Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2021.67.79

Research Article
Determination of Nutrients by SEM-EDS and ICP-OES in Mexican Pine *Pinus greggii* var. *greggii*

[1,2]Rebeca Casique-Valdés, [2]Fernando Galindo-García, [2]Willian Narvaez-Ortiz, [2]Rosalinda Mendoza-Villarreal and [3]Eduardo San Martin-Martinez

[1]Instituto de Ciencias y Humanidades Lic. Salvador González Lobo, Universidad Autónoma de Coahuila, México
[2]Department of Horticulture, Universidad Autónoma Agraria Antonio Narro, México
[3]Instituto Politecnico Nacional, Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada Unidad Legaria, Ciudad de México, México

Abstract

Background and Objective: *Pinus greggii* var. *greggii*, an endemic species of northern Mexico, adapts to nutritionally poor soil conditions. Despite its importance, the information on the nutritional needs of this species is scarce. This study evaluates the applicability of the X-ray Energy Dispersion Spectroscopy method (SEM-EDS) versus the Inductively Coupled Plasma Spectroscopy (ICP-OES) towards the elemental analysis of *P. greggii* grown in conventional management with Peat Moss (PM) and traditional management with forest soil (TM). **Materials and Methods:** Nutrient determination, in most cases, is performed by wet digestion, a process that becomes laborious and dangerous for the person who develops the laboratory technique. Six consecutive samples were taken every 25-35 days to analyze by SEM-EDS and ICP-OES methods. A t-student analysis and Pearson correlation were performed using the *P. greggii* nutrient percentage results. **Results:** It was demonstrated, that the nutritional absorption in both detection methods showed a highly significant Pearson correlation for P, K, Ca and S nutrients. Seedlings grown in PM had higher dry biomass content than results found in TM. The substrate based on PM induced higher percentages of mineral absorption against TM. **Conclusion:** The elemental composition of *P. greggii* seedlings can be evaluated more precisely via SEM-EDS technique, avoiding the use of corrosive materials, which damages the environment, when the ICP-OES analysis technique is used.

Key words: SEM-EDS, greenhouse, *Pinus greggii*, peat moss, soil forest

Citation: Casique-Valdés, R., F. Galindo-García, W. Narvaez-Ortiz, R. Mendoza-Villarreal and E. San Martin-Martinez, 2021. Determination of nutrients by SEM-EDS and ICP-OES in Mexican pine *Pinus greggii* var. *greggii*. Asian J. Plant Sci., 20: 67-79.

Corresponding Author: Eduardo San Martin-Martinez, Instituto Politecnico Nacional, Biomateriales, CICATA Unidad Legaria Ciudad de México, México

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

An alternative to restore highly degraded soils is to establish plantations where survival success depends mainly on the use of plants that are adaptable to poor nutrient-soil[1]. *Pinus* greggii is an endemic Mexican pine that adapts to nutritional scarce soil conditions that are shallow and slightly alkaline, with a pH range of 7.0-8.0; this species has a great potential for forest plantations to help the recovery of eroded soils[2].

Most nurseries in Mexico use forest soil for *P. greggii* production, as the main component of the growing media[3], which is dense and often creates a compacted and poorly drained system inside the container[4]. More than 60% of nursery-grown seedlings are obtained under the traditional production method, by shoot transplant using forest soil in polybags. In Mexico, information on the nutritional demands of most forest species, specifical endemics, is meager; that delays the nutritional diagnosis and the initiation of nutrition-related programs and results in erroneous applications of fertilizer, involving economic losses and environmental degradation[5]. The determination of nutrients found in pine can be carried out by different techniques, among them, the most commonly used is the nitrogen determination by wet digestion, by the method of Kjeldahl[6]. Other macronutrients and micronutrients concentrations are determined by ICP-OES[7], or atomic absorption spectrometry[8]. These wet digestion procedures need the use of dangerous concentrated acids and time-consuming processes that can lead to systematic errors and failures[9,10]. The aim of the present work was, to study the applicability of the X-ray Energy Dispersive Spectroscopy (SEM-EDS) in comparison with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) methods. To study the elemental analysis of *P. greggii* var. *greggii* grew in traditional soil management (TM) and conventional management with Peat Moss (PM) in the greenhouse.

MATERIALS AND METHODS

Study area: The experimental design with pine seedlings was conducted in the Horticulture department of the University Autonomous Agrarian Antonio Narro, during the months of February-October, 2016, during this time the destructive samplings were carried out. Nutrient determination tests were carried out during the months of February, 2017 to June, 2017 at the biomaterials laboratory of CICATA-IPN.

Substrates: For conventional management, a mixture of peat moss and perlite was used in a 1:1 ratio (PM). In the case of traditional management, forest soil of horizon A (0-25 cm) (101° 1'37.32 W, 25° 20'44.68 N), silt and clay at 2:1:1 ratio (TM) was used. Both substrates were sterilized for 1 h in an autoclave at 6.803 kg pressure. The mixture of peat moss and perlite was fertilized with osmocote 14-14-14® (1 kg m^{-3})[11]. The forest soil mixture was not fertilized.

Plant material and culture conditions: *Pinus greggii* seeds were obtained from cones of the Cañón de Caballos stand, located in Saltillo, Coahuila, México (100° 54'46.61 W and 25° 14'47.13 N). Seeds were treated with hydrogen peroxide at 2% for 4 hrs and seeded into 77 germination trays cells previously filled with autoclaved peat moss and perlite (1:1). Trays were kept in a greenhouse under a 70% sunblock shade cloth for two months, before transplanting. Irrigation was every three days and kept at 30-35°C during the day and 20-25°C at night. The natural daytime length was approximately 14-16 hrs during the months of November-January (2016 and 2017).

Sampling: The substrates were distributed in 10×20 cm polybags. Two hundreds bags were filled with PM and the other 200 with TM. *Pinus greggii* seedling was transplanted on each polybag for a total of 400 seedlings. Tap water was adjusted to a 5.8 pH using nitric acid to irrigate plants every five days. Seedlings were covered with a 70% sunblock shade cloth in the same environmental conditions as described above. Six consecutive samplings of four replicates each (three plants as an experimental unit) per treatment (PM and TM), were taken every 25-35 days for analysing, by removing them from the bag for the following eight months. Samples were taken after 85 days of being transplanted inside the polybags for eight months (210 days from February to October, 2016). Seedlings were separated into the root and shoot with for a total of 12 plants (12 roots and 12 shoots) yielding 72 plants (144 roots and 144 shoots) per treatment. Roots and shoots were cleaned with tap water to remove any remaining substrate and rinsed with distilled water. They were placed into separate paper bags afterward and dried in an oven at 52°C for 24-72 hrs. The dried biomass was weighed.

Elemental analysis by SEM-EDS

Sample preparation: For the elemental analysis, two repetitions per treatment (three plants per experimental unit) with a total of 6 dried plants (shoot and root separately), were ground from each consecutive sample. Approximately 1 g of milled sample was taken into a crucible and calcined in a muffle furnace at 500°C for 3 hrs according to Lohse[12].

After the time in the furnace, the crucibles were coolled at room temperature. The ash was weighed and placed into a 1 cm of the diameter sample holder. The ash was retained on a conductive double-sided carbon tape (NEM TAPE Nisshin Co., Ltd., USA). Samples were not coated.

Two spectrum readings of the two replicas were measured for the elements N, P, Mg, K, Ca and S using X-ray Dispersive Energy (EDS) Spectroscopy. The SEM-EDS equipment was an INCAx-sight model 7582 (Oxford Instruments, Oxfordshire, England) with a resolution of 5.9 keV at 137 eV and a detection area of 10 mm^2 coupled with SEM scanning electron microscope column JEOL JSM-639OLV Noran Six (Jeol Ltd., Japan). The electron beam emission was generated from a tungsten filament with a spectrum range of 0-20 KeV, 11 mm of Work Distance (WD), voltage acceleration of 20 kV, electron beam diameter 60-70 SS (SpotSize) and a magnification of 5000x, the exposure for each spectrum was 120 s.

The spectrum showed the percentage of each mineral found in the ash. With this data, the percentage of each mineral in the plant was extrapolated with the amount of dry weight taken to produce ash.

Elemental analysis by ICP-OES: For elemental analysis by ICP-OES, four repetitions per treatment (three plants per experimental unit) separated into the shoot and root, were ground from each consecutive sample, 1 g of the milled sample was taken and calcined as previously described. Thereafter 10 mL of nitric acid (65%) was added to each crucible and boiled. Once the sample was cold, 1 mL of hydrogen peroxide (30%) was added and simmered[13]. The solution was coolled and filtered using Whatman® 42 paper in a 25 mL volumetric flask. The crucible and filter paper were washed with deionised water (Milli-Q 18.2 MΩ cm; Millipore, Bedford, MA, USA) and the sample was diluted to the mark with deionised water and mix. The sample was analysed by ICP-OES Optima 8300 (Perkin Elmer, Inc., PA, USA) for Mg, P, Ca, S and K[14].

Nitrogen determination: Nitrogen in roots and shoots was quantified using the micro-Kjeldahl procedure[15]. Briefly, 0.5 g of the ground sample from each replicate was placed in a digestion tube with 0.4 g of $CuSO_4$, 3 g of anhydrous Na_2SO_4 and 10 mL of concentrated H_2SO_4 according to Vanden-Heuvel[16]. The sample was processed in the micro-Kjeldahl distillation unit (Rapid Still II, 65200, Labconco). Digestion was completed when the solution turned colour green. Two aliquots from the digestion (between 6-11 mL approx.) were taken and the evolved ammonia was trapped in HCl 0.1 N.

Statistical analysis: The comparisons of dried biomass between both treatments (PM and TM), were performed with the t-student test. Plant nutrient percentages determined by both techniques (ICP-OES and SEM-EDS), were performed with the t-student test and Pearson correlation (trials from all samples were used). Charts of the nutrient extraction curves were obtained with Sigma Plot 11.0. The analyses were conducted with the statistical software R, version 3.3.0[17] ($p<0.05$).

RESULTS

Dried biomass of *P. greggii* var. *greggii*: The dry biomass of both shoot and root was greater ($p<0.05$) in plants grown in the peat-based (PM) media (Table 1). It was shown that after 210 days of transplantation (300 days after seed germination), the dry mass weighed 3.491±0.52 g $shoot^{-1}$ in PM compared to 0.662±0.14 g $shoot^{-1}$ in TM. Values of 1.1242±0.27 g $root^{-1}$ in PM compared to 0.3038±0.16 g $root^{-1}$ in TM were obtained.

Nutrient determination by SEM-EDS and ICP-OES: The percentage of the nutrient concentration is given in an average of four replicates for each consecutive sample in shoots (Table 2) and roots (Table 3). It is important to recall that N determination was made with micro Kjeldahl method instead of ICP-OES.

The obtained percentages from shoots samples show that there are no statistical differences between the evaluated analytical methods (ICP-OES and SEM-EDS) for P, S and Mg concentration evaluated at the final sample. A highly significant difference was found between| micro-Kjeldahl and SEM-EDS methods for N concentration, however, in TM substrate, no differences were shown for the first and final sampling. The highest N percentage was obtained during the final sample by micro Kjeldahl technique in PM substrate with 13.29±2.07% of N, in comparison to 1.66±0.24% in SEM-EDS. For evaluation on the TM substrate, an N concentration of 1.52±0.98% was obtained by micro-Kjeldahl in comparison to 0.27±0.12% by the SEM-EDS. The phosphorus percentage in shoots grown in PM substrate, varied from 0.50±0.28% analysed by SEM-EDS and 0.53±0.14% by the ICP-OES method at the final sample. This percentage was 25-50 times lower in TM substrate results (0.01-0.02%) (Table 2). It was demonstrated that *P. greggii* shoots absorbed more nutrients from PM substrate than TM.

The concentration of nutrients analyzed in the roots of *P. greggii* is shown in Table 3. Nitrogen concentration was analyzed using the SEM-EDS and micro-Kjeldahl techniques; It was observed that there were no statistical differences

Table 1: Dried biomass of *Pinus greggii* var. *greggii* as affected by the substrate at varying days after transplant sampling dates

Treatment	Shoot/root	60 DAT (g)	90 DAT (g)	120 DAT (g)	150 DAT (g)	180 DAT (g)	210 DAT (g)
(PM)		0.23	0.517	0.912	2.823	2.536	3.491
(TM)	Shoot	0.151	0.164	0.292	0.699	0.352	0.662
p-value		p<0.05	p<0.001	p<0.01	p<0.001	p<0.001	p<0.001
(PM)		0.159	0.2191	0.367	0.8215	0.81328	1.1242
(TM)	Root	0.148	0.1545	0.1419	0.326	0.1711	0.3038
p-value		0.661	0.058	p<0.05	p<0.001	p<0.001	p<0.01

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), DAT: Days after transplant, n: 12 seedlings

Table 2: Average shoot nutrient concentration in *P. greggii* seedlings of all the trials grown on two substrates after 210 days of transplant

		PM (%)			TM (%)		
Nutrient	Sample	SEM-EDS	ICP-OES		SEM-EDS	ICP-OES	
N2	1	0.08±0.02	0.48±0.05	**	0.09±0.02	0.17±0.07	NS
	2	0.09±0.019	1.56±0.26	**	0.11±0.05	0.51±0.14	**
	3	0.13±0.024	1.94±0.44	**	0.15±0.02	1.45±0.19	**
	4	0.75±0.19	5.61±1.22	**	0.23±0.02	0.88±0.01	**
	5	0.34±0.10	4.69±1.50	*	0.08±0.009	0.94±0.44	*
	6	1.66±0.24	13.29±2.07	**	0.27±0.12	1.52±0.98	NS
P	1	0.05±0.02	0.03±0.006	NS	0.02±0.006	0.02±0.006	NS
	2	0.06±0.01	0.04±0.006	*	0.02±0.01	0.02±0.01	NS
	3	0.09±0.01	0.04±0.009	**	0.03±0.01	0.02±0.004	NS
	4	0.46±0.06	0.48±0.11	NS	0.01±0.002	0.03±0.003	**
	5	0.26±0.05	0.12±0.03	*	0.004±0.001	0.01±0.001	**
	6	0.50±0.28	0.53±0.14	NS	0.01±0.003	0.02±0.01	NS
K	1	0.10±0.01	0.10±0.01	NS	0.09±0.03	0.09±0.028	NS
	2	0.15±0.03	0.13±0.01	*	0.13±0.06	0.11±0.06	NS
	3	0.26±0.03	0.20±0.04	NS	0.23±0.02	0.13±0.02	**
	4	1.03±0.20	0.91±0.38	NS	0.34±0.05	0.23±0.01	*
	5	0.77±0.21	0.60±0.16	NS	0.09±0.003	0.09±0.01	NS
	6	2.94±0.52	2.02±0.43	*	0.27±0.13	0.17±0.07	NS
Ca	1	0.129±0.029	0.088±0.01	NS	0.13±0.04	0.12±0.03	NS
	2	0.109±0.01	0.07±0.012	*	0.22±0.10	0.17±0.08	NS
	3	0.13±0.01	0.11±0.02	NS	0.20±0.04	0.22±0.03	NS
	4	1.38±0.29	1.68±0.63	NS	0.39±0.01	0.31±0.01	**
	5	0.47±0.14	0.28±0.07	NS	0.19±0.03	0.12±0.01	**
	6	1.64±0.14	1.06±0.29	*	0.40±0.20	0.24±0.10	NS
S	1	0.03±0.006	0.03±0.006	NS	0.04±0.01	0.038±0.01	NS
	2	0.02±0.003	0.02±0.006	NS	0.07±0.08	0.04±0.02	NS
	3	0.02±0.008	0.03±0.006	NS	0.12±0.01	0.05±0.008	**
	4	0.52±0.18	0.69±0.34	NS	0.09±0.01	0.078±0.007	NS
	5	0.06±0.02	0.07±0.02	NS	0.09±0.01	0.029±0.003	**
	6	0.20±0.08	0.34±0.07	NS	0.08±0.04	0.06±0.02	NS
Mg	1	0.047±0.01	0.03±0.006	NS	0.06±0.01	0.04±0.01	NS
	2	0.047±0.005	0.03±0.004	*	0.05±0.02	0.05±0.02	NS
	3	0.05±0.008	0.044±0.009	NS	0.05±0.01	0.06±0.008	NS
	4	0.23±0.09	0.24±0.05	NS	0.12±0.01	0.09±0.007	*
	5	0.21±0.05	0.11±0.02	*	0.03±0.006	0.03±0.002	NS
	6	0.53±0.13	0.51±0.06	NS	0.06±0.02	0.06±0.03	NS

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), N_2: Nitrogen, micro Kjeldahl method was used instead ICP-OES. t-student analysis, n: 4 replicates, **p<0.01, *p<0.05

between the evaluated methods except for the last sample (p<0.05) cultivated in TM. Higher concentrations of N were detected using the micro-Kjeldahl (1.13±0.35%) than SEM-EDS (0.41±0.09%). Samples 1-4 had greater statistical differences between techniques (p<0.01) of N concentrations when they were cultured in PM. No significant differences were found in samples 5 and 6.

The highest concentration of N was observed in sample 6 of the PM substrate (1.62±2.02 SEM-EDS, 4.21±1.14 micro-Kjeldahl).

After 210 days of transplantation, the roots that were cultivated in PM substrate presented P values of 0.51±0.60% obtained by ICP-OES and 0.61±0.52% by the SEM-EDS method; roots grown in PM substrate absorbed sixty times

Table 3: Average root nutrient concentration (%) in *P. greggii* seedlings of all the trials grown on two substrates after 210 days of transplant

		PM (%)			TM (%)		
Nutrient	Sample	SEM-EDS	ICP-OES		SEM-EDS	ICP-OES	
N2	1	0.09±0.03	0.45±0.08	**	0.18±0.05	0.46±0.27	NS
	2	0.07±0.03	0.50±0.06	**	0.19±0.03	0.37±0.13	NS
	3	0.29±0.10	0.78±0.23	*	0.20±0.05	0.28±0.03	NS
	4	0.55±0.07	1.41±0.19	**	0.28±0.11	1.07±0.80	NS
	5	1.21±0.47	1.76±0.22	NS	0.28±0.04	0.50±0.27	NS
	6	1.62±2.02	4.21±1.14	NS	0.41±0.09	1.13±0.35	*
P	1	0.07±0.04	0.04±0.01	NS	0.04±0.01	0.03±0.01	NS
	2	0.07±0.03	0.04±0.02	NS	0.06±0.02	0.03±0.01	NS
	3	0.24±0.15	0.14±0.05	NS	0.05±0.06	0.03±0.007	NS
	4	0.39±0.10	0.20±0.02	*	0.03±0.01	0.028±0.01	NS
	5	0.58±0.29	0.46±0.15	NS	0.04±0.01	0.027±0.003	*
	6	0.61±0.52	0.51±0.60	NS	0.01±0.019	0.02±0.009	NS
K	1	0.07±0.02	0.08±0.03	NS	0.11±0.029	0.08±0.035	NS
	2	0.07±0.01	0.08±0.04	NS	0.15±0.06	0.08±0.02	NS
	3	0.34±0.15	0.23±0.08	NS	0.10±0.05	0.06±0.009	NS
	4	0.19±0.05	0.16±0.02	NS	0.15±0.01	0.09±0.02	*
	5	0.37±0.13	0.50±0.13	NS	0.08±0.02	0.09±0.01	NS
	6	0.44±0.45	0.34±0.38	NS	0.05±0.02	0.07±0.02	NS
Ca	1	0.16±0.06	0.11±0.03	NS	0.37±0.11	0.32±0.16	NS
	2	0.13±0.05	0.10±0.03	NS	0.44±0.08	0.37±0.10	NS
	3	0.44±0.17	0.40±0.15	NS	0.35±0.09	0.37±0.06	NS
	4	1.27±0.11	0.67±0.10	**	0.81±0.24	0.69±0.18	NS
	5	2.99±1.68	1.80±0.71	NS	0.60±0.16	0.43±0.05	NS
	6	3.48±4.11	3.32±4.2	NS	1.01±0.31	0.78±0.23	NS
S	1	0.02±0.005	0.02±0.004	NS	0.06±0.03	0.03±0.01	NS
	2	0.012±0.002	0.02±0.01	NS	0.03±0.01	0.03±0.01	NS
	3	0.12±0.06	0.12±0.04	NS	0.03±0.01	0.04±0.006	NS
	4	0.61±0.03	0.24±0.07	**	0.14±0.04	0.11±0.02	NS
	5	1.95±1.12	0.82±0.32	NS	0.07±0.01	0.05±0.007	NS
	6	2.56±3.41	1.70±2.19	NS	0.07±0.03	0.11±0.03	NS
Mg	1	0.03±0.01	0.03±0.01	NS	0.08±0.02	0.04±0.02	NS
	2	0.03±0.01	0.02±0.01	NS	0.09±0.02	0.05±0.01	*
	3	0.06±0.02	0.06±0.02	NS	0.05±0.04	0.04±0.007	NS
	4	0.09±0.06	0.06±0.01	NS	0.11±0.05	0.07±0.02	NS
	5	0.13±0.14	0.16±0.03	NS	0.07±0.009	0.05±0.006	NS
	6	0.10±0.09	0.16±0.18	NS	0.05±0.03	0.05±0.01	NS

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), N_2: Nitrogen, micro Kjeldahl method was used instead ICP-OES. t-student analysis, n: 4 replicates, **p<0.01, *p<0.05

more P concentration than those grown in TM (Table 3). No significant differences were shown between the evaluated methods for the concentrations of K nutrients; A statistical difference ($p<0.05$) was only observed in sample four when it was cultivated on a TM substrate with higher results in SEM-EDS (0.15±0.01%) compared to ICP-OES (0.09±0.02%).

Samples at PM substrate present K concentrations of 0.44±0.45 and 0.34±0.38% analyzed by the SEM-EDS and ICP-OES methods respectively, after 210 days of transplantation.

The highest concentrations of nutrient K obtained in the TM substrate were observed in sample 4 (approximately 145 days after transplantation).

No significant differences were shown for Ca concentration between analytical methods, using all root samples on the TM substrate. The highest Ca concentration was present after 210 days of transplantation (1.01±0.31% SEM-EDS, 0.78±0.23% in ICP-OES). Regarding the PM substrate, it was shown that sample 4 had a statistical difference between techniques ($p<0.05$) with higher calcium results in SEM-EDS (1.27±0.11%) comparing ICP-OES (0.67±0.10%).

The highest Ca results were recorded after 210 days of transplantation (3.48±4.11%, SEM-EDS, 3.32±4.2% ICP-OES). This trend was also observed in S concentrations between the analytical methods that evaluate both substrates. Sample 4

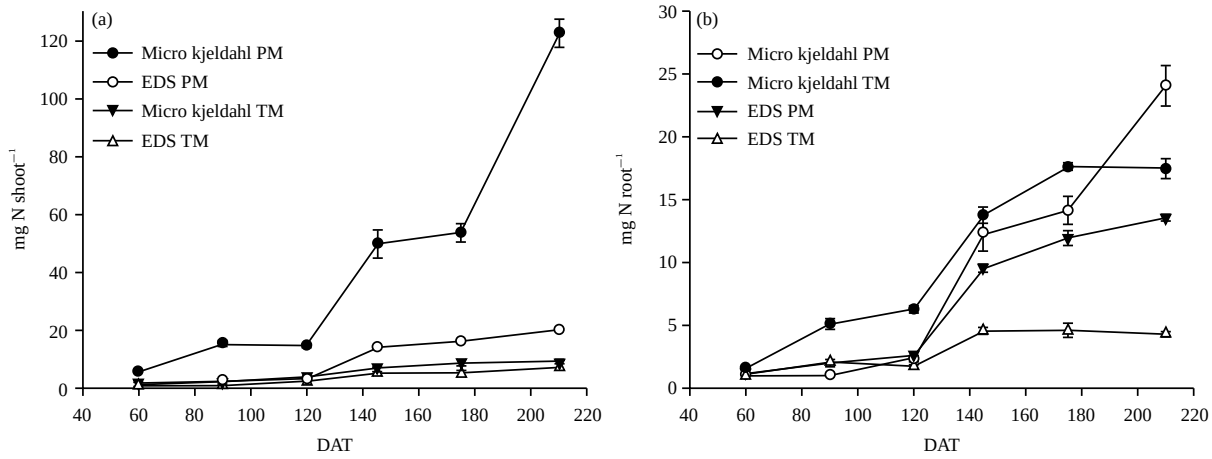

Fig. 1(a-b): Nitrogen extraction curve (mg plant^{-1}) in (a) Shoot and (b) Root in *Pinus greggii* var. *greggii* seedlings after 210 days after transplant (DAT)

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), SEM-EDS: Energy dispersive X-rays spectroscopy

showed a statistical difference between the techniques ($p<0.05$) with higher sulfur results in SEM-EDS (0.61±0.03%) comparing ICP-OES (0.24±0.07%).

The roots grown in PM substrate registered S concentrations of 2.56±3.41% in SEM-EDS and 1.70±2.19% in ICP-OES after 210 days of transplantation. The roots grown in TM substrate did not show significant differences between the evaluated methods; the highest results were presented in sample 4 (approximately 145 days after transplantation).

Regarding the concentration of Mg in roots, it was found that there were no significant differences between the techniques, in PM substrate samples. The highest Mg concentration was recorded after 170 days of transplantation (0.13±0.14% SEM-EDS, 0.16±0.03% ICP-OES). Regarding the samples grown on the TM substrate, sample 2 showed a difference in concentration between the techniques ($p<0.05$), SEM-EDS determined a higher concentration of Mg (0.09±0.02%) compared to ICP-OES (0.05±0.01%).

The uptake of nutrients by seedlings (Fig. 1-6), showed higher concentrations in samples grown in PM treatment than samples grown in TM treatment.

The uptake of N in the shoots in PM substrate after 210 days of transplantation, obtained by the micro-Kjeldahl method was 122.38±4.88 mg shoot^{-1}, the determination by SEM-EDS showed a concentration of 20.22±0.49 mg shoot^{-1} (Fig. 1a). Regarding N uptake in plants on TM substrate, 9.18±0.416 mg shoot^{-1} were obtained by micro-Kjeldahl compared to 7.44±0.90 mg shoot^{-1} in SEM-EDS (Fig. 1a). For nutrient N, higher results were observed by the micro-Kjeldahl method.

Figure 1b demonstrates the N uptake in *P. greggii* roots. The highest concentration was obtained in PM substrate (23.99±1.59 mg root^{-1} in micro-Kjeldahl) and 12.44±0.78 mg root^{-1} in EM-EDS). Regarding TM substrate, the nitrogen uptake varied from 4.30±0.16 mg root^{-1} in SEM-EDS to 17.52±0.25 mg root^{-1} in micro-Kjeldahl.

The highest P extraction was recorded after 210 days of cultivation on both substrates; in the shoot, PM samples showed values of 6.79±0.25 mg shoot^{-1} obtained by ICP-OES and 7.77±0.215 mg shoot^{-1} obtained by SEM-EDS (Fig. 2a). Plants grown in TM obtained less than 0.46 mg shoot^{-1} in both techniques (Fig. 2a). In *P. greggii* roots, values of 11.27±1.08 and 7.12±0.38 mg P root^{-1} were obtained by SEM-EDS and ICP-OES respectively for PM treatment (Fig. 2b). In TM treatment plants acquired 2.24±0.26 mg P root^{-1} by ICP-OES compared to 1.52±0.07 mg P root^{-1} by SEM-EDS (Fig. 2b).

For K element concentration, after 210 days of transplant, seedlings obtained 23.61±2.55 mg K shoot^{-1} determined by ICP-OES, compared to 34.59±2.84 mg K shoot^{-1} performed by SEM-EDS, for plants grown on the PM substrate (Fig. 3a). These concentrations were almost ten times lower in TM treatment, where the highest reading was 3.58±0.20 mg K shoot^{-1} realised by ICP-OES and 2.11±0.20 K shoot^{-1} by SEM-EDS after 210 days of transplant (Fig. 3a). Roots grown in PM media absorbed 3.46±0.18 mg K root^{-1} analyzed by

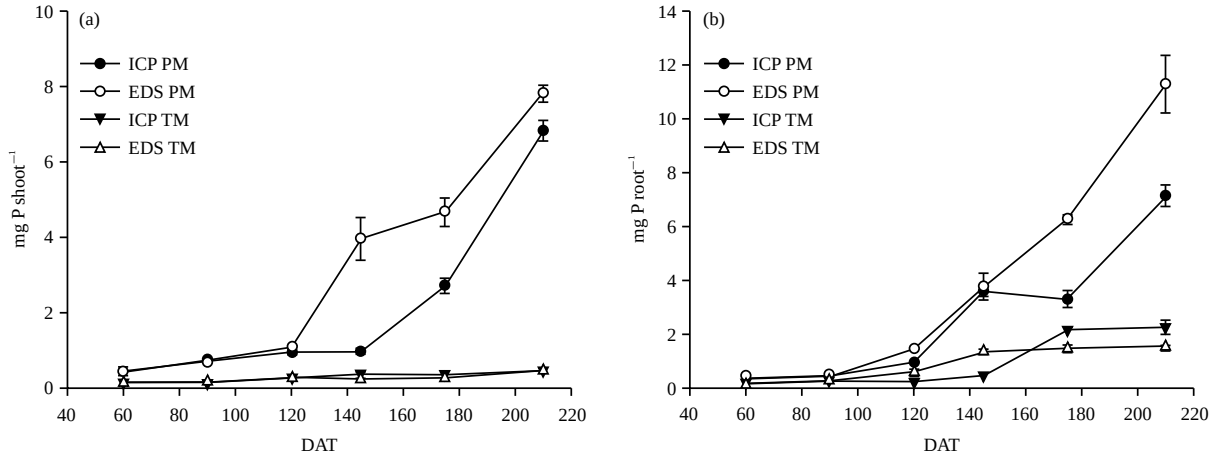

Fig. 2(a-b): Phosphorus extraction curve (mg plant^{-1}) in (a) Shoot and (b) Root in *Pinus greggii* var. *greggii* seedlings after 210 days after transplant (DAT)

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), SEM-EDS: Energy dispersive X-rays spectroscopy, ICP-OES: Inductively coupled plasma-optical emission spectrometry

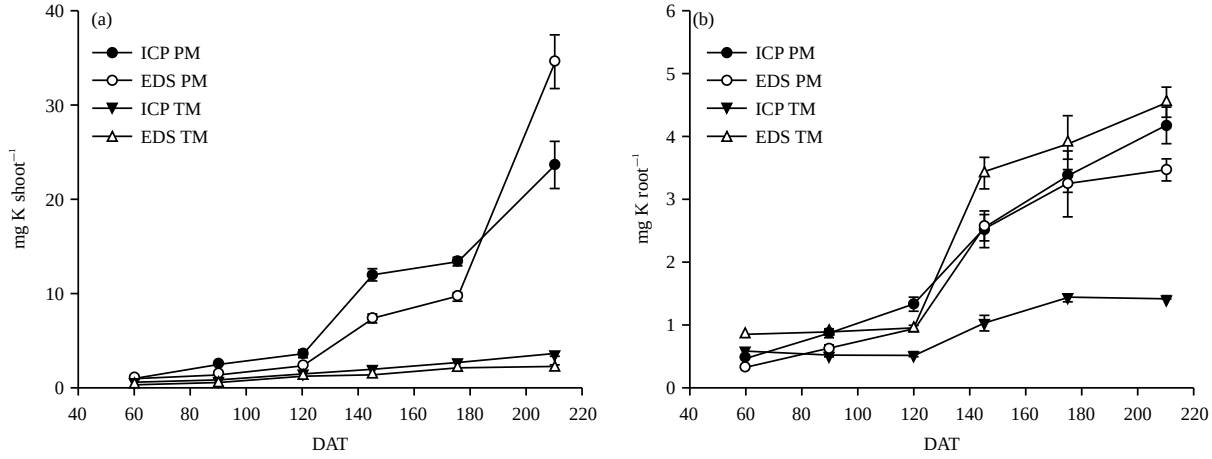

Fig. 3(a-b): Potassium extraction curve (mg plant^{-1}) in (a) Shoot and (b) Root in *Pinus greggii* var. *greggii* seedlings after 210 days after transplant (DAT)

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), SEM-EDS: Energy dispersive X-rays spectroscopy, ICP-OES: Inductively coupled plasma-optical emission spectrometry

SEM-EDS and 4.17±0.28 mg K root^{-1} by ICP-OES, versus 4.54±0.24 mg K root^{-1} by SEM-EDS and 1.41±0.004 mg K root^{-1} by ICP-OES from plants grown in TM media (Fig. 3b).

The maximum amount of sulfur obtained in PM treatment was 2.15±0.46 mg S shoot^{-1} (SEM-EDS) and 3.80±0.33 mg S shoot^{-1} (ICP-OES). Plants grown in TM treatment presented values of 1.68±0.26 mg S shoot^{-1} determined by SEM-EDS and 1.32±0.08 mg S shoot^{-1} analysed by ICP-OES (Fig 4a).

The maximum accumulation of S element in the root, from PM samples, was 24.78±1.06 mg S root^{-1} (ICP-OES method) and 13.20±0.53 mg S root^{-1} (SEM-EDS) (Fig. 4b).

The amount of Ca was higher in seedlings grown in PM treatment, with values of 18.00±0.76 mg Ca shoot^{-1} (SEM-EDS) and 11.12±0.81 mg Ca shoot^{-1} (ICP-OES) compared to TM treatment, 6.84±0.56 mg Ca shoot^{-1} (SEM-EDS) and 4.54±0.37 mg Ca shoot^{-1} (ICP-OES) (Fig. 5a). Concentrations of Ca were higher in roots, when grown in PM

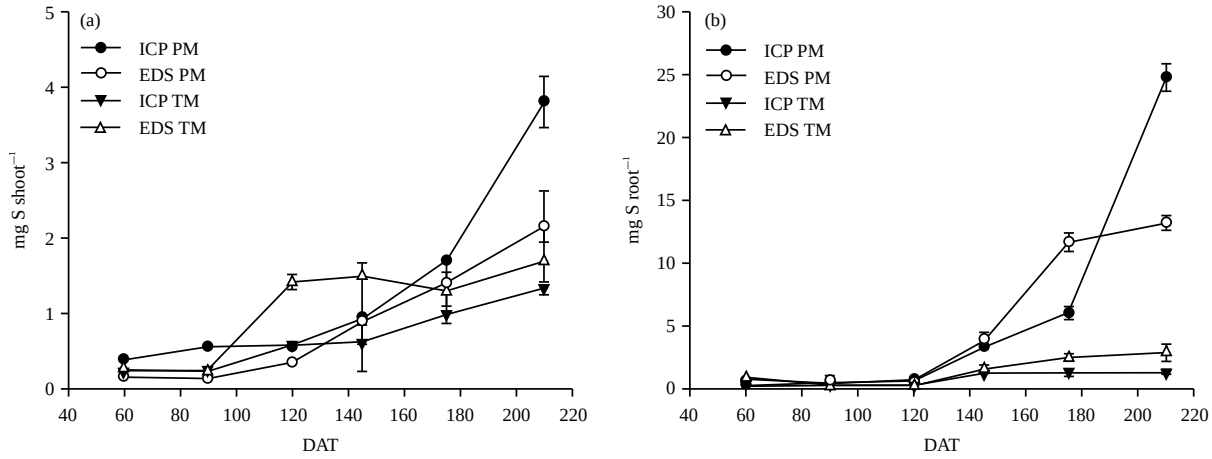

Fig. 4(a-b): Sulphur extraction curve (mg plant^{-1}) in (a) Shoot and (b) Root in *Pinus greggii* var. *greggii* seedlings after 210 days after transplant (DAT)

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), SEM-EDS: Energy dispersive X-rays spectroscopy, ICP-OES: Inductively coupled plasma-optical emission spectrometry

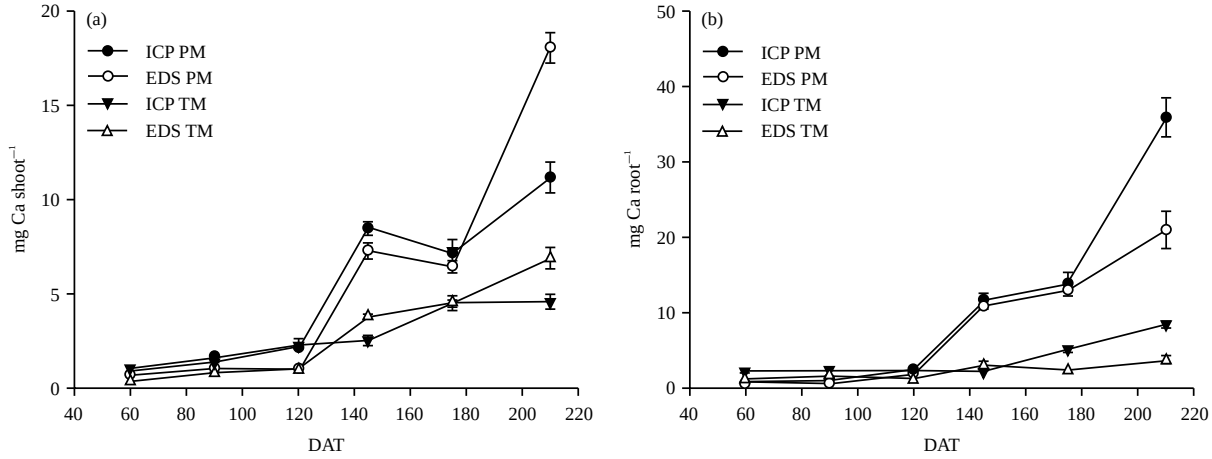

Fig. 5(a-b): Calcium extraction curve (mg plant^{-1}) in (a) Shoot and (b) Root in *Pinus greggii* var. *greggii* seedlings after 210 days after transplant (DAT)

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), SEM-EDS: Energy dispersive X-rays spectroscopy, ICP-OES: Inductively coupled plasma-optical emission spectrometry

treatment, the plant extracted 20.93±2.4 mg Ca root^{-1} determined by SEM-EDS and 35.88±2.66 mg Ca root^{-1} by ICP-OES (Fig. 5b). The maximum Ca concentration found in roots grown in TM treatment was 3.63±0.54 mg Ca root^{-1} by the SEM-EDS method and 8.40±0.50 mg Ca root^{-1} by the ICP-OES method (Fig. 5b). Figure 6a illustrates the accumulation curve of Mg nutrient. Plants absorbed between 4.5-5.6 mg Mg shoot^{-1} in PM treatment samples in comparison to 1.3-3.32 mg Mg shoot^{-1} from TM treatment samples. It is shown in Fig. 6b that roots absorbed 4.71±1.06 mg Mg root^{-1} at 210 days of transplant in PM obtained by ICP method in comparison with the value 2.13±0.317 mg Mg root^{-1} obtained by EDS, for the TM treatment, lower results were obtained, 1.21±0.16 and 0.378±0.07 mg Mg root^{-1} by ICP and EDS methods respectively.

Correlation between SEM-EDS and ICP-OES: Figure 7 contains the Pearson correlation coefficients between SEM-EDS and ICP-OES percentage readings. No correlation was found among the evaluated techniques for nitrogen concentration (-0.28), obtaining a negative correlation

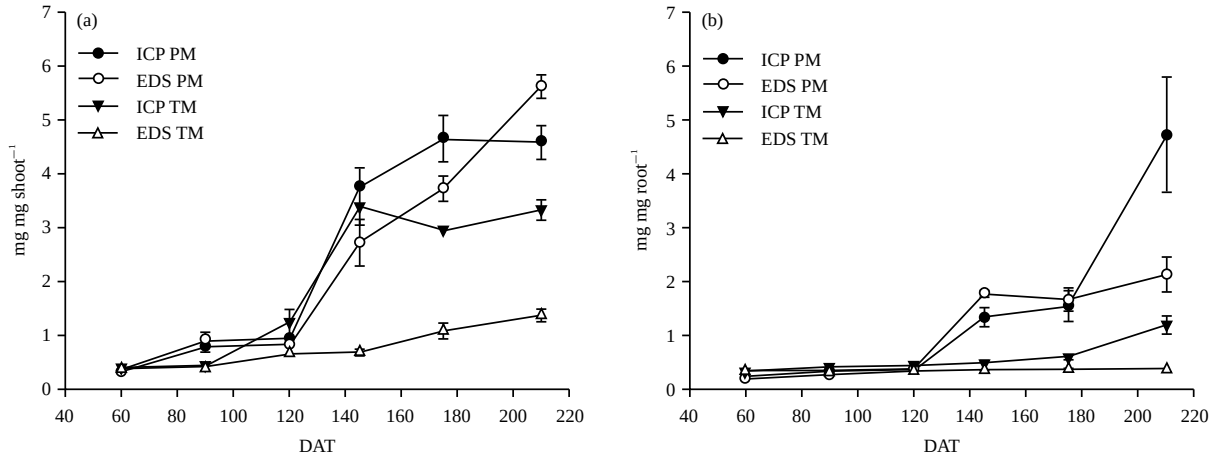

Fig. 6(a-b): Magnesium extraction curve (mg plant^{-1}) in (a) Shoot and (b) Root in *Pinus greggii* var. *greggii* seedlings after 210 days after transplant (DAT)

PM: Peat moss: perlite (1:1), TM: Forest soil, sand and silt (2:1:1), SEM-EDS: Energy dispersive X-rays spectroscopy, ICP-OES: Inductively coupled plasma-optical emission spectrometry

(Fig. 7a). The correlation test was highly significant ($p<0.001$) between ICP-OES and SEM-EDS techniques in the determination of P (0.759) (Fig. 7b), S (0.56) (Fig. 7c), K (0.55) (Fig. 7d) and Ca (0.79) (Fig. 7e). No significant correlation was observed in the concentration of Mg between the evaluated methods (Fig. 7f) (0.14).

DISCUSSION

The results of dry biomass found in PM substrate samples are similar to those reported by Oliet *et al.*[5], in which seedlings of *P. strobus* L. produced with slow-release fertilizers (like osmocote® used in PM substrate), ranged from 2.6-2.8 g shoot^{-1} at 210 days after transplant; in another study, Arteaga-León *et al.*[6] reported shoot dry weight of 1.05 g in *P. ayacahuite* seedlings after 2 years growth in the control, in TM substrate (used without fertilization), the results were inconsistent against the reported in a greenhouse study, carried by Sigala *et al.*[18], Liu *et al.*[19], where *P. pinaster* seedlings gained 2.43 g shoot^{-1} and 0.41 g root^{-1} of dry biomass from unfertilized plants after 205 days of emergence.

The levels of Ca, K and Mg are much lower in PM substrate compared to TM. The traditional substrate presented 3.92% of organic matter compared to 50% in peat-moss; in forest plantations, these plants are transplanted into poor soil conditions with low organic matter and high pH (7.0-8.3)[20].

Nitrogen concentration (%) results, were higher when analysed by the micro-Kjeldahl technique against the SEM-EDS method, the difference could lie in the quantification of total nitrogen determined by SEM-EDS, that passed through ash where the inorganic compounds suffer alteration (melting, decomposition or volatilization)[21]. It is not clear what is the optimal temperature for obtaining ashes, usually, is carried out in the laboratory at temperatures up to 500°C (norm for determining ash content in wood, Bakisgan *et al.*[22]); Although 580-600°C is the temperature range utilized, a significant portion of the inorganic material volatilizes.

Figure 1-6 show the elemental absorption per plant (mg) from each evaluated treatment according to the method of analysis; it can be observed that both techniques follow the same trend, with the exception of nitrogen, as previously mentioned. Higher concentrations were obtained using the micro-Kjeldahl extraction (Fig. 1). These results are similar to those reported by Arteaga-León *et al.*[6], where after 720 days of *P. ayacahuite* plant emergence, values of 12.28±2.66 mg plant^{-1} were obtained. The N concentration (%) determined by the micro-Kjeldahl method from shoots grown in PM substrate was 13.29±2.07% and for SEM-EDS method 1.6±0.24%. For seedlings grown in TM substrate, 1.52±0.98% was obtained by the micro-Kjeldahl method while SEM-EDS presented values of 0.27±0.12% N shoot^{-1}.

Albaugh *et al.*[23] states, that the optimal nitrogen range for pine, is between 0.12-1.33%. Therefore, *P. greggii* has a good amount of N, even after growing up in the forest soil. It is possible to see that after 140 days of transplant, this species of pine, needed almost three times the amount of N for its growth, than what it was observed 120 days after transplant. Deficiencies of this nutritional element will lead to low survival, low drought resistance and low development in the plantation field[18].

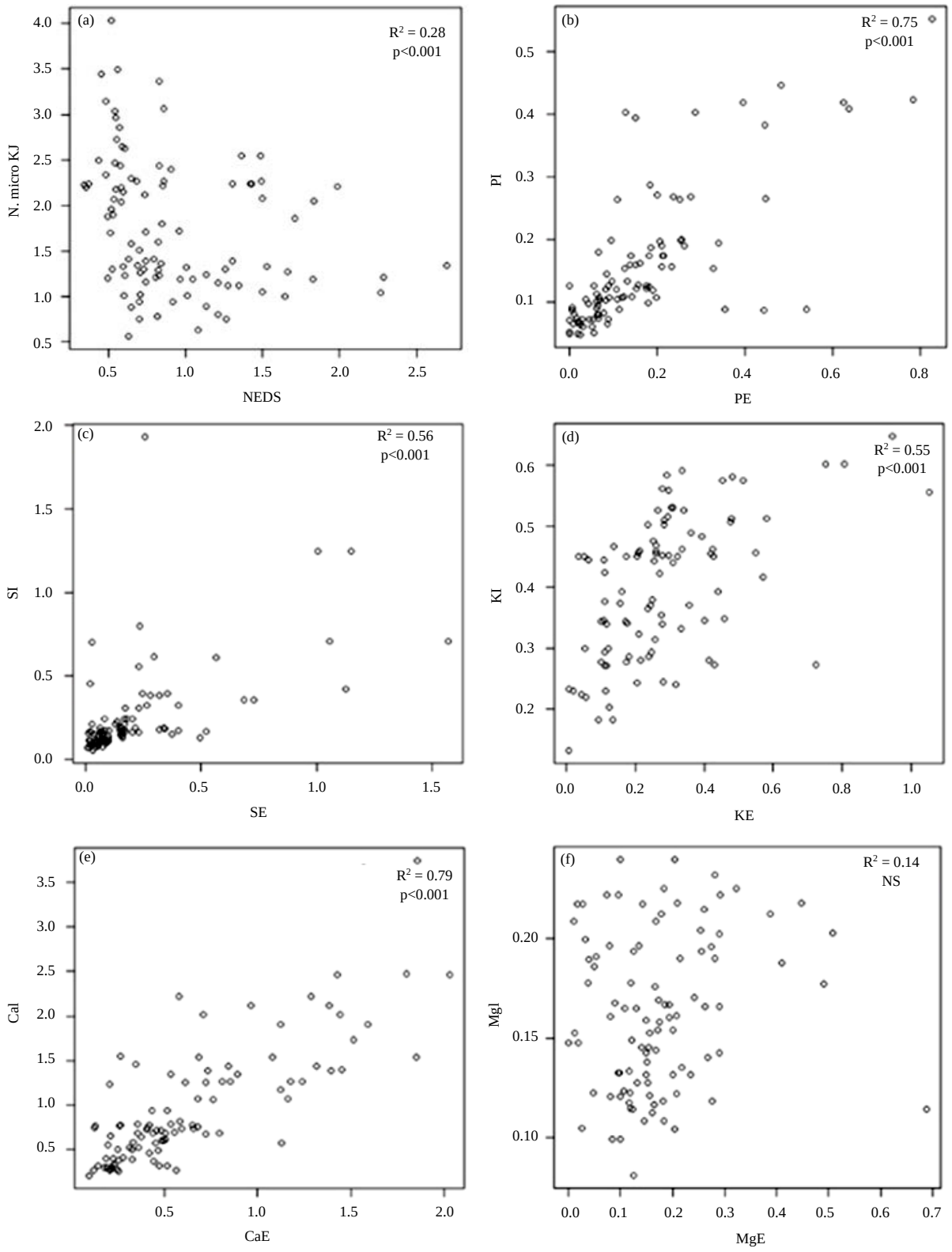

Fig. 7(a-f): Scatter plots from correlation between SEM-EDS technique and ICP-OES, (a) Nitrogen concentration (%) * for nitrogen correlation was made with Micro Kjeldahl and SEM-EDS, (b) Phosphorus concentration (%), (c) Sulphur concentration (%), (d) Potassium concentration (%), (e) Calcium concentration (%) and (f) Magnesium concentration (%)

As for the P absorption in *P. greggii* seedlings, 0.05-0.61% P plant^{-1} was obtained by SEM-EDS (Table 2, 3). The P is a limiting nutrient for the early growth of *P. greggii* and the availability of Ca in the substrate may hinder soil P and K uptake[24].

The obtained shoot concentration of K plant^{-1} by both techniques (Fig. 3), is comparable to that it was obtained by Liu *et al.*[19] who reported 31.97±3.05 mg K plant^{-1} in *P. pinaster* and higher than referred by Arteaga-León *et al.*[6] in *P. ayacahuite* (6.40±1.27 mg K plant^{-1}).

Typically, in young conifers, potassium deficiency takes the form of yellowing of terminal needles. This deficiency causes important diameter growth reduction and it also affects height growth, reflected in the dry biomass weight where TM treatment had weight values four to five times lower than PM (Table 1).

A concentration between 0.2-0.3% of sulfur is essential for conifers growth[25], concentrations of S in roots grown in PM media, varied from 0.02-2.56% in SEM-EDS and 0.02-1.70% in ICP-OES and roots grown in TM media, varied from 0.03-0.14% analysed by SEM-EDS in contrast to 0.03-0.11% by ICP-OES. The concentrations acquired from TM substrate samples, follow the S concentrations found in conifers, reported by Garrison *et al.*[26].

The standards of Ca ranged between 0.28-1.57% of forest species[27]. The foliar nutrient concentration was higher that reported Garrison *et al.*[26]. The highest extraction of Ca was presented after 210 days of transplant (35.88±2.66 mg Ca root^{-1}) in ICP-OES and 20.93±2.49 mg Ca root^{-1} in SEM-EDS.

The Mg plant^{-1} absorption by both described techniques, varied from 0.13-0.18%, compared to that reported by Albaugh *et al.*[23] in some forest species. Figure 6 illustrates the accumulation curve of this nutrient. Shoots absorbed between 4.5-5.6 mg Mg plant^{-1} (0.17-0.19%) in PM treatment in comparison to 1.3-3.32 mg plant^{-1} in TM treatment (0.13-0.14%). Garrison *et al.*[26] found concentrations of Mg in conifer species between 0.05-0.09%, the obtained concentrations, fall between the limits of essential macronutrients in conifers reported by Burdon[28]. Readings by SEM-EDS and ICP-OES methods are consistent with what was achieved by Bai *et al.*[29], where, the concentrations of minerals in SEM-EDS and ICP-OES were evaluated and found no significant differences between techniques, with the exception of K, who reports to be underestimated by ICP-OES.

Marguí *et al.*[9] states, that the SEM-EDS technique can provide accurate concentration data for Al, Fe, Mg, Ca and K. The SEM-EDS method proved to be an efficient tool for the determination of metals in plants since it allows the elimination of the digestion stage and minimizes the mistakes in the recovery of some elements.

Studies of elemental content in *P. greggii* seedlings are scarce, even less, the use of different techniques to evaluate the nutritional composition in forest plants. Although SEM-EDS analysis is generally used to provide a qualitative proportion of elements present in the samples, it measures the relative abundance of emitted x-rays versus their energy and it is possible to determine the elemental composition of the volume sample[30]. This study demonstrates that it can also be used to obtain quantitative elemental analysis and has the advantage of being fast and easier. The procedure involves the obtaining of ash, to then be analysed in the equipment. This method avoids dangerous reagents that require high care and individual protection, time and experience.

Although it is known that the ICP-OES technique is more sensitive and precise than SEM-EDS[31], Alomari *et al.*[32] reported similar results with SEM-EDS than obtained by the ICP-OES.

CONCLUSION

For the production of *P. greggii* seedlings, a conventional management substrate (peat-based) is recommended, since plants exhibit a higher mineral absorption concerning plants grown in forest soil. It was demonstrated, that the nutrient uptake in both detection methods (SEM-EDS and ICP-OES) followed the same tendency with a highly significant Pearson correlation for P, K, Ca and S elements. Percentage of nitrogen in the plants were higher when analysed by micro-kjeldahl in comparison with SEM-EDS. It is possible to determine the elemental composition of *P. greggii* seedlings using the SEM-EDS technique, evading the use of corrosive materials, laborious and tedious procedures, that are used in wet digestion for the mineral extraction by ICP-OES analysis.

ACKNOWLEDGMENT

R. Casique especially thanks to CONACYT for the financial support under scholarship CVU 315450 and CICATA-Legaria research center for their hospitality and contribution towards laboratory experiments.

SIGNIFICANCE STATEMENT

This study discovered the applicability of the SEM-EDS method in the analysis of the concentration of nutrients in plants, which could be beneficial for students and research groups. Because they save time and costs in reagents. The method is friendly to the environment because they avoid the use of dangerous reagents such as hydrochloric, sulfuric and nitric acids, among others.

REFERENCES

1. Steiner, K.G. and R. Williams, 1996. Causes of Soil Degradation and Development Approaches to Sustainable Soil Management. Margaf Verlag, Weikersheim, Germany, Pages: 96.
2. Hodge, G.R. and W.S. Dvorak, 2000. Differential responses of Central American and mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. New Forests, 19: 241-258.
3. Burney, O., A. Aldrete, R.A. Reyes, J.A.P. Ruíz, J.R.S. Velazquez and J.G. Mexal, 2015. México-addressing challenges to reforestation. J. Forest., 113: 404-413.
4. Kozlowski, T.T., 1999. Soil compaction and growth of woody plants. Scand. J. For. Res., 14: 596-619.
5. Oliet, J., R. Planelles, M.L. Segura, F. Artero and D.F. Jacobs, 2004. Mineral nutrition and growth of containerized *Pinus halepensis* seedling under controlled-release fertilizer. Scientia Horticulturae, 103: 113-129.
6. Arteaga-León, C., J. Pérez-Moreno, D. Espinosa-Victoria, J.J. Almaraz-Suárez, H. Silva-Rojas and A. Delgado-Alvarado, 2018. Ectomycorrhizal inoculation with edible fungi increases plant growth and nutrient contents of *Pinus ayacahuite*. Revista Mexicana de Biodiversidad, 89: 1089-1099.
7. Stein, B.R., V.A. Thomas, L.J. Lorentz and B.D. Strahm, 2014. Predicting macronutrient concentrations from loblolly pine leaf reflectance across local and regional scales. GISci. Remote Sens., 51: 269-287.
8. Farhadi, M., M. Tigabu, M. Pietrzykowski, D. Danusevičius and P.C. Odén, 2017. Application of near infrared spectroscopy for authentication of *Picea abies* seed provenance. New For., 48: 629-642.
9. Marguí, E., I. Queralt, M.L. Carvalho and M. Hidalgo, 2005. Comparison of EDXRF and ICP-OES after microwave digestion for element determination in plant specimens from an abandoned mining area. Anal. Chim. Acta, 549: 197-204.
10. Welna, M., A. Szymczycha-Madeja and P. Pohl, 2013. A comparison of samples preparation strategies in the multi-elemental analysis of tea by spectrometric methods. Food Res. Int., 53: 922-930.
11. Altland, J.E., M.G. Buamscha and D.A. Horneck, 2008. Substrate pH affects nutrient availability in fertilized douglas fir bark substrates. HortScience, 43: 2171-2178.
12. Lohse, G., 1982. Microanalytical azomethine H method for boron determination in plant tissue. Commun. Soil Sci. Plant Anal., 13: 127-134.
13. Belay, K.T., 2014. Analysis of lead (Pb), cadmium (Cd) and chromium (Cr) in Ethiopian spices after wet (Acid) digestion using atomic absorption spectroscopy. The Global J. Sci. Front. Res., Vol. 14.
14. Tafur-Marinos, J.A., M. Ginepro, L. Pastero and V. Zelano, 2016. Digestion procedures for the elemental analysis of wood by inductively coupled plasma optical emission spectrometry. Anal. Lett., 49: 1722-1733.
15. Reyes-Mendez, A.I., C. Figueroa-Hernandez, G. Melgar-Lalanne, H. Hernandez-Sanchez, G. Dávila-Ortiz and C. Jiménez-Martínez, 2015. Production of calcium-and iron-binding peptides by probiotic strains of *Bacillus subtilis*, *B. clausii* and *B. coagulans* GBI-30. Rev. Mex. Ing. Chim., 14: 1-9.
16. Vanden-Heuvel, R.C., 1965. Elemental Analysis by X-Ray Emission Spectrography. In: Methods of Soil Analysis, Part 2 Chemical and Microbiological Properties 9.2, Norman A.G., (Ed.)., The American Society of Agronomy Inc, United States, pp: 771-821.
17. Venables, W. and D. Smith, 2014. An Introduction to R. Notes on R: A programming environment for data analysis and graphics. Version 4.0.2. R Core Team.
18. Sigala, J.A., M. Uscola, J.A. Oliet and D.F. Jacobs, 2020. Drought tolerance and acclimation in *Pinus ponderosa* seedlings: The influence of nitrogen form. Tree Physiol., 40: 1165-1177.
19. Liu, F., H. Ma, S. Xing, Z. Du, B. Ma and D. Jing, 2013. Effects of super-absorbent polymer on dry matter accumulation and nutrient uptake of Pinus pinaster container seedlings. J. For. Res., 18: 220-227.
20. Wall, A. and J. Heiskanen, 2003. Effect of air-filled porosity and organic matter concentration of soil on growth of *Picea abies* seedlings after transplanting. Scand. J. For. Res., 18: 344-350.
21. Thiex, N., L. Novotny and A. Crawford, 2012. Determination of ash in animal feed: AOAC official method 942.05 revisited. J. AOAC Int., 95: 1392-1397.
22. Bakisgan, C., A.G. Dumanli and Y. Yürüm, 2009. Trace elements in Turkish biomass fuels: Ashes of wheat straw, olive bagasse and hazelnut shell. Fuel, 88: 1842-1851.
23. Albaugh, T.J., H.L. Allen and T.R. Fox, 2008. Nutrient use and uptake in *Pinus taeda*. Tree Physiol., 28: 1083-1098.
24. Colpaert, J.V., K.K. Van Tichelen, J.A. Van Assche and A. Van Laere, 1999. Short-term phosphorus uptake rates in mycorrhizal and non-mycorrhizal roots of intact *Pinus sylvestris* seedlings. New Phytol., 143: 589-597.

25. Beaton, J.D., A. Moss, I. MacRae, J.W. Konkin, W.P.T. McGhee and R. Kosick, 1965. Observations on foliage nutrient content of several coniferous tree species in British Columbia. The For. Chron., 41: 222-236.
26. Garrison, M.T., J.A. Moore, T.M. Shaw and P.G. Mika, 2000. Foliar nutrient and tree growth response of mixed-conifer stands to three fertilization treatments in northeast Oregon and north central Washington. For. Ecol. Manage., 132: 183-198.
27. Kiilsgaard, C.W., S.E. Greene and S.G. Stafford, 1987. Nutrient concentrations in litterfall from some western conifers with special reference to calcium. Plant Soil, 102: 223-227.
28. Burdon, R.D., 1976. Foliar macronutrient concentrations and foliage retention in radiata pine clones on four sites. The New Zealand J. For. Sci., 5: 250-259.
29. Bai, R., L. Wan, H. Li, Z. Zhang and Z. Ma, 2007. Identify the injury implements by SEM/EDX and ICP-AES. Forensic Sci. Int., 166: 8-13.
30. Goldstein, J., D.E. Newbury, D.C. Joy, C.E. Lyman and P. Echlin *et al*., 2003. Scanning Electron Microscopy and X-Ray Microanalysis. 3rd Edn., Springer, USA.
31. Pye, K. and D. Croft, 2007. Forensic analysis of soil and sediment traces by scanning electron microscopy and energy-dispersive X-ray analysis: An experimental investigation. For. Sci. Int., 165: 52-63.
32. Alomary, A., S. Belhadj and S.M. Obeidat, 2012. A comparison of SEM-EDS with ICP-OES for the quantitative elemental determination of Algerian mediterranean sea sediments. Jordan J. Chem., 146: 383-391.