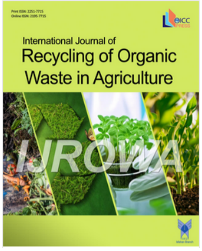

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Recycling waste biosolids to forest propagation of *Pinus leiophylla* Schltdl. & Cham.

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Original research

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Abstract:

Purpose: Analyze the use of biosolids, mixed with substrates, as an alternative in the nursery and field production of forest species such as *Pinus leiophylla*.
Method: Biosolids mixed with commercial substrates and chemical fertilizers were tested. The experiment was carried out under nursery conditions, using a randomized experimental design in a $2 \times 2 \times 3$ factorial arrangement. Two substrates were tested in this experiment: 60:30:10 (S1) and 50:40:10 (S2) of peat moss, perlite and vermiculite, respectively. Two doses of controlled release fertilizer: 2 g/L and 5 g/L. And three doses of biosolids: 0 L/L, 0.13 L/L and 0.26 L/L. To test the nursery treatments, a field quality test was conducted. The response was evaluated with morphological variables and leaf nutrient concentration.
Results: The diameter and the height of plants were favored using biosolids. The high dose of fertilization exceeded the low by 1.24 mm and 2.97 cm in diameter (D) and height (H), respectively. The S1 substrate, recorded higher values of D and H than the S2 substrate. Differences in leaf concentrations were found. The high biosolids dose showed higher means for N, P and Mn. High fertilization produced higher means in P and Mn concentration, and low fertilization only in Mg. In contrast, nutrient concentrations were not affected by the substrate factor.
Conclusion: Derived from the results obtained in this research, it is likely that with an adequate dose of biosolids and a balanced mixture, quality plants can be produced for reforestation, enhancing the development of forest species. The data observed, both for nursery propagation and for the field planting stage, indicate that the use of biosolids provides minerals that assign characteristics that improve the adaptability of the species in the natural environment.

Keywords: Nursery; Sludge waste; Nutrient concentration; Survival in reforestation; Chemical fertilization

1. Introduction

Forest fires, climate change and land use changes have been the main causes of natural forest destruction. These alterations have led to the need to increase nursery plant production to reforest and restore degraded areas. For example, at the end of the 2010´s, an average of 81 million nursery plants were produced in Mexico (Comisión Nacional Forestal, 2020) and the current trend is upward. One of the critical factors in tree seedling production is the substrate mix. Teat moss is one of the most widely used organic substrates due to its physical and chemical characteristics, water holding capacity, porosity and pH control (Pane et al., 2011). However, in recent years there has been an effort to reduce its use to compensate for the damage to the ecosystem caused by its extraction, as well as by the inflation of the cost (Vandiver-Taylor et al., 2015). An alternative to reduce the use of peat and mineral substrates is the use of biosolids generated in wastewater treatment, which can be an economical and environmental option. Biosolids are organic by products generated in large quantities by wastewater treatment plants. Worldwide, biosolids is approximately 20 million tons

(Ping and Jing-Yuan, 2016), while in Mexico it is 480 thousand tons (Alvarado-Ibarra et al., 2017). By 2021, there were 2 872 wastewater treatment plants in Mexico, of which 818 corresponded to the sewage sludge process, representing 73% of the flow treated in the country (Comisión Nacional del Agua, 2021). Actions for the use and deposition of biosolids are regulated by the Mexican Official Standard NOM-004-SEMARNAT-2002 (Secretaría del Medio Ambiente y Recursos Naturales, 2002), where the maximum permissible limits of heavy elements such as As, Cd, Cr, Cu, Pb, Hg, Ni and Zn are specified. However, the problem with the production of biosolids is that they do not have subsequent use that allows for reuse; most of them are placed in open fields deposits which represent an environmental concern (Kumar et al., 2022; Elgarahy et al., 2024).

Benefits have been observed for the use of biosolids in forestry. For instance, prolonged application significantly increased the organic C reserves in the soil of coastal dune forests (Wang et al., 2021). In one research by Alaoui-Sossé et al. (2018) noted a significantly increased species in the ecosystem, two years after it is biosolids application on the understory. Moreover, its addition to the soil improved the nutrient condition of four New Zealand native species (Gutiérrez-Ginés et al., 2017); while in adult individuals of *Eucalyptus globulus* Labill improved their water status (Donoso et al., 2016). The application of 20 Mg/ha significantly increased the concentrations of nutrients and the N content in the needles of *Pinus sylvestris* L. (Bramryd, 2013). Its repeated application for 19 years in a *Pinus radiate* D. Don plantation increased the N in its needles; the microbial community and the organic N increased in the soil (Wang et al., 2017).

Regarding the use of biosolids as substrates for nursery plant production. For *Ceiba speciose* in 280 cm^3 containers, biosolids used as the only substrate, favored greater development in height and biomass (Alonso et al., 2018). A similar effect was found in *Pinus sylvestris*, where the additions of biosolids with diatomite accelerated growth in the nursery and yield in the field (Kose et al., 2020). In *Schinus terebinthifolius* Raddi, the combination with fertilizers is recommended for nursery production (Silva-Melo et al., 2019). This contrariety of results makes sense considering the differences between species in growth behavior according to environmental conditions, especially in the growth medium, where root growth and development is primarily affected, especially when containers are used, in addition to the particularities in the composition of the biosolids used. In this sense, continue being necessary to evaluate the feasibility of using biosolids in a greater number of species so that define better guidelines for plant production specially in those that are native and economic and ecologically important. This is the case of *Pinus leiophylla* Schl. & Cham. *Pinus leiophylla* is a species native to North America, found naturally along the Sierra Madre Oriental, Sierra Madre Occidental and Trans-Mexican Volcanic Belt, and it is one of the most widely distributed species in coniferous forests (Ramírez-Orozco et al., 2022; Ricker et al., 2022; Flores et al., 2023). It is known for its resistance to salinity, can grow under restricted humidity conditions and has the ability to sprout after fire (Jimenez-Casas and Zwiazek, 2014; Barton et al., 2023). To continue research on whether or not the use of biosolids in forest species is viable or not, the objective of this study was to determine the effect of biosolids as a substrate to grow *Pinus leiophylla* in nursery, mainly on the nutritional condition of foliage. To complement the nursery effect, plant growth and survival in the field were analyzed with and without the use of biosolids.

2. Material and methods

The nursery stage was carried out from November 2021 to September 2022, in the experimental forest nursery of the Universidad Autónoma Chapingo in Mexico at 19°29′21″N and 98°52′16″W. The site has a temperate climate, with an average temperature of 16.4 °C (±1.0 °C) and annual rainfall of 616.6 mm (Instituto Mexicano de Tecnología del Agua, 2013). *Pinus leiophylla* seeds were collected in a natural stand in Tlaxcala, Mexico; 19°29′05″N and 98°35′40″W, at 2500 meters above sea level. Seeds were disinfected with 0.5% active chlorine and soaked for 24 hours before sowing. Sowing was carried out in seedbeds with perlite in germination trays. On December 15, transplanting was carried out using 305 cm^3 tubes.

A completely randomized experimental design was used, with a $2 \times 2 \times 3$ factorial treatment arrangement. The experimental unit was a table with 25 individuals and each treatment was repeated five times. The first factor was the substrate with two levels: "S1" with 60% peat moss, 30% perlite and 10% vermiculite; "S2" with 50% peat moss, 40% perlite and 10% vermiculite. The second factor was the application of chemical fertilization with two doses: low 2 g/L (Fb) and high 5 g/L (Fa) of Osmocote® brand-controlled release fertilizer with formula 14-14-14. The third factor was biosolids with three levels: 0 L/L (Bn), 0.13 L/L (Bb) and 0.26 L/L (Ba), which were uniformly incorporated with the corresponding substrate and fertilizer.

Biosolids were collected from the Atotonilco Wastewater Treatment Plant (PTARA) located in Hidalgo, Mexico. The PTARA meets the parameters and maximum permissible limits for heavy metals, pathogens and parasites established in the Mexican Standard (Secretaría del Medio Ambiente y Recursos Naturales, 2002). Controlled release fertilizer and biosolids were the only sources of nutrition during the entire nursery period. The effects of the treatments were evaluated with morphological and nutrimental variables. The morphological variables were root collar diameter (D), total height (H), root dry weight (RDW), aboveground weight (ADW), and total dry weight (TDW). D was measures with a Steren® Her-411 digital Vernier Caliper; and height with a flexometer graduated in cm and mm. The dry weights were determined in September 2022, the plants were separated into root and stem and placed in s Shel Lab® at 70 °C for 48 h. Individual growth rate was evaluated by the Daily Relative Growth Rate of Diameter (RDG) and Height (RHG) (Pallardy, 2008) with equation:

$$RG = \frac{\ln(x_2) - \ln(x_1)}{\Delta t} \quad (1)$$

where x_1 is the variable measured at the first date, and x_2 at the last date, and Δt is the time between both measurements. The Aboveground/Root Ratio (ARR) was evaluated, dividing ADW and RDW; also, indices such as the Slenderness Index (SI) dividing H by D (Johnson and Cline, 1991) and the Dickson Quality Index (DQI) (Dickson et al., 1960), with equation:

$$DQI = \frac{\text{TW (g}}{\frac{\text{H (cm)}}{\text{D (mm)}} + \frac{\text{ADW (g)}}{\text{RDW (g)}}} \quad (2)$$

For leaf concentrations, composite samples were prepared from needles of five plants each, three samples were taken per treatment giving a total of 36. Nitrogen concentration was determined with dried and ground plant tissue, using the Semimicro-Kjeldahl method (Bremmer, 1965). The concentrations of P, K, Ca, Mg and Mn were determined in the extract resulting from the HNO_3:HCl_4 (2:1, v:v) digestion of dried and ground plant tissue, with an inductively coupled plasma atomic emission spectrophotometer (ICP-OES 725 Series, Agilent) (Alcántara and Sandoval, 1999). The factorial effects on morphological and nutritional variables was analyzed by means of an ANOVA using R software (R Core Team, 2022), with reliability of 95% ($p < 0.05$). To identify differences between factors, a comparison of means was performed with the Tukey ($\alpha = 0.05$). The diagnosis of nutritional conditions was made using the vector graphic method (Timmer and Stone, 1978), and with the interpretation of the nomograms (López-López and Alvarado-López, 2010).

To test the nursery treatments, a field quality test was conducted in September 2022. The plantation is located in Tlaxcala, Mexico, 19°30′17″N and 98°32′57″W, at an elevation 2,756 masl. It has an average temperature of 10.8 °C and an average annual rainfall of 756.5 mm, with a rainy period from June to September (Instituto Mexicano de Tecnología del Agua, 2013). The field trial was established under a completely randomized experimental design. The experimental unit was a set of four individuals per treatment, with four replications per treatment. For planting, common strains of 40 × 40 × 40 cm were made, with a true frame distribution at a distance between trees of 2.5 m. A composites soil sample was taken from the top, middle and bottom of the first 20 cm of the surface for fertility analysis. A wedge rain gauge was placed in the plantation to measure precipitation and values were taken daily.

The response variables were survival and plant development with morphological variables. The increase in basal diameter (DRG, mm) with equation (1), and the percentage of survival were estimated. Four measurements were made: September 2022, December 2022, March 2023 and June 2023. The DRG analysis was performed using the ANOVA test. This analysis was only performed in the growth period between September 2022 and December 2022. This was because from that date onwards there was a high percentage of mortality that affected all treatments. Survival was analyzed with the Long-Rank statistical test (Harrington, 2005).

3. Results and discussion

Nursery stage

The results of the ANOVA and Tukey tests are described in Table 1. The three factors studied (substrate, fertilization and biosolids) showed a significant effect on diameter (D), height (H), aboveground dry weight (ADW) and total dry weight (TDW) and relative growth in both diameter (RDG) and height (RHG). Substrates showed no significant effect on root dry weight (RDW). Fertilization had no significant effect on the aboveground part-root ratio (ARR), nor on the slenderness index (SI). In the case of the Dickson Quality Index (DQI), the model showed no significant effect on the three factors ($p < 0.0846$).

Specifically, in the biosolids factor, seedlings with the low dose (Bb = 0.13 L/L) showed significantly higher values in D and RDW. The high dose (Ba = 0.26 L/L) presented significantly higher values in RHG, and SI. For H, ADW, TDW, RDG and ARR, the application of biosolids showed significantly higher values compared to the treatments without biosolids.

In this study, the diameter at root collar and the height of *Pinus leiophylla* plants were favored using biosolids as part of the substrate in nursery. The treatment that included 0.13 L/L biosolids S2xFaxBb, showed larger diameters and height (Fig. 1). On the other hand, the S2xFbxBn treatment, which did not include biosolids, showed lower height and diameter values (Fig. 1). Seedlings grown with the low dose of biosolids, reached a diameter of 9.66 mm and a height of 26.64 cm, while those grown with the high does, reached a diameter of 8.47 mm and height of 27.7 cm. These values area above 4 mm and 15.25 cm, which are the high-quality plants values recommended by the Comisión Nacional Forestal to guarantee greater plant survival in the field (Comisión Nacional Forestal, 2010).

The high dose of fertilization exceeded the low by 1.24 mm and 2.97 cm in diameter and height, respectively. This reflects that the use of fertilization in the nursery directly favors plant morphology (Heras-Marcial et al., 2022). The S1 substrate, recorded higher values of D and H than the S2 substrate. It is observed that the combination of biosolids with a substrate with a higher organic proportion reflects higher morphological values, which is consistent with the results observed by Silva-Melo et al. (2019).

In research with *P. leiophylla*, the average values recorded for D were higher, than those produced in a technified system (3.5 mm); but in the height range, seedlings were below (Palacios-Romero et al., 2017). On the other hand, the values of D, H and DQI were higher than those reported by Buendía-Velázquez et al. (2017) and Buendia et al. (2020). The average height reached was lower compared to the height recorded in a production system with bags (28.4 cm) (Pineda-Ojeda et al., 2020). DQI values of all treatment were higher than 0.5, which is indicative of the quality of the plant produced, according to (Sáenz-Reyes et al., 2018). In this research, all slenderness index (SI) are below the

Table 1. Results of the ANOVA on morphological variables of *P. leiophylla* in nursery stage.

Variable p-value	Source of variation	Treatment	Mean*	Variable p-value	Source of variation	Treatment	Mean*
	Substrate	S1	8.67 **a**		Substrate	S1	0.0095 **a**
		S2	8.17 b			S2	0.0093 b
D	Fertilization	Fb	7.81 b	RDG	Fertilization	Fb	0.0091 b
(mm)		Fa	9.04 **a**	(mm/day)		Fa	0.0097 **a**
$p < 0.0001$	Biosolids	Bn	7.14 c	$p < 0.0001$	Biosolids	Bn	0.0087 b
		Bb	9.66 **a**			Bb	0.0099 **a**
		Ba	8.47 b			Ba	0.0096 **a**
	Substrate	S1	25.15 **a**		Substrate	S1	0.0068 **a**
		S2	22.36 b			S2	0.0066 b
H	Fertilization	Fb	22.27 b	RHG	Fertilization	Fb	0.0064 b
(cm)		Fa	25.24 **a**	(cm/day)		Fa	0.007 **a**
$p < 0.0001$	Biosolids	Bn	16.92 b	$p < 0.0001$	Biosolids	Bn	0.0052 c
		Bb	26.64 **a**			Bb	0.0071 b
		Ba	27.70 **a**			Ba	0.0077 **a**
	Substrate	S1	1.81 **a**		Substrate	S1	2.17 **a**
		S2	1.88 **a**			S2	1.78 b
RDW	Fertilization	Fb	1.76 b	RHG	Fertilization	Fb	1.9 **a**
(g)		Fa	1.93 **a**	(cm/day)		Fa	2.06 **a**
$p < 0.0047$	Biosolids	Bn	1.68 b	$p < 0.0001$	Biosolids	Bn	1.49 b
		Bb	2.01 **a**			Bb	2.15 **a**
		Ba	1.83 ab			Ba	2.29 **a**
	Substrate	S1	3.87 **a**		Substrate	S1	3.27 **a**
		S2	3.31 b			S2	2.96 b
ADW	Fertilization	Fb	3.28 b	RHG	Fertilization	Fb	3.11 **a**
(g)		Fa	3.91 **a**	(cm/day)		Fa	3.12 **a**
$p < 0.0001$	Biosolids	Bn	2.55 b	$p < 0.0001$	Biosolids	Bn	2.54 c
		Bb	4.12 **a**			Bb	3.21 b
		Ba	4.1 **a**			Ba	3.6 **a**
	Substrate	S1	5.68 **a**		Substrate	S1	1.08 **a**
		S2	5.19 b			S2	1.14 **a**
TDW	Fertilization	Fb	5.03 b	RHG	Fertilization	Fb	1.06 **a**
(g)		Fa	5.84 **a**	(cm/day)		Fa	1.16 **a**
$p < 0.0001$	Biosolids	Bn	4.24 b	$p < 0.0001$	Biosolids	Bn	1.06 **a**
		Bb	6.13 **a**			Bb	1.19 **a**
		Ba	5.94 **a**			Ba	1.07 **a**

Different letters within the same factor mean indicate significant differences between means (Tukey´s test $p < 0.05$). S1 = 60:30:10, S2 = 50:40:10 peat moss, perlite and vermiculite. Fb = 2 g/L, Fa = 5 g/L. Bn = 0 L/L, Bb = 0.13 L/L, Ba = 0.26 L/L.

valor of 6, which indicates a high quality plant; the ARR showed values between 2 and 2.4 in plants with biosolids treatments, in the high dose of fertilization and in the S1 substrate. The previous ranges classifies the seedlings as medium quality (Rueda-Sánchez et al., 2014).

Treatment S1xFaxBa has the highest means in the mor-

Figure 1. Comparison of development and root size between S2xFaxBb and S2xFbxBn treatments.
S1 = 60:30:10, S2 = 50:40:10 peat moss, perlite and vermiculite. Fb = 2 g/L, Fa = 5 g/L. Bn = 0 L/L, Bb = 0.13 L/L, Ba = 0.26 L/L.

phological variables RHG (Fig. 2 (g)), ADW (Fig. 2 (d)), TDW (Fig. 2 (e)) and ARR (Fig. 2 (h)). The second interaction with the highest recorded means is S2xFaxBb, with higher means in D (Fig. 2 (a)), RDG (Fig. 2 (f)) and RDW (Fig. 2 (c)). The results of S2xFbxBn interaction, showed the lowest means for the variables D, H, RDG, RHG, ARR and SI (Fig. 2 (i)). While the S1xFbxBn treatment, showed the lowest means in RDW, TDW and DQI (Fig. 2 (j)). These last treatment agreed in having the lowest fertilizer dose and no biosolids application. The mean range of D was from 4.75 to 10.25 mm and for H was from 11.16 to 29.55 cm. High values of total dry weights may indicate a better physiological condition of the plants, as they have a larger leaf areas as observed by Moreno-Gabira et al. (2021).

The greatest differences in N, P, Ca, Mg and Mn concentrations occurred in the biosolids factor (Table 2). The high biosolids dose showed higher means for N, P and Mn, while the low biosolids dose only in Ca and Bn in Mg. Fertilization only showed significant differences in P, Mg and Mn. High fertilization produced higher means in P and Mn concentration, and low fertilization only in Mg. In contrast, nutrient concentrations were not affected by the substrate factor. In the case of K, the model showed no significant differences (0.1749) among any of the three factors.

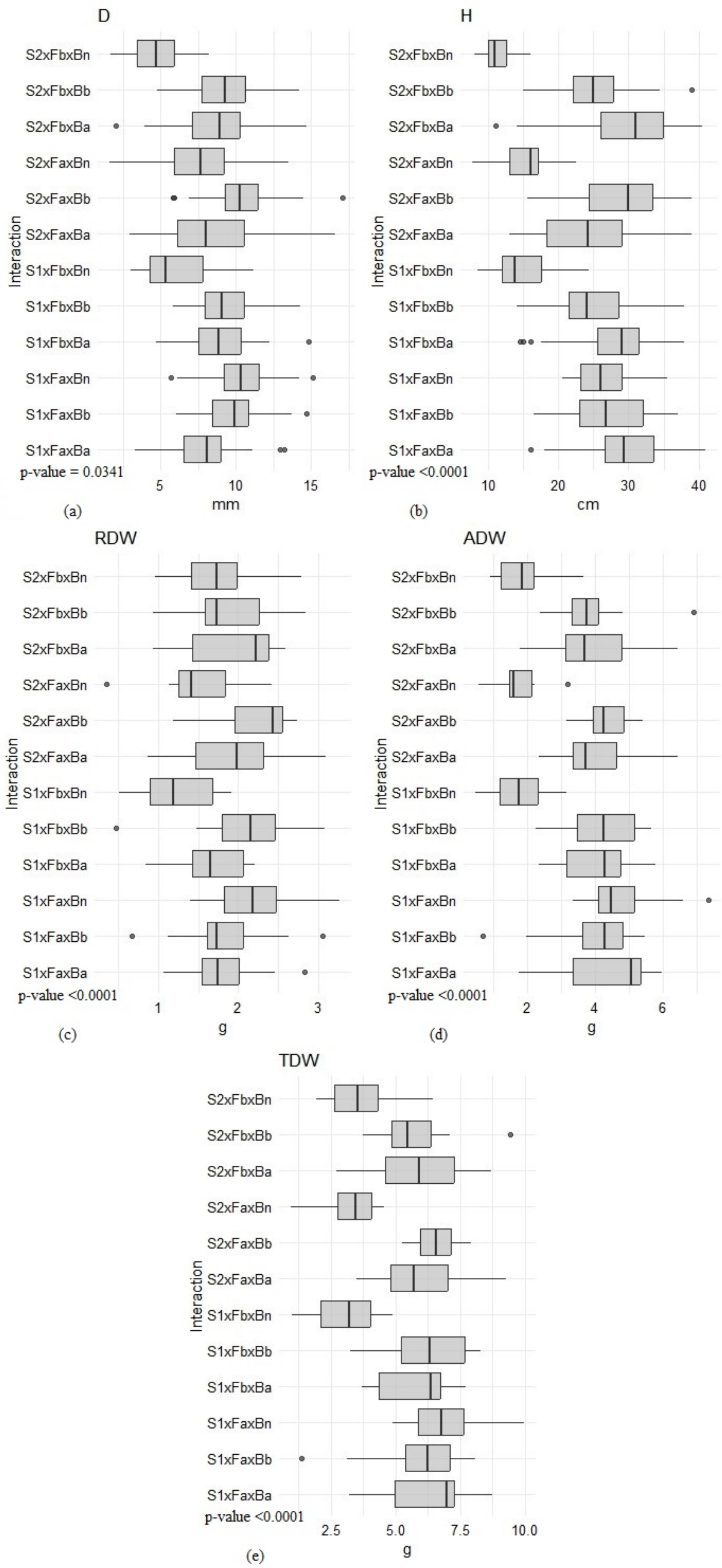
In relation to other research on *P. leiophylla*, the leaf concentration of seedlings with the high dose of biosolids, showed lower values of N, P and K compared to chemically fertilized seedlings (Buendía-Velázquez et al., 2017). This is repeated in another study, where N is lower by 1.37%, 0.06 in P, but K was higher by up to 0.66% compared to plants produced with the high dose of biosolids (Buendia et al., 2020). In comparison with conifer species, the leaf N and P concentrations resulting from the low and high biosolids doses exceeded the average value reported for Pinus greggii in nursery, but not those of K (Vicente-Arbona et al., 2019). With Pinus patula, N concentrations are lower by 0.3%, and Mn by up to 190 mg/Kg, while the concentrations of K, P, Ca and Mg found in this research are higher (Aguilera-Rodríguez et al., 2021). The higher fertilization dose reflected an increase in all morphological characteristics of the seedlings (Table 1), this result coincides with Madrid-Aispuro et al. (2020). In general, the addition of nutrients sources such as controlled release fertilizers and biosolids was beneficial for leaf N and P concentrations.

Vector plots are shown in Fig. 3. The nutrient level of plants in the S1xFbxBn interaction was taken as a reference point because it was the treatment with the low dose of fertilizer of controlled release, with no addition of biosolids and the most used substrate for plant production in the nursery.

Similar trends are observed for N (Fig. 3 (a)), P (Fig. 3 (b)) and Mn (Fig. 3 (f)), the others show an increase in biomass and concentrations, obtaining luxury consumption in seedlings. S1xFbxBa treatment shows a slight growth in biomass bur a large increase in nutrient concentration. For S2xFaxBn treatment a decrease in growth accompanied by an increase in concentration was observed. This may be because nutrients were not limiting in growth and there was an excess, which may suggest a toxic effect.

Most treatments indicated an increase in needle weight while K concentration decreased, which is interpreted as nutrient dilution, which became deficient after treatment (López-López and Alvarado-López, 2010).

Leaf Ca concentrations tended to increase or remain constant with the high dose of fertilization and to decrease with the low dose of the same material and applying biosolids of 100 needles (Fig. 3 (d)).


D
H
RDW
ADW
TDW
S2xFbxBn
S2xFbxBb
S2xFbxBa
S2xFaxBn
S2xFaxBb
S2xFaxBa
S1xFbxBn
S1xFbxBb
S1xFbxBa
S1xFaxBn
S1xFaxBb
S1xFaxBa
Interaction
p-value = 0.0341
p-value <0.0001
mm
cm
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(a)
(b)
(c)
(d)
(e)

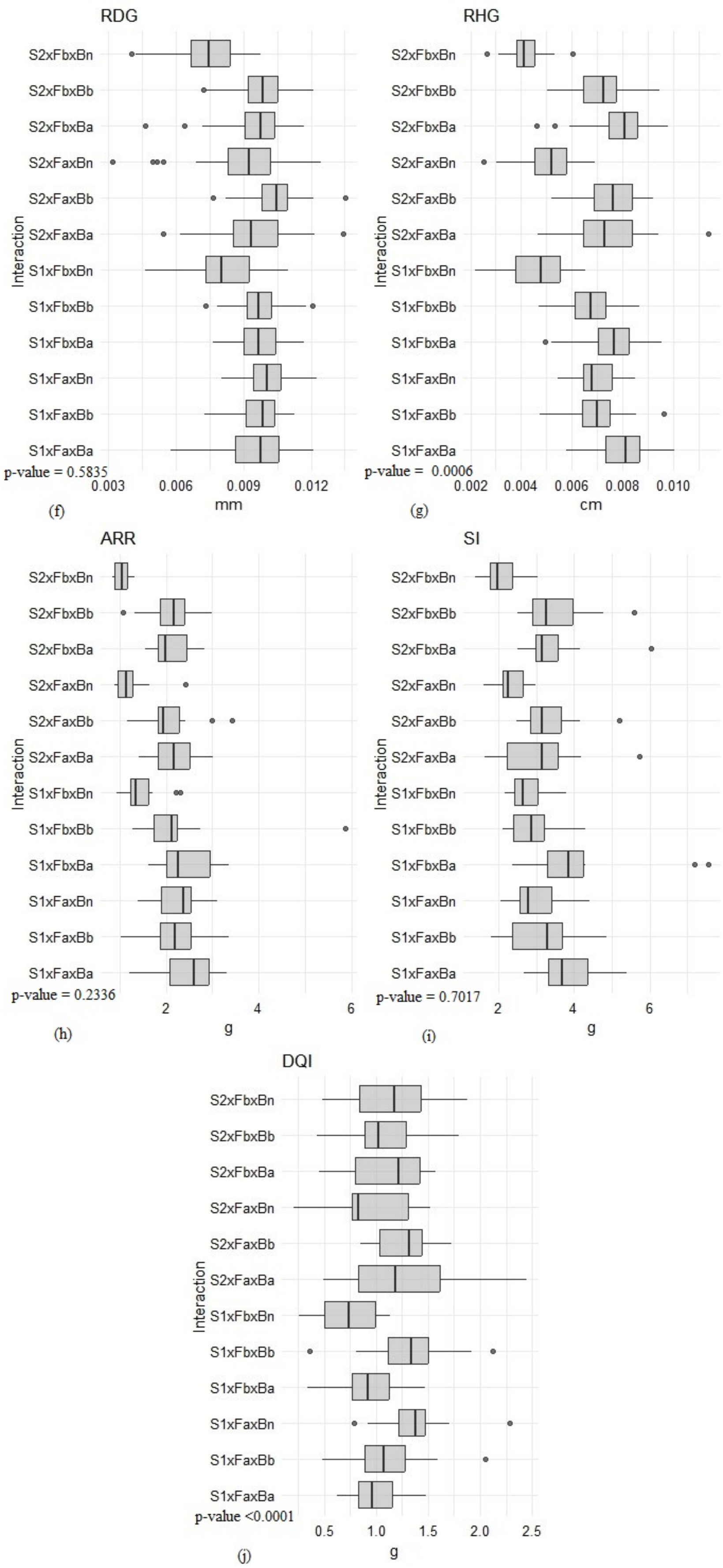

Figure 2. Interactions of substrates and doses of fertilization and biosolids on the morphological variables of *Pinus leiophylla* in nursery. S1 = 60:30:10, S2 = 50:40:10 peat moss, perlite and vermiculite. Fb = 2 g/L, Fa = 5 g/L. Bn = 0 L/L, Bb = 0.13 L/L, Ba = 0.26 L/L.

Table 2. Results of ANOVA of leaf concentration of *Pinus leiophylla* in nursery stage, subject to a combination of substrate and doses of fertilization and biosolids.

Variable p-value	Source of variation	Treatment	Mean	Variable p-value	Source of variation	Treatment	Mean
	Substrate	S1	0.91 **a**		Substrate	S1	2,624.9 **a**
		S2	0.95 **a**			S2	2,633.1 **a**
N	Fertilization	Fb	0.89 **a**	Ca	Fertilization	Fb	2,660.9 **a**
%		Fa	0.97 **a**	(mg/kg)		Fa	2,597.1 **a**
P < 0.0001		Bn	0.75 b	p < 0.1455		Bn	2,448.2 **a**
	Biosolids	Bb	0.96 **a**		Biosolids	Bb	2,741.9 **a**
		Ba	1.09 **a**			Ba	2,696.9 **a**
	Substrate	S1	0.13 **a**		Substrate	S1	1,185.6 **a**
		S2	0.14 **a**			S2	1,239.7 **a**
P	Fertilization	Fb	0.13 b	Ca	Fertilization	Fb	1,274.1 **a**
%		Fa	0.14 **a**	(mg/kg)		Fa	1,151.2 b
P < 0.0001		Bn	0.1 b	p < 0.1455		Bn	1,359.1 **a**
	Biosolids	Bb	0.15 **a**		Biosolids	Bb	1,157.1 b
		Ba	0.16 **a**			Ba	1,121.8 b
	Substrate	S1	1.01 **a**		Substrate	S1	75.87 **a**
		S2	1.04 **a**			S2	74.33 **a**
K	Fertilization	Fb	1.05 **a**	Ca	Fertilization	Fb	54.73 b
%		Fa	0.1 **a**	(mg/kg)		Fa	95.47 **a**
P = 0.1749		Bn	0.96 **a**	p < 0.1455		Bn	58.26 b
	Biosolids	Bb	1.05 **a**		Biosolids	Bb	69.15 b
		Ba	1.07 **a**			Ba	97.89 **a**

Different letters within the same factor indicate significant between means (Tukey´s test $p < 0.05$). S1 = 60:30:10, S2 = 50:40:10 peat moss, perlite and vermiculite. Fb = 2 g/L, Fa=5 g/L. Bn = 0 L/L, Bb = 0.13 L/L, Ba = 0.26 L/L.

Finally in Mg, except for S2xFbxBn and S2xFaxBn, the interactions showed growth in biomass while the Mg concentration decreased (Fig. 3 (e)), showing again a dilution effect with probable deficiency of the nutrient as a consequence of the treatment. S2xFaxBn treatment shows a decrease in both growth and concentration, indicating an antagonistic effect and excess of Mg.

Field stage

Soil fertility analysis results showed a silt loam texture, with a pH of 7.21, bulk density of 1.45 g/cm, 0.46 dS/m electrical conductivity, a cation exchange capacity of 42.33 cmol/Kg and 4.12% organic matter. The site has a concentration of N of 0.46%, 9.9 mg/Kg of P and 1.22 cmol/Kg of K. Total precipitation recorded from September 2022 to June 2023 was 443 mm. September was the highest precipitation month with 95.9 mm, while no precipitation was recorded in January, and only 2 mm pp was recorded in February.

For the recording period between September and December 2022, the Relative Diameter Growth showed statistical differences ($p = 0.0457$) (Table 3), in this case the substrate was the factor that showed differences between its means ($p = 0.0189$). Relative Height Growth showed no difference between treatments ($p = 0.9071$). However, Table 3 shows that trees with high fertilization and use of biosolids grew more in diameter, which may be a positive reflection of nursery management (Grossnickle, 2018; Grossnickle and MacDonald, 2018).

The average overall survival rate for the first three months after planting was 98% (Table 4), with eight treatments having 100% survival and only four having a mortality rate of only 6%. Mortality increased considerably in the following three months, with the average overall survival decreasing to 43%, and there were even treatments with up to 75% mortality. This trend continued after nine months, when the average overall survival was 30%. It is noteworthy that no interaction exceeded 50% survival, with S1xFbxBa having the highest survival rate. The Long-Rank test showed no differences in tree survival between treatments over time ($p = 0.515$). The highest mortality was observed in S1xFbxBn and S2xFaxBn treatments.

The average survival rate in the field was 30% nine months

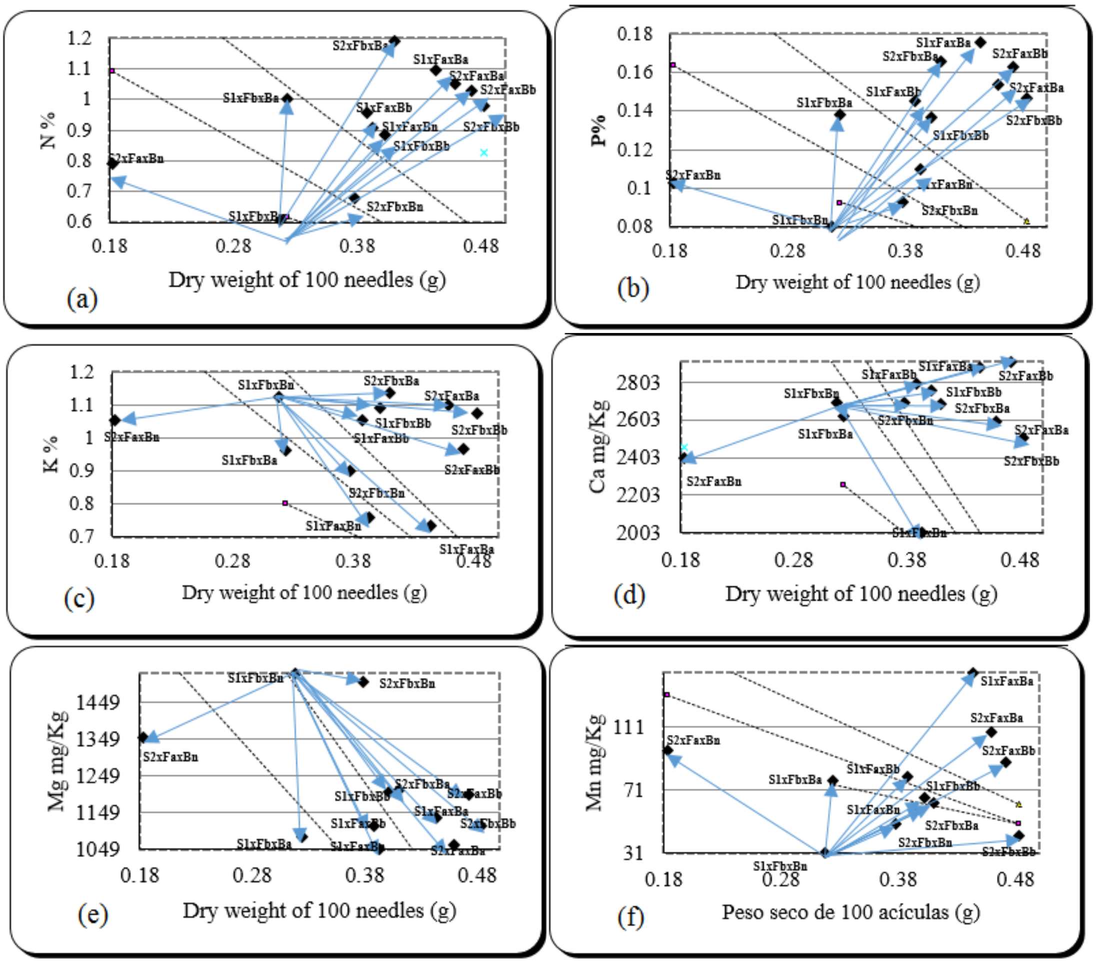

Figure 3. Timmer nomograms of foliar N, P, K, Ca, Mg and Mn concentrations in *Pinus leiophylla* seedlings in nursery stage, subject to a combination of substrates and doses of fertilization and biosolids.

after planting, this value is below the national average survival rate recorded in Mexico in the last 10 years (Comisión Nacional Forestal, 2020). Only S1xFbxBa exceeded this average, while treatments T1 and T10, which did not receive biosolids, recorded the lowest survival. This result makes it highly probable that the number of nutrient reserves in the plant is an important factor in reducing mortality. The plants that received the null dose of biosolids had a survival of 25%, while the low and high doses had a survival of 30% and 34%, respectively. The use of the fertilization technique in nursery and field is favorable for forest species (Fig. 4), decreases mortality, increases growth, and

Table 3. Results of the ANOVA of RDG and RHG of *Pinus leiophylla* in field stage nine months after establishing the plantation.

Variation	Treatment	RDG mm/month		RHG cm/month	
		Mean	p-value	Mean	p-value
Model			0.0457		0.9071
Substrate	S1	0.09 **a**	0.0189	0.01 **a**	0.3253
	S2	0.06 b		0.01 **a**	
Fertilization	Fa	0.06 **a**	0.2026	0.01 **a**	0.8909
	Fb	0.08 **a**		0.01 **a**	
Biosolids	Bn	0.06 **a**	0.266	0.01 **a**	0.9882
	Bb	0.08 **a**		0.01 **a**	
	Ba	0.08 **a**		0.01 **a**	

*Different letters within the same factor indicate significant differences between means (Tukey´s test $p < 0.05$). S1 = 60:30:10, S2 = 50:40:10 peat moss, perlite and vermiculite. Fb = 2 g/L, Fa = 5 g/L. Bn = 0 L/L, Bb = 0.13 L/L, Ba = 0.26 L/L.

Figure 4. Field development of an individual of Treatment S2xFaxBb six months after planting.

helps the plant to face adverse climatic situations such as drought (Bernaola-Paucar et al., 2022; Paz et al., 2023).

The main factor that directly affected survival in the plantation was climate, specifically precipitation. This study coincides with the fact that high mortality began to be recorded after December 2022. In the month of January 2023, no precipitation was recorded in the area and in the month of February only 2 mm of rainfall was reported. These values are below the historical monthly average recorded in January (8 mm) and February (7 mm) (Instituto Mexicano de Tecnología del Agua, 2013). This lack of rain was reflected in high mortality in March. Rainfall began to occur precisely in March, probably being too late to be used in the plantation.

Table 4. Survival of *Pinus leiophylla* in field stage.

Interaction	Months		
	Three	**Six**	**Nine**
S1xFbxBn	94%	25%	13%
S1xFaxBb	94%	56%	31%
S1xFbxBa	100%	56%	44%
S1xFaxBn	94%	50%	38%
S1xFbxBb	100%	31%	25%
S1xFaxBa	100%	38%	25%
S2xFbxBn	100%	50%	38%
S2xFaxBb	100%	44%	25%
S2xFbxBa	94%	38%	31%
S2xFaxBn	100%	25%	13%
S2xFbxBb	100%	56%	38%
S2xFaxBa	100%	44%	38%
Average	98%	43%	30%

S1 = 60:30:10, S2 = 50:40:10 peat moss, perlite and vermiculite. Fb = 2 g/L, Fa = 5 g/L. Bn = 0 L/L, Bb = 0.13 L/L, Ba = 0.26 L/L.

4. Conclusion

Wastewater treatment contributes to reducing water stress. Solid waste (biosolids) derived from the treatment process is a material that can be used in the agricultural sector, mainly for cotton, forestry and flower crops. The use of biosolids in the forestry sector, as nursery material, in propagation and specifically in pines, as a results of this research, conclusions of suitability can be reached if a balanced mixture is achieved that enhances the development of the forest species. Derived from the results obtained in this research, both for propagation in the nursery and for the star of planting in the field, the use of biosolids provides minerals that assign characteristics that improve the adaptability of the species in the natural environment. There is a potential in volume and mineral content with useful capacities in the production of pines plants and their propagation for reforestation purposes and with a perspective of timber production, which would be the research perspective.

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Authors contributions
The authors confirm the study conception and design: P.S.R.T., J.F.V., M.A.L.L.; data collection: P.S.R.T.; analysis and interpretation of results: P.S.R.T.; M. A. L. L.; draft manuscript preparation: P. S. R. T., J. F. V., M. A. L. L., A. K. G., C. R. A., E. B. V. The results were evaluated by all authors, and the final version of the manuscript was approved.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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.

References

Aguilera-Rodríguez M, Arnulfo A, Trejo Téllez L, Ordaz-Chaparro VM (2021) Sustratos con aserrín de coníferas y latifoliadas para producir planta de *Pinus patula* Schiede Ex Schltdl. et Cham. *Agrociencia* 55 (8): 719–732. DOI: https://doi.org/10.47163/agrociencia.v55i8.2664.

Alaoui-Sossé B, Bourioug M, Benbrahim M, Carnus JM, Clert S, Bourgeade P, Aleya L (2018) Influence of different sludge compositions on understorey vegetation in an amended *Pinus pinatser* forest plantation. *Sci Total Environ* 640–641:1082–87. DOI: https://doi.org/10.1016/j.scitotenv.2018.05.371.

Alcántara GG, Sandoval VM (1999) Manual de análisis químico de tejido vegetal. Guía de muestreo, preparación, análisis e interpretación. *Sociedad Mexicana de la Ciencia del Suelo A.C. Universidad Autónoma Chapingo, México*

Alonso JM, Marques AH, Amaral-Melo L, Santos-Leles PS, Valkinir-Cabreira G (2018) Biosolids as substrate for the *production of Ceiba* speciosa seedlings. *CERNE* 24 (4): 420–29. DOI: https://doi.org/10.1590/01047760201824042568.

Alvarado-Ibarra J, Aguilar-Gastelum I, Nubes-Ortiz G, Velázquez-Contreras (2017) Contaminación potencial por biosólidos depositados en un campo deportivo. *Biotecnia* 19 (2): 13–18. DOI: https://doi.org/10.18633/biotecnia.v19i2.379.

Barton AM, Poulos HM, Koch GW, Kolb TE, Thode AE (2023) Detecting patterns of Post-Fire pine regeneration in a Madrean Sky Island with field surveys and remote sensing. *Sci Total Environ* 867:161517. DOI: https://doi.org/10.1016/j.scitotenv.2023.161517.

Bernaola-Paucar RM, Ruiz-Blandon B, Salcedo-Pérez E, Zapata-Hernández I (2022) Nursery management factors that influence growth and survival of *Pinus douglasiana* in Mexico. *Bosque* 43 (2): 101–115. DOI: https://doi.org/10.4067/S0717-92002022000200101.

Bramryd T (2013) Long-term effects of sewage sludge application on the heavy metal concentrations in acid pine (*Pinus sylvestris* L.) forests in a climatic gradient in Sweden. *For Ecol Manag* 289:434–44. DOI: https://doi.org/10.1016/j.foreco.2012.08.045.

Bremmer JM (1965) Total Nitrogen. In: Methods of Soil Analysis. Part 2. *American Society of Agronomy. Madison, WI, USA*

Buendia M, Lopez M, Cetina V, Diaz S, Sanchez O (2020) Producción en vivero y respuesta morfofisiológica en campo de *Pinus leiophylla* Schl. & Cham. *Rev Cub Cien For* 8 (2): 358–374.

Buendía-Velázquez MV, López-López MA, Cetina-Alcalá VM, Diakite L (2017) Substrates and nutrient addition rates affect morphology and physiology of *Pinus leiophylla* seedlings in the nursery stage. *IForest* 10 (1): 115–120. DOI: https://doi.org/10.3832/ifor1982-009.

Comisión Nacional del Agua (2021) Inventario Nacional de Plantas Municipales de Potabilización y de Tratamiento de Aguas Residuales En Operación. *Ciudad de México,* https://www.gob.mx/conagua/documentos/inventario-de-plantas-municipales-de-potabilizacion-y-de-tratamiento-de-aguas-residuales-en-operacion

Comisión Nacional Forestal (2020) El Sector Forestal Mexicano En Cifras 2019. Bosques para el Bienestar Social y Climático. *México: Secretaría del Medio Ambiente y Recursos Naturales,* http://www.conafor.gob.mx:8080/documentos/docs/1/7749El\%20Sector\%20Forestal\%20Mexicano\%20en\%20Cifras\%202019.pdf

——— (2010) Prácticas de reforestación. Manual Básico. Comisión Nacional Forestal. *Secretaría del Medio Ambiente y Recursos Naturales. Zapopan, México,* https://www.conafor.gob.mx/BIBLIOTECA/MANUAL_PRACTICAS_DE_REFORESTACION.PDF

Dickson A, Leaf AL, Hosner JF (1960) Quality appraisal of white spruce and white pine seedling stock in nurseries. *For Chron* 36 (1): 10–13. DOI: https://doi.org/10.5558/tfc36010-1.

Donoso S, Peña-Rojas K, Galdames E, Pacheco C, Espinoza C, Durán S, Gangas R (2016) Evaluación de la aplicación de biosólidos en plantaciones de Eucalyptus globulus, en Chile Central. *Rev Facul Cien Agra* 48 (2): 107–119.

Elgarahy AM, Eloffy MG, Priya AK, Yogeshwaran V, Yang Z, Elwakeel KZ, Lopez-Maldonado EA (2024) Biosolids management and utilizations: A review. *J Clean Prod* 451:141974. DOI: https://doi.org/10.1016/j.jclepro.2024.141974.

Flores A, Buendía-Rodríguez E, Pineda-Ojeda T, Flores-Ayala E, Méndez-González J (2023) Genetic conservation and use of genetic resources of 18 Mexican pine species. *Diversity* 15 (6): 735. DOI: https://doi.org/10.3390/d15060735.

Grossnickle S (2018) Seedling establishment on a forest restorarion site - An ecophysiological perspective. *Reforesta* 6:110–139. DOI: https://doi.org/10.21750/REFOR.6.09.62.

Grossnickle S, MacDonald J (2018) Seedling Quality: History, Application, and Plant Atributes. *Forests* 9:83. DOI: https://doi.org/10.3390/f9050283.

Gutiérrez-Ginés MJ, Robinson BH, Esperschuetz J, Madejón E, Horswell J, McLenaghen R (2017) Potential use of biosolids to reforest degraded areas with New Zealand native vegetation. *J Environ Qual* 46 (4): 906–14. DOI: https://doi.org/10.2134/jeq2017.04.0139.

Harrington D (2005) Linear rank tests in survival snalysis. *Ency Biostati Wiley,* DOI: https://doi.org/10.1002/0470011815.b2a11047.

Heras-Marcial M, Aldrete A, Gómez-Guerrero A, Rodríguez-Trejo DA (2022) Influencia de la fertilización en la supervivencia y crecimiento de Pinus patula Schiede Ex Schltdl. & Cham. en vivero y campo. *RCHSCFA* 29 (1) DOI: https://doi.org/10.5154/r.rchscfa.2022.03.019.

Instituto Mexicano de Tecnología del Agua (2013) Estractor Rápido de Información Climatológica-ERIC III. Instituto Mexicano de Tecnología del Agua. *Secretaría del Medio Ambiente y Recursos Naturales. Jiutepec, México*

Jimenez-Casas M, Zwiazek JJ (2014) Adventitious sprouting of *Pinus leiophylla* in response to salt stress. *Ann For Sci* 71 (7): 811–819. DOI: https://doi.org/10.1007/s13595-014-0379-z.

Johnson JD, Cline ML (1991) Seedling quality of southern pines. In: Forest regeneration manual. *Kluwer Academic Publisher,* 143–59. DOI: https://doi.org/10.1007/978-94-011-3800-0"¨8.

Kose M, Ugurlu C, Oncul O, Demirci F, Angin I (2020) The use of sewage sludge and diatomite as growing medium in scots pine (*Pinus sylvestris* L.) seedling production and evaluation of its Land Performance. *Sumar List* 144 (11): 559–564. DOI: https://doi.org/10.31298/SL.144.11-12.3.

Kumar R, Vuppaladadiyam AK, Antunes E, Whelan A, Fearon R, Sheehan M, Reeves L (2022) Emerging contaminants in biosolids: Presence, fate and analytical techniques. *Emerg Contam* 8:162–194. DOI: https://doi.org/10.1016/j.emcon.2022.03.004.

López-López MÁ, Alvarado-López J (2010) Interpretación de Nomogramas de Análisis de Vectores Para Diagnóstico Nutrimental de Especies Forestales. *Madera Bosques* 16 (1): 99–108. DOI: https://doi.org/10.21829/myb.2010.1611182.

Madrid-Aispuro RE, Prieto-Ruíz JÁ, Aldrete A, Hernández-Díaz JC, Wehenkel C, Chávez-Simental JA, Mexal JG (2020) Alternative substrates and fertilization doses in the production of *Pinus cembroides* Zucc. in nursery. *Forests* 11 (1) DOI: https://doi.org/10.3390/f11010071.

Moreno-Gabira M, Gomes-daSilva RB, Almeida-Prado FP, DAndrea-Mateus CM, Villas-Boas RL, Rossi S, Montoro-Girona M, Ribeiro M daSilva (2021) Composted sewage sludge as an alternative substrate for forest seedlings production. *IForest* 14 (6): 569–575. DOI: https://doi.org/10.3832/ifor3929-014.

Palacios-Romero A, Rodríguez-Laguna R, Razo-Zárate R, Meza-Rangel J, Prieto-García F, Hernández-Flores ML (2017) Supervivencia de plantas de *Pinus leiophylla* Schiede Ex Schltdl. & Cham., al adicionar reservorios de agua al momento de trasplante en invernadero. *RCHSCFA* 23 (1): 35–45. DOI: https://doi.org/10.5154/r.rchscfa.2015.10.046.

Pallardy SG (2008) Physiology of Woody Plants. *Academic Press*

Pane C, Spaccini R, Piccolo A, Scala F, Bonanomi G (2011) Compost amendments enhance peat suppressiveness to *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia minor*. *Biol Control* 56 (2): 115–24. DOI: https://doi.org/10.1016/j.biocontrol.2010.10.002.

Paz M, Rodríguez-Trejo DA, Villanueva-Morales A, Borja-De la Rosa MaA (2023) Fertilization, plant quality and field survival of *Pinus spp*. in Ixtlán de Juárez, State of Oaxaca. *Rev Mex Cien For* 14 (76): 71–92. DOI: https://doi.org/10.29298/rmcf.v14i76.1324.

Pineda-Ojeda T, Flores-Ayala E, Flores-García A, Buendía-Rodríguez E, Cruz V Guerra-de la, Islas-Gutiérrez F (2020) Calidad de planta de seis especies del Género *Pinus* producidas en bolsas de polietileno. *Rev Mex Cien For* 11 (62) DOI: https://doi.org/10.29298/rmcf.v11i62.809.

Ping W, Jing-Yuan W (2016) Comprehensive characterisation of sewage sludge for thermochemical conversion processes - Based on Singapore Survey. *Waste Manag* 54:131–142. DOI: https://doi.org/10.1016/j.wasman.2016.04.038.

Ramírez-Orozco CL, Hernández-Díaz JC, Carrillo-Parra A, Wehenkel C, Quiñones-Pérez CZ, López-Sánchez CA, Bailón-Soto CE (2022) The centre–periphery model, a possible explanation for the distribution of some *Pinus spp*. in the Sierra Madre Occidental, Mexico. *Forests* 13:1–18. DOI: https://doi.org/10.3390/f13020215.

R Core Team (2022) R: A language and environment for statistical computing. r foundation for statistical computing. Vienna, Austria.

Ricker M, Calónico J, Castillo-Santiago M, Galicia A, Kleinn C, Martínez-Salas EM, Mondragón E (2022) Mexico's Forest Diversity: Common tree species and proposed forest-vegetation provinces. *Forests* 13 (10): 0–23. DOI: https://doi.org/10.3390/f13101598.

Rueda-Sánchez A, Solorio JDB, Trinidad-Saenz RJ, Flores-Hipólito JM, Ruiz-Prieto JA, Gutiérrez-Orozco G (2014) Quality of plants produced in forest nurseries of nayarit. *Rev Mex Cien For* 5 (22): 58–73.

Secretaría del Medio Ambiente y Recursos Naturales (2002) Norma Oficial Mexicana NOM-004-SEMARNAT-2002 Protección Ambiental. Lodos y Biosólidos. Especificaciones y Límites Máximos Permisibles de Contaminantes Para Su Aprovechamiento y Disposición Final. *Diario Oficial de la Federación. Ciudad de México. México*

Silva-Melo FA, Margueri-Nunes G, Zanon JA, Villas-Boas RL, Barboza-Silva R (2019) Granulated and biosolid fertilizers on the quality of *Schinus terebinthifolius* Raddi Seedlings. *FLORAM* 26 (3): 1–10. DOI: https://doi.org/10.1590/2179-8087.110417.

Sáenz-Reyes JT, Muñoz-Flores HJ, Pérez CMA, Rueda-Sánchez A, Hernández-Ramos J (2018) Calidad de planta de tres especies de pino en el Vivero 'Morelia', Estado de Michoacán. *Rev Mex Cien For* 5 (26): 98–111. DOI: https://doi.org/10.29298/rmcf.v5i26.293.

Timmer VR, Stone EL (1978) Soil characteristics determine soil carbon and nitrogen availability during leaf litter decomposition regardless of litter quality. *Soil Sci Soc Am J* 42 (1): 125–130. DOI: https://doi.org/10.2136/sssaj1978.03615995004200010027x.

Vandiver-Taylor A, Fain GB, Anna-Marie M, Gilliam CH, Sibley JL (2015) Distilled eastern redcedar (*Juniperus virginiana* L.) as an alternative substrate in the production of greenhouse-grown annuals. *J Environ Hortic* 33 (4): 166–72. DOI: https://doi.org/10.24266/0738-2898-33.4.166.

Vicente-Arbona JC, Carrasco-Hernández V, Rodríguez-Trejo DA, Villanueva-Morales A (2019) Seedling quality of *Pinus greggii* produced in sawdust-based growing media. *Madera Bosques* 25 (2): 1–14. DOI: https://doi.org/10.21829/myb.2019.2521784.

Wang M, Xue J, Horswell J, Kimberley MO, Huang Z (2017) Long-term biosolids application alters the composition of soil microbial groups and nutrient status in a pine plantation. *Biol Fertil Soils* 53 (7): 799–809. DOI: https://doi.org/10.1007/s00374-017-1219-8.

Wang M, Zhang Y, Huang Z, Xue J, Gutierrez-Gines MJ (2021) Limited potential of biosolids application for long-term soil carbon stabilization in coastal dune forests. *Geoderma* 403:115384. DOI: https://doi.org/10.1016/j.geoderma.2021.115384.