# Comparing Soil Substrates of Low Cost for the Production of Calabrian Pine (*Pinus brutia* Ten) Seedlings Resilient to Unfavorable Conditions having in Mind the Climatic Change Phenomenon

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*Abstract:* For the production of *Pinus brutia* seedlings resilient to dry climatic conditions of Mediterranean ecosystems and better adapted to climatic change, the laboratory of Forest Soil seedlings of *P. brutia* on the first year of their growth, replanted in bigger sized plastic pots. As fulfilled material used forest soil from 90% gneiss rock and 10% from different low-cost materials like cow manure, goat manure, forest floor of broadleaved forests and Calabrian pine. The research was conducted to the greenhouse of the Laboratory of Forest Soils. To evaluate the results the development of the seedlings and conciseness of different nutrients were measured. The measurements were analyzed with One Way Anova test and the results indicate the soil substrate most suitable for the production of second year Calabrian pine seedlings with greater probability of survival in dry climate conditions.

Key-Words: - nutrients, soil substrates, Calabrian pine seedlings, One Way Anova Test

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## **1** Introduction

Soil fertility constitutes the capacity of the soil to supply nutrients to growing plants [1]. This capability of forest soils is not stable, and it relies on the fact that nutrients are accessible through the release of decomposing organic matter. precipitation, activity of microorganisms and dissolution of minerals [2]. Every nutrient is paramount for the plant metabolism and each nutrient is physiologically irreplaceable. Proper growth of the vegetation is feasible only if each nutrient can be accessed in a satisfactory amount and in a chemical form that can be absorbed by the plants [3]. This is more apparent when a major element N, P, Ca, Mg, S and less obvious when a trace element is in shortage [4]. The uneven distribution in the supply of nutrients contributes in the abnormal development of vegetation and make plants susceptible to pathogens [5].

Nitrogen together with Potassium and Phosphorus are the three most basic elements in the soil. Deficiencies of nitrogen result in stunted growth [6]. Plants can absorb nitrogen mainly in the form of nitrate ions and secondarily in the form of ammonium ions from the soil. In situations that don't allow the supply of the needed amount of nitrogen, the leaves are smaller and take a yellow color while also having smaller amounts of chlorophyll [7]. Branches are thinner with reduced photosynthesis occurring in their leaves and production of organic matter is decreased while in comparison, the root system is developed further and the development cycle being reduced [3].

Phosphorus can be found in the soil in both organic and inorganic forms, while both forms of Phosphorus originate exclusively from magmatic and metamorphic mineral [8].

P most commonly creates bonds with Al, Fe, Ca and Mg. Forest soil is loaded with great amounts of P (2,5-12 kg/ha) due to the decomposition of organic matter [9] with only a small portion of Phosphorus being soluble in water while also being absorbable with the rest of the P being inaccessible to the vegetation [10]. In the case of phosphorus deficiency in pine trees, the tree's oldest needles have a red- chestnut color [11]. K that is contained in the soil originates from the dissolution of minerals containing it, with large amounts of K being released after the decomposition of organic matter [12]. K has a great role in the metabolic process, particularly in enzymes that metabolize nitric components and hydrocarbons [3].

K takes place in the process of lignification of the seedlings and increases resistance to both drought and cold [13]. Ca while having an important influence in the traits of the soil it also nullifies the acidity of acidic organic compounds that are produced by the decomposing organic matter. With abundance of Ca resulting in smaller absorption of K [14].

Organic matter has a positive in maintaining fertility while also being a material composing the soil, source of energy for the microorganisms inhabiting it and as reservoir of water. The organic material is composed of vegetation, manure of animals e.tc. [15].

Organic material in favorable conditions while in the presence of a variety of microorganisms breaks down and becomes humus [16].

The forest floor constitutes one of the major variables in the improvement of the physical, chemical and biological traits of the soil [17]. In rural environments the use of peat is particularly popular for the improvement of parks. The effectiveness of peat diminishes in forest areas of urban and peri-urban soils [18] especially in dry conditions when peat droughts totally during the development cycle of the seedlings [3].

Manure is fairly affordable and is used by farmers to fertilize their crops to provide nutrients and organic matter. It is a cheap and affordable fertilizer that can be obtained by farmers with great ease [19]. The fertilizer greatly improves the quality of the soil in the long-term [20]. The chemical composition of manure depends on the diet and species of animal and the straw covering the living spaces of the animals. Manure contains 50% of both organic matter and nitrogen while also 60% of P and K originating from the animal feed [14]. In general, Phosphorus is contained in small amounts, with continuous use of manure in particular situations creating symptoms of Phosphorus malnutrition in vegetation [21].

Use of peat intensifies the activity of microorganisms of soil with high corrosion while achieving an increase in fertility [22].

An alternative method for the production of seedlings with resilience to the harsh conditions with high survivability is the use of inoculated with mycorrhizae seedlings [23-26].

An early start in the absorption of nutrients, water increase the chances of survival of the seedlings [27].

The use of inoculated with mycorrhizae seedlings is going to increase the success of the efforts made by the country to reinstall tree vegetation in areas with harsh climatic conditions [1].

The process can be considered to be an imitation of the natural process of regeneration [27] with research proving the positive impact of symbiotic fungi at the first stages of acclimatization of forest species [10].

The current conditions have led to the creation of advanced methods of seedling production, which was achieved through experimentation and the study of alternative methods of seedling production. The study described in this paper is one of them.

The aim of the study was the use of organic material (forest floor, manure) to improve the properties of inorganic soil and to inoculate the root system of the seedlings with mycorrhizae originated by the use of forest floor.

# 2 Materials and Methods

## 2.1 Study Area

The study was conducted in the research facilities of the laboratory of forest soils in the School of Forestry and Natural Environment, Aristotle University of Thessaloniki, in which the data were collected (photo 1).

### 2.2 Experimental Design

Water is very valuable to Mediterranean ecosystems [28-29]. For multitude of reasons like climatic change and eroded soils, in Greece the half million of one year seedlings planted after wild fires are not succeeded in high degree [30]. The approach of this research was developed by the need to develop plants tolerant to the conditions of the Mediterranean forest environment:

1. In order to replace peat that has some particular positive qualities (low weight, can hold a large amount of water, does not contain weeds, has large alternative capacity) and a major setback (in dry environment shrinks and separates with the soil resulting in air entering in the mass of the soil, not allowing any humidity increase.

2. The production of plants with greater resilience, capable of overcoming the problems that appear when peat is used.

3. In order to decrease the cost by using solid mixed with organic materials instead of peat, and increase the totally volume of the substrate the plant will have during reforestation.



Photo 1. Seedlings of Calabrian pine in the laboratory of forest soils, in Aristotle University of Thessaloniki

The seedlings of Calabrian pine (*Pinus brutia*) were used for the experiment of the study originated from the nursery of Halkidona, 37.2 km near the city of Thessaloniki, with the plants being one year old. The soil was used, originated from gneiss and was collected from the area of Agia Anastasia (Basilika village, 31 km near Thessaloniki). The cow and goat manure originated from the area of Gomati located in Chalkidhiki. The hummus of deciduous evergreen trees and hummus of *Pinus brutia* were collected by the area of Gomati and Thasos respectively.

The hummus was used for a multitude of reasons a) In order to improve the natural properties of soil, especially to the absorption of water.

b) For providing nutrients

c) In order to colonize the soil with mycorrhiza, that increases the uptake of nutrients

The experiment was performed at the 10<sup>th</sup> of February 2017, with the measurements of height starting at the 15<sup>th</sup> of March 2017.

1. Analysis of the sub layer at the start of the study

2. Both the height of every plant and after a while the diameter above the root system were measured every 15 days.

3. When the study ended every plant was separated in its over ground and underground part. The two parts were weighted being both fresh and dry. The plant tissue was analyzed macronutrients (N, P, Ca, Mg, K, Na) and micronutrients (Fe, Cu, Zn, Mn) The plants were separated to above ground and underground parts on the root node.

The seedling of *Pinus brutia* where grown in 3lt bags. With 5 experimental treatments used as sub layer for the seedlings

- 1. 90% gneiss +10 cow manure
- 2. 90% gneiss +10% goat manure
- 3. 90% gneiss + 10% humus of evergreen broadleaf
- 4. 100% gneiss (control group)
- 5. 90% gneiss + 10% Pinus brutia humus

The experiment was performed at 10-2-17, with the measurements of height starting at 15-2-17.

These measurements were about:

- 1. Analysis of the sub layer at the start of the experiment.
- 2. Both the height of every plant and after a while the diameter above the root system were measured every 15 days.
- 3. When the study ended every plant was separated in its over ground and underground part. The two parts were weighted being both fresh and dry. The plant tissue was analyzed macronutrients (N, P, Ca, Mg, K, Na) and micronutrients (Fe, Cu, Zn, Mn).

The plants were separated to aboveground and underground parts on the radical node. The analyses of the aboveground part of the seedlings, needles and stem were included, while the underground part constituted by the root system

### 2.3 Laboratory Analyses

The plants tissue was made in a powdery and homogeneous material that was created after the grinding of the dry sample. To the samples the total N was measured using the Kjeldahl method [31]. The chemical elements Ca, K, Na, Fe, Mn, Zn and Cu were measured with the use of an Atomic Absorption Spectrophotometer in a diluted solution that was produced after the dissolution by H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>  $\kappa\alpha$ t HClO<sub>4</sub> of powdered sample. In the same solution P was determined by the method Molybdenum Blue.

The alkalinity (pH) of the soil suspended in water (1:1 ration) was determined using a potentiometer [31]. For the measurement of Carbon the method of liquid oxidation was used [32]. Organic N was measured with the Kjeldahl method [29]. For the measurement the extractable P Olsen method was used. The alternative cations Ca, Mg, K, Na, were measured with the use of a solutions of CH<sub>3</sub>COONH<sub>4</sub> 1N, pH 7 [35]. With the trace elements Fe, Mn, Zn and Cu being measured with the use of DTPA, pH 7.3. And the extractable ions Ca, Mg, K, Na, K, Na, Fe, Mn, Zn and Cu were measured

with the use of Atomic Absorption Spectrophotometer [34].

#### 2.4 Statistical Analyses of the Data

The data of the study was collected, categorized and statistically modified with SPSS. The results of the study were depicted in diagrams with the use of excel. The data was analyzed using both one way anova and two step cluster analysis.

In particular one way anova was used to examine if there is significant deference in a dependent variable between individuals that differentiate from one another in one independent variable [36]. The method uses the F distribution to compare the estimation of the dispersion between samples [37]. Particularly in the comparison of more than one sample means.

By comparing the five samples means (five layers)  $\mu 1$ ,  $\mu 2$ ,  $\mu 3$ ,  $\mu 4$  and  $\mu 5$  we formulate the H<sub>o</sub> and H<sub>1</sub> in the following way:

- H<sub>o</sub> There is no significant difference between the five means
- $H_1$  There is significant difference between at least to a couple of means.

The assumptions of one way anova according to [38] are: a) Randomness and independence, b) normality and variance equality.

The Shapiro-Wilk test was used to check normality while in order to test assess the equality of variances for a variable Levene test was used [38].

## **3** Results

We used a one-way anova to assess differences in variables (length shoot, and diameter e.tc.) among the different substrate treatments.

The results of the analysis are presented on table 1.

The null hypothesis was tested in order to determine if it was true of false, and the statistical difference between substrates was tested with Tukey's range test and testing of assumptions.

Variables with green color are statistically significant, with a significance level of 0.05.

Kitikidou et al. [39], didn't find any significant interaction between seedlings and substrates while researching a similar topic in four different forest tree species seedlings

In order to check if there is any significant statistical difference between the different substrates the Tukey test is used. The results of the test are demonstrated on table 2. Means that are underlined indicate statistical deference. Means that are accompanied by bold letters are statistically different (a=0.05) according to the Tukey test. The

letter b and less transparent coloring indicates the group with statistically greater values, while the letter and the transparent coloring indicate the group with statistically smaller values. When a substrate is placed in both groups it is represented with ab and the according coloring. The substrates that produce optimal results should be considered to be the ones with less transparent coloring and the letter b for the factors concerning plant growth (the fresh and dry weight).

In the first line of table 2 there is no statistical difference between the substrates with all of them being placed in the same group.

The variable dry weight gr, underground presents significant variance between substrate 5 (90% gneiss + 10% *Pinus brutia* humus), substrate 2 (90% gneiss + 10% goat manure), substrate 3 (90% gneiss + 10% humus of evergreen broadleaf) and substrate 1 (90% gneiss + 10 cow manure). All in substrate 5 is statistically superior to substrates 1, 2 and 3 for the variable fresh weight. While substrate 4 (control group) is placed in both groups cause.

There is a significant difference between substrates 5 and 2 for the variables dry weight for both over and underground plant tissue. Substrates 3, 1 and 4 are grouped and placed in both groups and they don't have any statistical difference.

N % concentration for the above ground tissue of the seedlings is significantly differentiated between the group of substrates 5, 4, 2, 1 and substrate 3. With all substrates being statistically superior to layer 3 for the variable above ground with lower concentration of N %.

N % concentration for the above ground tissue of the seedlings is significantly differentiated between the group of substrates 5, 4, 2, 1 and substrate 3. With all substrates being statistically superior to layer 3 for the variable above ground N % concentration.

N % concentration for the underground tissue if the seedlings is significantly differentiate between the group of substrate 5 and substrate 3. With substrate 5 being statistically superior to substrates 3 while substrates 1,2 and 4 are placed in both groups.

The variable concentration of P mg/gr of the above ground tissue of the seedlings creates 2 groups of substrates with one being substrate 1 and the other group being substrate 4 and 5. With substrate 1 being statistically superior in the concentration of P mg/gr to substrates 4 and 5 while substrates 2 and 3 are placed in both groups

Respectively the variable concentration of P mg/gr of the underground tissue of the seedlings creates 2 groups of substrates with one being substrate 1 and with the other group being substrate

5 concentration of P mg/gr rate 5. With substrate 1 and 4 being statistically superior to substrate 5, while substrates 2 and 3 are placed in both groups.

The variable concentration of Mg mg/gr of the above ground tissue of the seedlings creates 2 groups of substrates with one being substrate 1, 2 and the other group being substrate 3 and 4. With substrates 1 and 2 are statistically superior to substrates 3 and 4 while substrate 5 is placed in both groups.

To the variable concentration of Mg mg/gr of the underground tissue there is sufficiently separation between substrates 1 and 3. This means that statistically substrate 1 contains more quantity of Mg from the substrate 3 Substrates 3 and 4 and 5 are placed in both groups.

The variable concentration of K mg/gr of the above ground tissue of the seedlings creates 2 groups of substrates with one being substrate 1, and the other group being substrate 3. With substrate 1

being statistically superior to substrate 3 while substrates 2, 4 and 5 are placed in both groups.

The variable concentration of Ca mg/gr of the above ground tissue of the seedlings creates 2 groups of substrates with one being substrates 5, 1 and 2 and the other group being substrates 4 and 3. With substrate 5, 1 and 2 being statistically superior (contain more quantity of Ca) comparing to substrates 3 and 4 while substrates 3 and 4 are placed in group 3 of substrates.

Concentration of Cu ppm of the above ground tissue of the seedlings there is differentiation between substrate 4 and substrate 2. With substrate 4 being statistically superior to substrate 2, while substrates 1, 3 and 5 are placed in both groups.

Adding up the results from Table 2 and 3, we can extrapolate a relationship between the growth characteristics of and the nutritional situation of the plants of P. brutia with the soil analyses of substrates.

	Table 1. Concentrated results of dispersion analysis with one factor														
	Characterists	Observing level of	Statistically significant difference of substrates (Tukey method)									of	Check of assumptions		
		statistical significance	1-2	1-3	1-4	1-5	2-3	2-4	2-5	3-4	3-5	4-5	Randomness - Independence	Regulavity	Dispersion equality
Ι	ength shoot 26 of June	sign. = 0.485 > 0.05													
Di	ameter (mm) 26 of June	sign. = 0.321 > 0.05													
	Fresh weight (gr)	sign. = 0.048 < 0.05							ν				yes	sign. 1,2,3,4,5 > 0.05	sign. $= 0.071 > 0.05$
	Dry weight (gr)	sign. $= 0.034 < 0.05$							ν				yes	sign. 1,2,3,4,5 > 0.05	sign. = 0.135 > 0.05
	N%	sign. = 0.0002 < 0.05		ν			ν			ν	ν		yes	sign. 1,2,3,4,5 > 0.05	sign. = 0.843 > 0.05
ч	P mg/gr	sign. = 0.004 < 0.05		ν	ν	ν							yes	sign. 1,2,3,4,5 > 0.05	sign. = 0.633 > 0.05
Aboveground	Mg mg/gr	sign. = 0.0002 < 0.05		ν	ν		ν	ν					yes	sign. 1,2,3,4,5 > 0.05	sign. $= 0.090 > 0.05$
grc	K mg/gr	sign. = 0.031 < 0.05		ν									yes	sign. 1,2,3,4,5 > 0.05	sign. = 0.337 > 0.05
ove	Ca mg/gr	sign. = 0.00003 < 0.05		ν	ν			ν			ν	ν	yes	sign. 1,2,3,4,5 > 0.05	sign. = 0.078 > 0.05
Ab	Na mg/gr	sign. = 0.803 > 0.05													
	Cu ppm ή μg/ml	sign. = 0.0003 < 0.05						ν	ν	ν	ν		yes	sign. $2 = 0.012 < 0.05$	sign. $= 0.432 > 0.05$
	Fe ppm	sign. = 0.223> 0.05													
	Zn ppm	sign. = 0.118 > 0.05													
	Mn ppm	sign. = 0.00003 < 0.05		ν	ν	ν	ν	ν	ν				yes	sign. $1 = 0.008 < 0.05$	sign. = 0.233 > 0.05
	Fresh weight (gr)	sign. = 0.003 < 0.05				ν			ν		ν		yes	sign. 1,2,3,4,5 > 0.05	sign. $= 0.470 > 0.05$
	Dry weight (gr)	sign. = 0.027 < 0.05							ν				yes	sign. 1,2,3,4,5 > 0.05	sign. = 0.375 > 0.05
	N%	sign. = 0.018 < 0.05									ν		yes	sign. 1,2,3,4,5 > 0.05	sign. $= 0.151 > 0.05$
	P mg/gr	sign. = 0.003 < 0.05				ν						ν	yes	sign. 1,2,3,4,5 > 0.05	sign. = 0.625 > 0.05
pu	Mg mg/gr	sign. = 0.029 < 0.05		ν									yes	sign. 1,2,3,4,5 > 0.05	sign. = 0.168 > 0.05
Underground	K mg/gr	sign. = 0.332 > 0.05													
erg	Ca mg/gr	sign. = 0.086 > 0.05													
Jnd	Na mg/gr	sign. = 0.002 < 0.05	ν	ν	ν	ν							yes	sign. 1,2,3,4,5 > 0.05	sign. $= 0.009 < 0.05$
	Cu ppm ή μg/ml	sign. = 0.012 < 0.05						ν					yes	sign. 1,2,3,4,5 > 0.05	sign. $= 0.171 > 0.05$
	Fe ppm	sign. = 0.073 > 0.05													
	Zn ppm	sign. = 0.013 < 0.05								ν	ν		yes	sign. 1,2,3,4,5 > 0.05	sign. $= 0.029 < 0.05$
	Mn ppm	sign. = 0.041 < 0.05											yes	sign. 1 = 0.038 < 0.05 sign. 2 = 0.025 < 0.05	sign. = $0.055 > 0.05$

Table 1. Concentrated results of dispersion analysis with one factor

for significance level a = 0.05

	Substrate							
	90% gneiss + 10% cow manure	90% gneiss + 10% goat manure	90% gneiss +10% humus of evergreen broadleaf	100% gneiss (control group) from St. Anastasia	90% gneiss from St. Anastasia +10% Pinus brutia humus			
Fresh weight (gr) - Aboveground	27.9448	19.2300	20.8500	32.2675	42.6040			
Fresh weight (gr) - Underground	23.0700 <b>a</b>	16.0700 <b>a</b>	17.3920 <b>a</b>	25.7650 <b>ab</b>	45.9066 <b>b</b>			
Dry weight (gr) - Aboveground	10.1940 <b>ab</b>	7.5580 <b>a</b>	8.2740 <b>ab</b>	12.8125 <b>ab</b>	16.6700 <b>b</b>			
Dry weight (gr) - Underground	4.5840 <b>ab</b>	3.3180 <b>a</b>	3.9140 <b>ab</b>	6.2025 <b>ab</b>	8.4260 <b>b</b>			
N% - Aboveground	1.2978 <b>b</b>	1.3262 <b>b</b>	0.9917 <b>a</b>	1.3713 <b>b</b>	1.4805 <b>b</b>			
N% - Underground	1.2506 <b>ab</b>	1.1473 <b>ab</b>	0.8433 <b>a</b>	1.0627 <b>ab</b>	1.3551 <b>b</b>			
P mg/gr - Aboveground	1.5367 <b>b</b>	1.2647 <b>ab</b>	1.2294 <b>ab</b>	1.1808 <b>a</b>	1.07275 <b>a</b>			
P mg/gr - Underground	1.2505 <b>b</b>	1.0885 <b>ab</b>	1.1029 <b>ab</b>	1.2546 <b>b</b>	0.8505 <b>a</b>			
Mg mg/gr - Aboveground	1.7165 <b>b</b>	1.7080 <b>b</b>	1.1132 <b>a</b>	0.9752 <b>a</b>	1.3924 <b>ab</b>			
Mg mg/gr - Underground	2.4835 <b>b</b>	1.9818 <b>ab</b>	1.4365 <b>a</b>	1.8710 <b>ab</b>	1.996 <b>ab</b>			
K mg/gr - Aboveground	6.3123 <b>b</b>	5.7747 <b>ab</b>	4.3072 <b>a</b>	5.0271 <b>ab</b>	5.1812 <b>ab</b>			
Ca mg/gr - Aboveground	4.6169 <b>c</b>	3.9484 <b>bc</b>	3.3046 <b>ab</b>	2.5932 <b>a</b>	4.8173 <b>c</b>			
Cu ppmή μg/ml - Underground	13.3735 <b>ab</b>	7.6990 <b>a</b>	9.6931 <b>ab</b>	16.0150 <b>b</b>	10.6932 <b>ab</b>			

#### Table 2. Table of checking the totally homogeneity of the groups

for significance level a = 0.05

Table 3. Substrate analysis

	Substrate						
	90% gneiss + 10% cow manure		90% gneiss +10% humus of evergreen	100% gneiss (control group) from St.	90% gneiss from St. Anastasia +10% Pinus		
	5.96	6.25	broadleaf	Anastasia	brutia humus		
рН	5.86		6.27	6.14	6.29		
C%	1.189	1.494	1.565	0.908	1.621		
Organic matter	2.050	2.575	2.652	1.265	2.794		
N%	0.124	0.086	0.110	0.109	0.165		
C/N	9.562	17.417	14.014	8.359	9.824		
Pmg/100gr of soil	Pmg/100gr of soil     1.480     1.250		0.360	0.410	2.060		
Ca cmol/kg	9.931 10.403		11.695	9.417	10.883		
Mg cmol/kg	Mg cmol/kg 4.593 4.813		4.079	4.426	5.210		
K cmol/kg	K cmol/kg 0.476 0		0.146	0.183	0.850		
Na cmol/kg	0.609	0.332	0.251	0.229	0.300		
Cu ppm or µg/ml	0.376	0.242	0.250	1.858	1.934		
Fe ppm	37.42	20.06	44.74	62.38	78.63		
Zn ppm	0.906	1.048	0.950	0.568	0.596		
Mn ppm	10.98	5.84	11.16	21.80	8.46		

In particular, for substrate 5 in table 2 the seedlings seem to have greater value of N% for both above and underground tissue while also having high values of N% in analysis of soil (table 3). Also in substrate 5, Ca cmol/Kg presents statistically greater values in the over ground tissue while Ca cmol/Kg in the underground tissue doesn't differ with from any of the other substrates (table 3).

## **4** Conclusion

Taking into account the results of table 2 and 3 in come to the following conclusions.

The study used one way anova and found major differences between the manifested characteristics of the seedlings. More specifically the fresh weight of the shoot and diameter of the seedlings of the substrates presented small differences with no substrate that can be considered superior.

Substrate 5 provides better results than substrates 1 2 and 3 in increase of fresh weight of shoot and leaves of the seedlings.

Substrate 4 (control group) provided average results.

With substrate 5 proving more effective than sub substrate 2 in the increase of dry weight of both length shoot, diameter and roots with neutral results in substrates 4,3 and 1

A reliable solution that can be used in *Pinus brutia* seedlings is substrates 5 using 90% gneiss and 10% humus of *Pinus brutia* with sub layer 3 (90% gneiss and 10% goat manure) being an acceptable alternative.

Use of *Pinus brutia* humus is beneficial cause of the existence of fungi that can create mycorrhizae. The main benefit is the easy absorption of nutrients needed by the seedlings. Substrates with results of average impact from best to worst are substrate 4 (100 % gneiss control group), substrate 1 (90 % gneiss + 10% cow manure) and substrate 3 (90 % gneiss + 10% humus of evergreen broadleaf).

The highest interest presents substrate 5 with plants cultivated in it having the largest amounts of N mg/gr for length shoot and diameter, above and underground and large amounts of Ca mg/gr for shoot and crown. With substrate presenting the lowest value for P mg/gr for root, shoot and crown even through the seedlings that grown on the particular layer had greater development (in weight) than their counterparts.

From the soil analysis of substrate 5 has high values of N cmol/Kg while values of Ca cmol/Kg are average in comparison to the other 5. Also substrate 5 has the greatest values for P mg/100gr, Mg cmol/Kg, K cmol/Kg and Cu ppm.

We should accept that the amount of nutrients has no impact in the fertility of the soil with the availability of those nutrients being of major importance.

These findings reveal that the approach used here is suitable for preliminary screening of the impact of a forestry species on soil, to aid in species selection and improve soil health for afforestation and reforestation projects [40].

The conclusions we take from the results constitutes preliminary results where we can based and organize a future research using only the substrate 5, with 90% gneiss and 10% *Pinus brutia* hummus .and checking the kind of mycorrhiza there are in the particular hummus in the area and its contribution to the development of the seedlings.

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#### **Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)**

-Tampakis Antonios designed the research and coordinated all the phases for its effective implementation.

-Papaionou Evgenia, designed the methods and materials used for the research.

-Hatzistathis Theoharis designed and coordinated the data collection.

-Paraskevi Karanikola was responsible for the implementation of fieldwork.

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