventional fertilization'' was used as a control treatment, since plantations with satisfactory productivity should receive nutrition. Nutrition in forestry production normally comes from mineral fertilizers based on NPK with the addition of micronutrients, which were used in the control treatment of this study. The water management was determined based on the three soil water retention curves (soil only; soil and 25% volume of potting soil; soil and 50% volume of potting soil) (Figure 1) using the Richards pressure chamber method and adjusted by the model proposed by Van Genuchten (1980). Five tensiometers were installed per experimental plot in pots at a depth of 0.15 m, totaling 20 tensiometers. Soil water tension was monitored twice a day, in the morning (9 am) and afternoon (3 pm), using a digital tensiometer, and the tension values were converted to volumetric moisture content based on the fitting equation of the soil water retention curve. The total volume of water applied for the NPK treatment was 114.70 L per plant, and 98.76 L per plant for the WW treatment plants. The B25 treatment plants received 92.01 L per plant, and the B50 treatment plants received 75.80 L per plant.

All pots received drip irrigation, raising or maintaining soil moisture at a tension of 14 kPa, considered as tension corresponding to control moisture, close to field capacity. The WW treatment was irrigated exclusively with wastewater, while the other treatments were irrigated with tap water.

Variables measured

At the end of the 120 experimental days, five plants from each treatment were randomly selected for height (cm), stem diameter (mm) and leaf area collection. To measure the leaf area, all the leaves of each plant were collected, and their areas were measured by the leaf area integrator model Li-3100C, Li-COR®, whose results are expressed in cm^2. From the determination of growth parameters and plant biomass, the following growth indices were determined: SLA (Specific Leaf

Area), LWR (Leaf Weight Ratio), and LAR (Leaf Area Ratio) according to the method of Hunt (1982). The indices were analyzed at the end of the 120-day experiment.

For the dry matter analysis, four plants per treatment were separated into leaf, stem and root and dried in an oven with forced air circulation at 60 °C ± 5 until constant mass. After drying, the samples were ground and sent to the Laboratory of Mineral Nutrition of Plants at FCA/UNESP – Botucatu, SP, to determine macro (N, P, K, Ca, Mg and S) and micronutrients (B, Cu, Fe, Mn and ZN) according to Malavolta et al. (1997).

Data analysis

The experimental design was in randomized blocks, consisting of 4 treatments and 5 blocks, with each plot consisting of 10 pots with one plant per pot, totaling 50 pots per treatment. The data obtained were submitted to analysis of variance and the means were compared by the Tukey test, at 5% probability with the aid of the Minitab statistical package v.16. Based on the large number of variables generated in this study, a multivariate statistical analysis—Principal Component Analysis (PCA)—was performed using R software, version 4.0.0, in order to group the main components according to their responses to the treatments.

Conclusion

The plants treated with WW did not present growth parameters (height, diameter, leaf area) significantly superior to the control treatment (NPK). The application of biosolids significantly improved the growth parameters and nutritional parameters of *Eucalyptus urograndis* plants and proved to be an efficient source mainly of N, P, S and Zn. For plant management, it was concluded that the B25 treatment was sufficient to meet the nutritional demands of the hybrid *Eucalyptus urograndis*.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests.

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