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Growth of eucalyptus seedlings irrigated with different wastewaters

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This study aimed to evaluate the growth of two species and one eucalyptus hybrid irrigated with different wastewaters. The experiment was carried out in a greenhouse in a completely randomized split-plot experimental design with three replications. The effluent included: Urban source, beef cattle slaughterhouse, poultry/swine slaughterhouse and soy processing/dairy plant from the wastewater treatment plants (WWTPs). In the subplot, the wastewater was diluted to the concentrations of 0, 25, 50, 75 and 100%. In the subplot, two species (*Eucalyptus citriodora* and *Eucalyptus urophylla*) and a eucalyptus hybrid (*Eucalyptus urophylla* x *Eucalyptus grandis*) were tested. The following physiological attributes were evaluated: mortality, root area, leaf area, stem diameter, plant height, green matter mass and dry matter weight. The results showed that the water from the beef cattle slaughterhouse WWTP, at 50% concentration, impaired growth of genotypes and promoted higher mortality values. As the concentrations of the four wastewater types increased, there was a significant variation for all characteristics except for: leaf area, plant height and dry matter weight with the soy processing/dairy WWTP water. The tested waters might be used in seedling irrigation of the two Eucalyptus plant species and the hybrid.

Key words: Sewage sludge, water reuse, seedling production.

INTRODUCTION

The planned use of wastewater residues from treatment plants (WWTP) are an important way to reduce or eliminate the discharge of nutrients and toxic elements into water bodies. In agriculture, this approach also ensures the supply of water and nutrients for the crops, reducing costs with the use of mineral fertilizers (Carey

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and Migliaccio, 2009; Qadir et al., 2010).

Eucalyptus plantations are expanding rapidly in tropical regions, with about 20 million hectares worldwide (Grant et al., 2012; Booth, 2013). The area of these fast-growing plantations in Brazil increased from 3.4 million hectares in 2005 to 5.6 million ha in 2014 (ABRAF, 2006; IBA, 2015), which represents about 25% of the total eucalyptus plantation area in the world and almost 70% of planted forests in Brazil.

Eucalyptus is the most planted forest species in reforestation programs in Brazil, usually it grows in low fertility soils where the rainfall quantity and distribution limit the survival and growth of other trees (Gama-Rodrigues et al., 2005). The use of fast-growing species, as eucalyptus, for timber production is concerned with the fixation (sequestering) of carbon (C), that is, the mobilization of CO_2 in the forest biomass, and especially in the timber product that has, as a rule, a long duration (Lima, 2005).

The objective of this work was to evaluate the physiological attributes of seedlings of two species and a hybrid of eucalyptus irrigated with four different wastewater.

MATERIALS AND METHODS

This study was conducted in a vegetation house at the Universidade de Rio Verde-GO, located in the city of Rio Verde, whose coordinates are: 50° 57’ 54’’ west longitude and 17° 47' 15'' south latitude, with an average altitude of 784 m.

Eucalyptus seeds were acquired at the Institute of Instituto de Pesquisa Florestais-IPEF-Piracicaba-SP and had an 80 to 90% germination rate. The seeds were sown in 50 cm^3 tubes filled with Bioplant® substrate and remained for 15 days in the nursery of the Comigo II Forest Company. During germination, they were irrigated by an automatic system with water coming from the company reservoir and covered with shade cloth (40% shading) until the thinning time, which occurred 30 days after emergence, leaving only one plant per tubet.

The phytosanitary control was carried out with the application of the fungicide Ridomil® and the insecticide Enguel®, according the criteria adopted by the Comigo Florestal Company.

The residuary waters were collected after their treatment from WWTP I - Urban source, WWTP II -Beef cattle slaughterhouse, WWTP III - Poultry/swine slaughterhouse and WWTP IV - Soy processing/dairy plant.

Irrigation with the wastewater treatment effluents was carried out with 2 manual sprinklers three times a day, at 7 a.m., 1 p.m. and 6 p.m. for 75 days. The WWTP wastewaters were diluted with distilled water at concentrations of 0, 25, 50, 75 and 100%.

The chemical composition of the wastewater was determined by the soil laboratory at the Universidade de Rio Verde, according to the methodology cited by Silva (1999). Two samples were taken, the first, on the day the water was captured at its origin and the second, seven days after the first collection, in order to verify changes in composition during the storage period in polyethylene bottles (Table 1). The electrical conductivity- EC and pH were determined by the laboratory of the WWTP and WTP of the BRF agribusiness using a Metrotem Micro Processor-Condutivity Meter-TDS apparatus and a Texto portable pH meter (Table 1).

The experiment was conducted in a completely randomized split plot design with three replications. In the main plot, it was allocated the wastewater type; WWTP I - Urban source, WWTP II -Beef cattle slaughterhouse, WWTP III - Poultry/swine slaughterhouse and WWTP IV - Soy processing/dairy plant, in a subplot, the wastewater was diluted to the concentrations of: 0, 25, 50, 75 and 100% and also in a subplot the two eucalyptus species (*Eucalyptus citriodora*, *Eucalyptus urofila*) and the eucalyptus hybrid (*Eucalyptus urophylla* x *Eucalyptus grandis*). Each subplot consisted of 20 eucalyptus seedlings and the 10 central plants were considered the useful area.

The volume of water supplied for each subplot presented variation according to crop need detected by the symptoms of water stress. The water levels used were 7.5, 10, 15 and 20 mm, equivalent to 2.25, 3, 4.5 and 6 liters per day, respectively until the end of the experiment, divided into three daily applications.

Seventy-five days after sowing, the plants were harvested and their physiological attributes were evaluated: plant mortality, root area, stem diameter, plant height, shoot green matter mass, leaf area and shoots dry matter mass of the eucalyptus seedlings according to the treatments.

Mortality (number of dead plants) was obtained by the difference between the count of living seedlings and amount of seedlings used in each subplot (20 plants). Root area (cm^2) was determined from the average values obtained from six plants. This was accomplished prior to separation of soil from root system by washing using running water. The material was stored in plastic bags with 30% alcohol in order to maintain hydration of real roots, and quantified using the "QuantROOT version 1.0" software (UFV).
Plant leaf area (cm^2) was obtained from the mean values of six plants from each subplot determined by the digitalization of all leaves previously separated from the stem with scissors, and quantified using the "QuantROOT" software (UFV).

Stem diameter (cm) was obtained from average stem diameter of six plants from each subplot using a caliper, measured in the region of seedling collection. Plant height (cm) was obtained from the average height of six plants of each subplot, by measuring them from the collar until the last leaf using a millimeter ruler.

Fresh matter of the aerial part (g $plant^{-1}$) was obtained from the average weight of ten plants of each subplot. There was separation of the shoot at the collar region and subsequent weighing, using a digital scale with a precision of 0.001 g.

Dry matter of the aerial part (g $plant^{-1}$) was obtained from the average weight of ten plants of each subplot. There was a separation of the aerial part at the collar and the leaves, along with the stems, they were placed in paper bags and placed in an oven with forced-air circulation at 65°C, until constant weight. The material was then weighed on a 0.001 g digital scale.

Data of all the characteristics were subjected to statistical analysis. Regression by orthogonal polynomials was employed for the wastewater dilution factor, for the water types and species and they were analyzed by the Tukey test at 5% probability, using SISVAR statistical program (Ferreira, 2000).

RESULTS

The waste water from the beef cattle slaughterhouse WWTP presented the highest electrical conductivity, thus explaining the high mortality of plants irrigated at concentrations of 75 and 100%, respectively (Figure 1A).

The other types of wastewater also promoted significant plant mortality, a fact that must be associated with a gradual increase in nutrients as the wastewater concentration increased. The wastewater concentration increase was proportional to the number of dead plants with the use of all types of wastewaters (Figure 1A). The beef cattle slaughterhouse WWTP water stood out for

Table 1. Macronutrients and micronutrients contents in wastewaters and UniRV well water used in irrigation of eucalyptus seedlings in the various treatments. WWTP I (Urban source), WWTP II (beef cattle slaughterhouse), WWTP III (poultry and swine slaughterhouse), WWTP IV (soy processing and dairy plant).

Wastewater	Concentration	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	Electric conductivity	pH
		mg/L										μs/cm 25°C	
WWTP I	100%	14.50	1.25	15.1	14.61	3.62	20.58	0.321	0.022	0.001	0.001	577.00	8.43
	75%	10.90	0.95	11.7	16.75	3.85	15.91	0.241	0.017	0.001	0.001	464.00	8.47
	50%	7.24	0.64	8.35	18.90	4.08	11.25	0.161	0.012	0.001	0.001	399.00	7.30
	25%	3.62	0.33	4.98	21.04	4.30	6.58	0.081	0.006	0.001	0.001	285.00	7.90
	0%	0.00	0.03	1.60	23.18	4.53	1.91	N.D	N.D	N.D	N.D	N.D	N.D
WWTP II	100%	53.00	10.7	51.70	126.00	11.94	7.69	0.534	0.089	0.013	0.001	3.770	7.60
	75%	39.80	8.06	39.20	100.3	10.09	6.24	0.401	0.067	0.01	0.001	3.260	7.63
	50%	26.50	5.38	26.70	74.61	8.24	4.80	0.268	0.045	0.007	0.001	2.410	7.89
	25%	13.30	2.70	14.10	48.89	6.38	3.36	0.134	0.023	0.004	0.001	1.728	7.82
	0%	0.00	0.03	1.60	23.18	4.53	1.91	N.D	N.D	N.D	N.D	N.D	N.D
WWTP III	100%	22.00	5.54	54.00	37.97	4.97	41.72	5.972	0.254	0.001	0.001	1.245	7.21
	75%	16.50	4.16	40.90	34.27	4.86	31.77	4.479	0.191	0.001	0.001	953.00	7.13
	50%	11.00	2.78	27.80	30.58	4.75	21.81	2.987	0.128	0.001	0.001	764.00	7.04
	25%	5.49	1.40	14.70	26.88	4.64	11.86	1.494	0.064	0.001	0.001	503.00	7.33
	0%	0.00	0.03	1.60	23.18	4.53	1.91	N.D	N.D	N.D	N.D	N.D	N.D
WWTP IV	100%	1.17	3.19	20.00	27.66	2.37	222.00	0.19	0.001	0.001	0.001	999.00	9.86
	75%	0.88	2.40	15.40	26.54	2.91	167.00	0.143	0.001	0.001	0.001	890.00	10.00
	50%	0.58	1.61	10.80	25.42	3.45	111.9	0.096	0.001	0.001	0.001	710.00	9.77
	25%	0.29	0.82	6.20	24.30	3.99	56.92	0.048	0.001	0.001	0.001	491.00	8.40
FESURV WELL	0%	0.00	0.03	1.60	23.18	4.53	1.91	N.D	N.D	N.D	N.D	N.D	N.D

*ND: not shown.

presenting the death of almost all plants in the subplot when irrigated at the 100% concentration.

The root area, as a function of the wastewater concentration had linear positive behavior for the poultry/slaughterhouse WWTP and quadratic for the remaining treatments (Figure 1B). The wastewaters from the soy processing/dairy plant WWTP, beef cattle slaughterhouse WWTP and urban source WWTP, resulted in a maximum technical efficiency index of 75, 48, 67%, respectively. The inhibition of root growth can be linked to the high concentration of nutrients present in these treatments, with amounts of nutrients greater than required for eucalyptus seedlings. For the leaf area and stem diameter, it was observed that irrigation with most of the wastewaters promoted the highest average (Figure 1C and D) values, except the soy processing/dairy plant treatment, probably associated with its low N and P contents (Table 1).

The highest plant height (34.06 cm) was obtained for the urban source wastewater (Figure 1E) treatment, and the lowest average value was detected in plants irrigated with wastewater from soy processing/dairy plant and beef cattle slaughterhouse WWTPs.

For the shoot green and dry matter mass production, it was observed that the plants irrigated with wastewaters from the beef cattle slaughterhouse WWTP showed higher green and dry matter mass (Figure 1F and G). The leaf area, stem diameter, plant height and shoot green and dry matter mass presented similar behavior for the increasing wastewater concentrations. The WWTP-beef cattle slaughterhouse data were adjusted to a quadratic regression model and the remaining waters to positive linear model (Figure 1).

The WWTP-beef cattle slaughterhouse wastewater promoted the highest average values for leaf area (43%), stem diameter (34%), plant height (34%), shoot green matter mass (53%) and shoot dry matter mass (49%).

The mortality for both species and hybrid did not differ statistically from each other when subjected to treatments with waters from the soy processing and poultry/swine slaughterhouse. The wastewaters from WWTP-beef cattle slaughterhouse and WWTP-urban source presented significant differences between the species studied, showing that the hybrid was the most sensitive to both types of waters, whereas *E. citriodora* and *E. urofila* presented no significant differences between each other (Table 2).

The beef cattle slaughterhouse water presented higher plant mortality among the treatments, and this was

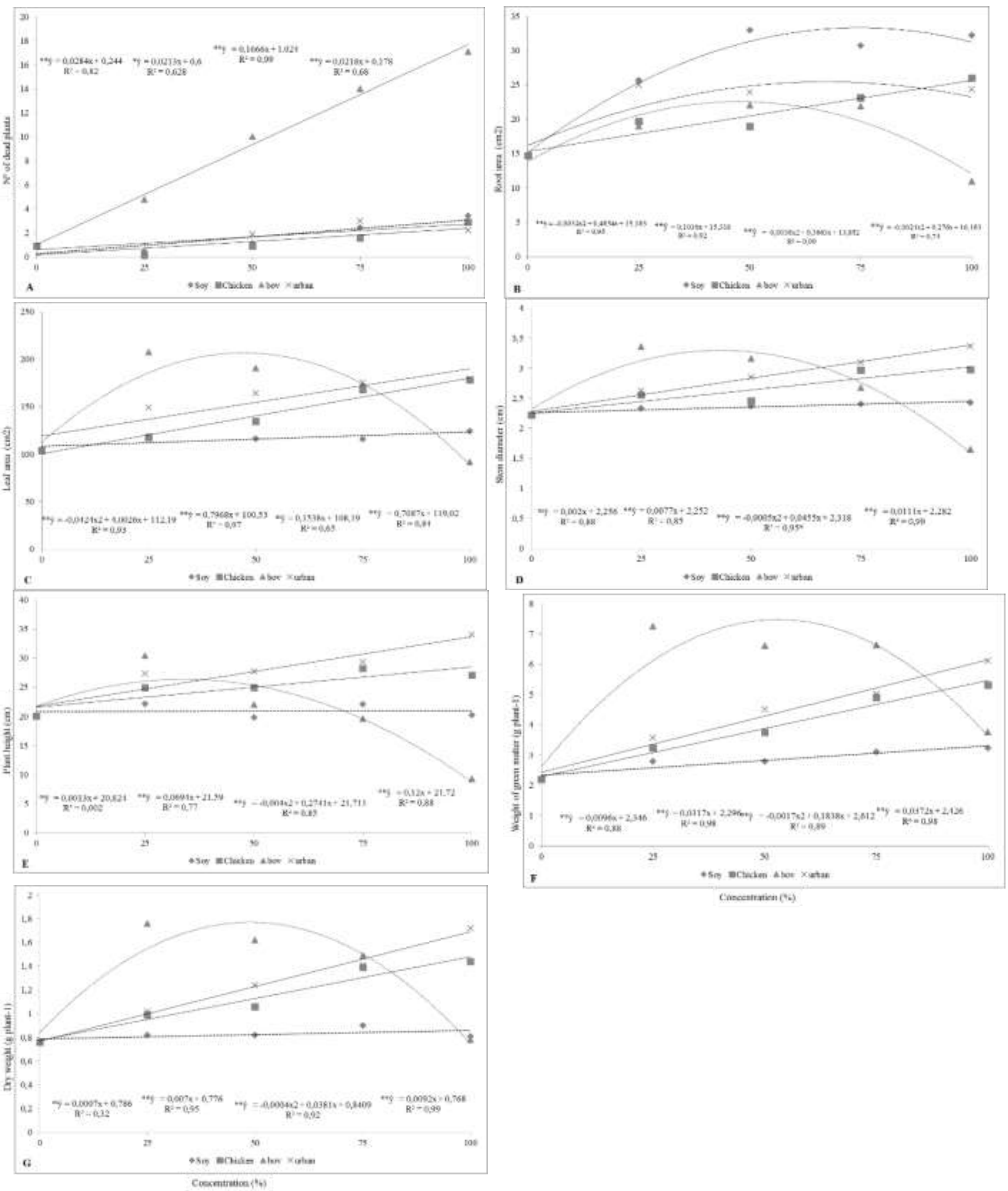

Figure 1. Number of dead plants, root area, leaf area, stem diameter, plant height, green matter mass, dry matter mass of seedlings of two eucalyptus species and a eucalyptus hybrid in function of irrigation with wastewater at different concentrations. **, *Significant at 1 and 5% probability, respectively.

Table 2. Mortality of plants and root area of seedlings of three eucalyptus genotypes within each wastewater type and behavior of the four types of water according to each species.

Genotype	WWTP I	WWTP II	WWTP III	WWTP IV
	Mortality (number of dead plants)			
Citriodora	0.87^{aA}	0.53^{aA}	8.27^{aB}	0.47^{aA}
Urofila	1.73^{abA}	1.33^{aA}	7.47^{aB}	1.53^{aA}
Urograndis	2.40^{bA}	1.93^{aA}	12.33^{bB}	3.00^{bA}
Root area (cm^2)				
Citriodora	24.47^{Ba}	21.59^{bAB}	16.85^{bC}	18.90^{bBC}
Urofila	28.98^{aA}	24.46^{aB}	19.60^{aC}	26.94^{aAB}
Urograndis	28.36^{aA}	15.46^{cC}	16.81^{bC}	20.99^{bB}
Leaf area (cm^2)				
Citriodora	123^{aA}	135^{abA}	128^{bA}	146^{aA}
Urofila	119^{aC}	157^{aB}	191^{aA}	158^{aB}
Urograndis	106^{aC}	129^{aBC}	141^{bAB}	160^{aA}
Stem diameter (cm)				
Citriodora	2.48^{AB}	2.79^{aAB}	2.37^{bB}	2.86^{aA}
Urofila	2.32^{aB}	2.63^{abAB}	2.91^{aA}	2.83^{aA}
Urograndis	2.27^{aB}	2.48^{bAB}	2.55^{bAB}	2.78^{aA}
Plant height (cm)				
Citriodora	22.85^{aB}	27.22^{aA}	19.21^{bC}	28.39^{abA}
Urofila	21.02^{abC}	26.51^{aAB}	23.67^{aBC}	28.68^{aA}
Urograndis	18.80^{bBC}	21.45^{bB}	18.00^{bC}	26.08^{bA}
Green matter mass (g plant^{-1})				
Citriodora	3.40^{aB}	4.46^{aA}	4.29^{bA}	4.59^{aA}
Urofila	2.73^{abC}	3.99^{abB}	6.70^{aA}	4.33^{aB}
Urograndis	2.36^{bC}	3.20b^{BC}	4.89^{bA}	3.93^{aB}
Dry matter mass (g plant^{-1})				
Citriodora	1.02^{aB}	1.36^{aA}	1.12^{bB}	1.39^{aA}
Urofila	0.77^{bC}	1.12^{bB}	1.61^{aA}	1.20^{abB}
Urograndis	0.67^{bC}	0.90^{cB}	1.13^{bA}	1.10b^{AB}

Means followed by the same lower case letter in the column and capital in the line do not differ by 5% with Tukey test.

associated with the chemical composition of the water (Table 2).

The root area of the three eucalyptus genotypes presented different behavior for all wastewater (Table 2). The species, E. urofila and the hybrid were more efficient when irrigated with soy processing/dairy plant water. The poultry/swine slaughterhouse water promoted larger root area for the species, *E. urofila*. The waters from the beef cattle slaughterhouse and urban source WWTPs had similar behavior.

The soy processing WWTP and the dairy WWTP promoted the formation of higher root area for the *E. citriodora,* species followed by the poultry/swine slaughter house WWTP, urban source WWTP and beef cattle slaughter house WWTP. The species *E. urofila* and the hybrid produced the highest mean root area when irrigated with treated wastewater from soy processing followed by the urban source WWTP, poultry slaughterhouse and beef cattle slaughterhouse WWTPs (Table 2). The wastewater from beef cattle slaughter-house proved to be the least efficient for root formation and the soy processing was the most efficient.

The leaf area of the three eucalyptus seedling genotypes was different, according to the water used for irrigation, and the *E. urofila* genotype presented the highest leaf area (Table 2). Seedlings of *E. urofila* irrigated with beef cattle slaughterhouse treated wastewater had the greatest leaf area. However, the *E. urograndis* eucalyptus hybrid seedlings had the highest leaf area when irrigated with wastewater from the urban source WWTP (Table 2).

It was observed that the stem diameter differed only for water coming from the beef cattle slaughterhouse, in which the species *E. urofila* was superior to others. When comparing the species within each type of water, there was a significant difference only for the *E. urofila* species and the hybrid presented the highest average when irrigated with poultry slaughterhouse, beef cattle slaughterhouse and urban source WWTP waters and the lowest average when irrigated with soy processing/dairy plant water (Table 2).

Plant height between species within each water type showed significant differences only for wastewater provided by the beef cattle slaughterhouse and poultry/swine slaughterhouse agro industries, where the *E. urofila* species was superior to the *E. citriodora* species and the *E. urograndis* hybrid. Comparing each species within each water type, it was observed that poultry/swine slaughterhouse and urban source WWTP waters were superior to the others for the species *E. citriodora.* The Soy processing water promoted the lowest plant height. The height of the *E. urofila* species irrigated with water from the urban source WWTP and poultry/swine slaughterhouse WWTP stood out, obtaining the highest average. The wastewater from urban source WWTP was shown to be superior for the *E. urograndis* hybrid plant height characteristic (Table 2).

The shoot green matter mass between species within each water type presented significant differences only for the water supplied by the beef cattle slaughterhouse. *E. citriodora* species stood out with the greatest mass weight dry for water originating from soy processing, poultry/swine slaughterhouse and urban source WWTPs. Otherwise, for the *E. urofila* species, height was the greatest for the beef cattle slaughterhouse WWTP water.

The shoot dry matter mass of *E. citriodora*, *E. urofila* and *E. urograndis* hybrid seedlings irrigated with wastewater from the soy processing/dairy plant WWTP were small. However, the seedlings irrigated with wastewater from the beef cattle slaughterhouse WWTP provided the highest mass for the *E. urofila* and *E. urograndis* hybrid species (Table 2).

Comparing the three eucalyptus genotypes as a function of the wastewaters types for the plant mortality, it was observed that irrigation with beef cattle slaughter-house water had differentiated behavior, obtaining the highest plant mortality, and this was associated with the chemical composition of this water (Table 1). Irrigation eucalyptus seedlings with wastewaters from the beef cattle slaughterhouse WWTP showed the highest eucalyptus seedlings mortality, independent of genotype evaluated.

DISCUSSION

The data demonstrate the possibility of using wastewater as an irrigation source for the eucalyptus seedlings. The application of the urban source WWTP effluent was significantly satisfactory for the growth of eucalyptus seedlings. These results are explained by many researchers, who claim that sewage effluents demonstrate a stimulating effect on the vegetative growth of the trees (Guo and Sims, 2000), for *Eucalyptus globules* (Bhati and Singh, 2003), for *Eucalyptus camaldulensis* (Ali et al., 2012) and for *Tipuana speciosa*.

Similar results are observed by Rebouças et al. (2010). These authors studied the growth of *Vigna unguiculata* (cowpea) under irrigation with treated domestic sewage wastewater, and observed an increase in the leaf area, number of leaves per plant and stem, leaf, root and total dry biomass.

According to Dechen and Nachtigall (2007), each nutrient present in the effluents has a specific role in plant metabolism and the imbalance between their proportions may cause disability or toxicity, limiting plant growth or even leading to their death.

Alves et al. (2009), studying leaf area irrigated with wastewater fertilized with nitrogen and phosphorus, observed that the application of the effluent did not affect the development of the cotton plants. The leaf area increased with increasing wastewater irrigation levels. The low concentration of N and P may impair the development of the plant aerial part and consequently present a leaf area decrease.

According to Missio et al. (2004), working with G*rápia,* showed an increase in the number of leaves and larger stem diameter in plants that received phosphorus fertilization, showing that this macronutrient is important for the development of these parts of the plant. According to Daniel et al. (1997) and Carneiro (1995), the increase in diameter of the stalk in general, is more important to indicate the seedling survivability in the field and should be more widely used in indications of fertilizer rates to be applied for seedling production.

Evaluating cotton crops irrigated with wastewater, Figueiredo et al. (2005), obtained positive results for plant height and Lemainski and Silva (2006), applying sewage sludge in corn, found better efficiency in the plant aerial part growth, when compared with mineral fertilizer. Augusto et al. (2003), evaluating the dry matter mass in forest species seedlings (*Croton floribundus* Spreng. (Capixingui) and *Copaifera langsdorffii* Desf. (Copaiba)) irrigated with wastewater, verified dry matter accumulation in the shoot as well as in the root system.

Figueiredo et al. (2005) and Azevedo and Oliveira (2005), working with wastewater on okra and cucumber crops, respectively, obtained similar results. Gomes et al. (2002) concluded that the stem diameter at the collar and plant height are the parameters most indicated for assessing the quality of eucalyptus seedlings.

According to Augusto et al. (2007), working with the production of *E. grandis* seedlings using wastewater in a continuous subirrigation system gave results that indicated that wastewater from sewage biological treatment systems can be used in nursery fertigation.

Conclusions

The tested waters might be used in seedling irrigation of the two *Eucalyptus* plant species and the hybrid. The use of wastewater WWTP as a source of water for irrigation of eucalyptus seedlings is an alternative viable for the final disposal of this waste, considering the fertilizer economy that this material can provide, in addition to the environmental benefit.

Conflict of interests

The authors have not declared any conflict of interests.

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