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Original Article

# Kinetics of growth, development and absorption of nutrients in stone pine (*Pinus pinea* L.) cultivated in the presence of NaCl

## Cinética de crescimento, desenvolvimento e absorção de nutrientes em pinheiro-manso (*Pinus pinea* L.) cultivado na presença de NaCl

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**Abstract**

The aim of the present work was to evaluate and analyze the growth and mineral nutrition response of stone pine (*Pinus pinea* L.) seedlings, an economically important forest species. We analyzed the salinity effects on the kinetics of growth, development, and absorption of nutrients of plants cultivated under controlled conditions on a solid organic substrate. *Pinus pinea* plants were able to tolerate 25 mM NaCl concentration without reduced growth compared to the non-saline control. However, the salt concentration of 50 mM significantly affected the seedling growth after two weeks of treatment. Root growth activity was decreased more than the aerial parts at applied NaCl concentrations. On the other hand, seedlings restricted the transport of $Na^+$ ions to the aerial parts and were strongly selective in favour of $K^+$ ions. The presence of NaCl in the culture medium decreased the absorption rate and the export of $K^+$ and $Na^+$ ions to the aerial parts. This was reflected in the accumulation way of these two ions in the whole plant.

**Keywords:** *Pinus pinea* L, salinity, growth, mineral nutrition, flow of absorption, flow of transport.

**Resumo**

O objetivo do presente trabalho foi avaliar e analisar a resposta de crescimento e nutrição mineral de mudas de pinheiro-manso (*Pinus pinea* L.), uma espécie florestal economicamente importante. Analisamos os efeitos da salinidade na cinética de crescimento, desenvolvimento e absorção de nutrientes de plantas cultivadas sob condições controladas em substrato orgânico sólido. As plantas de *P. pinea* foram capazes de tolerar a concentração de 25 mM de NaCl sem redução do crescimento em comparação com o controle não salino. No entanto, a concentração de sal de 50 mM afetou significativamente o crescimento das plântulas após duas semanas de tratamento. A atividade de crescimento das raízes foi mais reduzida do que as partes aéreas nas concentrações de NaCl aplicadas. Por outro lado, as plântulas restringiram o transporte de íons $Na^+$ para a parte aérea e foram fortemente seletivas em favor dos íons $K^+$. A presença de NaCl no meio de cultura diminuiu a taxa de absorção e a exportação de íons $K^+$ e $Na^+$ para as partes aéreas. Isso se refletiu na forma de acúmulo desses dois íons em toda a planta.

**Palavras-chave:** *Pinus pinea* L., salinidade, crescimento, nutrição mineral, fluxo de absorção, fluxo de transporte.

## 1. Introduction

Soil salinization is one of the major troubles affecting soil quality and limiting crop productivity in many agricultural regions all over the world (Mustafa et al., 2019). Tunisia is a semi-arid country due to its location between the Mediterranean Sea and the Sahara. In semiarid Tunisia about 50% of the irrigated land is considered as highly sensitive to salinization (Bouksila et al., 2013). Approximately, 20% of agricultural lands in Tunisia, as in Chott and Sebkha, have been destroyed as a result of rapid increase of salts accumulation due to poor quality of water used in these area for irrigation. This situation combined with the intensive evaporation will raise the surface salt concentration, which have a negative impact on agriculture, particularly in arid regions (Corwin, 2021). To improve agronomic yields, there are generally two possible two steps that can be taken to improve agronomic yields. The first involves either desalination of salted water or purification of land by installing drainage canal systems. But this operation is

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very expensive and requires significant investments. The second comprises developing an improved knowledge of the physiology and genetics of the plant tolerance to salinity, which is a prerequisite for realizing afforestation programs or selecting species and varieties that tolerate salt stress (National Research Council, 1990).

On the other hand, Stone pine, *Pinus pinea* is a native plant to the Mediterranean region. This plant is an important tree species, due to the Ancient use of its edible seeds (Küçüker and Baskent, 2017; Loewe and Delard, 2019), and its ability to adapt to different environmental conditions and its relatively fast growth. On the other hand, stone pine is prized for fruit production in addition to the industrial value of its wood. The seeds are oily and particularly rich in lipids, which comprise 45 –50% of the dry seed weight (Nasri and Triki, 2004), vitamins and mineral ions (Nergiz and Donmez, 2004). Its seeds have been used since antiquity times as a culinary product in the Mediterranean. In the Middle East and Tunisia, it is used in the pastry industry (Nasri and Triki, 2007). Stone pine, increased salt accumulation is one of the most challenges facing species (Ganatsas and Tsakaldimi, 2007; Khaldi et al., 2011). Therefore, the study of salt tolerance during germination and growth stages of the plant is important for determining saline limits at each stage of growth (Teobaldelli et al., 2004; Zapata et al., 2004; Deligoz and Gur, 2015).

The aim of the present work is to evaluate and analyze the growth and mineral nutrition response of stone pine (*Pinus pinea* L.) trees, an economically important forest species. Its culture in a nursery could expose it to saltwater irrigation. Moreover, in spite of the multitude of existing studies on the subject of salinity, very few data are available on the salt tolerance of this plant since forest grounds are rarely affected by this constraint (Epron et al., 1999). In the present study, we analyzed the salinity effects on the kinetics of growth, development, and absorption of nutrients of plants cultivated under controlled conditions on a solid organic substrate.

## 2. Materials and Methods

### *2.1. Plant material and culture conditions*

Two-month-old seedlings were obtained by direct sowing in plastic plates containing compost irrigated with tap water. This step was carried out at the pilot nursery of Wad El-Bir (Nabeul, Tunisia). Then, the cultures were grown under artificial light (fluorescent tubes cool-white of 40 W) with a photoperiod of 16 H and an average intensity of 150 μmol/m$^2$/s measured at the plant level. The temperature and relative humidity were 27°C and 60% during the day and 23°C and 80% at night, respectively. These two parameters were monitored using a thermo-hygrograph.

The seedlings, maintained on compost, were irrigated for 28 days with a complete nutrient solution enriched with NaCl at two concentrations (25 and 50 mM). NaCl was added in the culture medium, after one week of acclimatization of plants to the basic nutrient solution containing the following macronutrients (mM): $Ca(NO_3)_2.4H_2O$: 1.75; $KNO_3$: 0.5; $MgSO_4.7H_2O$: 0.5; $KH_2PO_4$: 0.5; $K_2HPO_4$: 0.25, as well as trace elements (ppm): B: 0.16 ; Fe: 1.4 ; Cu: 0.03; Zn: 0.03; Mn: 0.25; Mo: 0.01. The compost consisted of leafy branches of Acacia and pine bark. The pH was close to 6, its electric conductivity was 415 µS and its water content (WC) at saturation was 71.32%. Seedlings were irrigated every 3 days at a rate of 50 ml per plant.

### *2.2. Plant collection*

Five harvests were performed for every 10 plants per harvest: the first before salt irrigation and the others at weekly intervals during 28 days irrigation. The collected plants were separated into aerial parts (AP) and roots (R). The length of the most developed root and the number of verticils were measured. Aftert harvest, the fresh matter (FW) was determined by double weighing with a precision balance (0.1 mg). After drying plants in an oven at 80°C for 48 hours, dry matter (DW) was measured.The fresh matter (FW) was determined by double weighing at harvest using a precision balance (0.1 mg). Dry matter (DW) was measured after drying plants in an oven at 80°C for 48 h.

### *2.3. Extraction and mineral ion dosage*

Dried samples were ground to fine powder and digested with nitric acid 0.1 N $HNO_3$. The contents of $K^+$, $Na^+$ and $Ca^{2+}$ in the digestion extract were determined with an Eppendorf flame photometer (Wolf, 1982). The $Cl^-$ content was determined by a coulometric measurement with a Buchler Cotlove chloridometer (Zid and Grignon, 1985).

### *2.4. Growth and development*

Parameters used to study growth were FW and DW weights of roots and AP, expressed in mg/plant. The ratio of the organ biomasses of AP to R (AP/R) was calculated as a parameter used to determine the biomass distribution between the two plant organs, i.e., the allocation of their photosynthetic assimilates.

### *2.5. Water Content (WC)*

The WC of the organs was expressed in ml/g DW, and was defined as follows (Equation 1):

$$\mathbf{WC} = \left(\mathbf{FW} - \mathbf{DW}\right) / \mathbf{DW} \tag{1}$$

Where:
DW: Dry weight
FW: Fresh weight

### *2.6. Relative Average Growth (RAG)*

Average relative growth (RAG) indicates the biosynthesis activity of plant organs. This parameter was expressed as the DW produced per unit of biomass and unit of time, and is calculated according to the following formula (Equation 2):

$$RAG = \left(DW_{t1} - DW_{t2}\right) / \left[\left(DW_{t1} + DW_{t2}\right) / 2\right] x \, \Delta t \tag{2}$$

Where:
$DW_{t1}$: DW mass (mg) before salt application
$DW_{t2}$: DW mass (mg) at the end of treatment period

Δt: Duration of the treatment, in days.

*2.7. Balance of ionic transport*

The balance of ionic transport during the culture was determined by the difference in the amount of ions between the plants at harvest and before salt irrigation. The measured quantities in the roots allowed the root accumulation to be estimated. Those measured in the AP allowed the salt exported by the xylem to be estimated. The sum of the two quantities gave the net absorption. All of these quantities were expressed in μeq/plant.

*2.8. Rates of net uptake (J) and transport ($J_s$)*

*2.8.1. Rate of net uptake (J)*

The rate of net uptake of the ions can be estimated by the following formula from Pitman (1975) (Equation 3):

$$\mathbf{J} = (\mathbf{Q2} - \mathbf{Q1}) / \mathbf{WR}\ (\mathbf{T_2} - \mathbf{T_1}) \tag{3}$$

Where Q1 and Q2 are the ion contents of the whole plant at harvest times T1 and T2, respectively.

Equation 4:

$$\mathbf{WR} = (\mathbf{WR_2} - \mathbf{WR_1}) / \mathbf{logE}(\mathbf{WR_2} / \mathbf{WR_1}) \tag{4}$$

Where WR1 and WR2 are the weights of the root system at harvest times T1 and T2, respectively.

*2.8.2. Rate of net transport in AP ($J_s$)*

The rate of net transport in the AP was calculated according to the formula (Equation 5):

$$\mathbf{Js} = (\mathbf{Qs_2} - \mathbf{Q_{S1}}) / \mathbf{WR}\ (\mathbf{T_2} - \mathbf{T_1}) \tag{5}$$

Where Qs1 and Qs2 are the ions contents of the shoot at harvest times T1 and T2, respectively.

Equation 6:

$$\mathbf{WR} = (\mathbf{WR_2} - \mathbf{WR_1}\ /)\mathbf{log_E}(\mathbf{WR_2} / \mathbf{WR_1}) \tag{6}$$

Where WR1 and WR2 are the weights of the root system at harvest times T1 and T2, respectively

*2.9. Ionic Contents (IC)*

Given in meq/g DW, the ionic contents (IC) express the root and AP tissue enrichment and enable the plant nutritional state under the applied treatments to be diagnosed. It was calculated according to the following formula (Equation 7):

$$\mathbf{IC} = \mathbf{Ionic\ quantities / DW} \tag{7}$$

*2.10. $K^+/Na^+$ selectivity*

The equivalent ionic fraction $K^+/(K^+ + Na^+)$ is given on the IC of the AP and roots. Reported to the equivalent ionic fraction in the medium, this ratio defines the $K^+/Na^+$ selectivity in the plant (Glenn et al., 1994).

*2.11. Statistic analysis*

The statistical analyses were carried out by using the software Statsoft STATISTICA (Version 8.0, StatSoft Inc., Tulsa, USA). The various parameters under salt stress were followed on the basis of ten replications for each treatment. The measured parameters were subjected to an analysis of variance. The confidence interval was calculated at the threshold of 95%.

## 3. Results and Discussion

*3.1. Development*

In the absence of salt, the average number of twigs increased from 1.5 to 6.7 after 3 weeks of culture and remained constant until the 4th week. With 25 mM NaCl, the same result was reached but only after 5 weeks where development was slower during the first two weeks and accelerated thereafter. With 50 mM NaCl, the initial development remained the same for 2 weeks but then stopped totally (Figure 1A).

The evolution of the biomass ratio of AP to R (AP/R) is an indication of the allowance of the photosynthetic assimilates

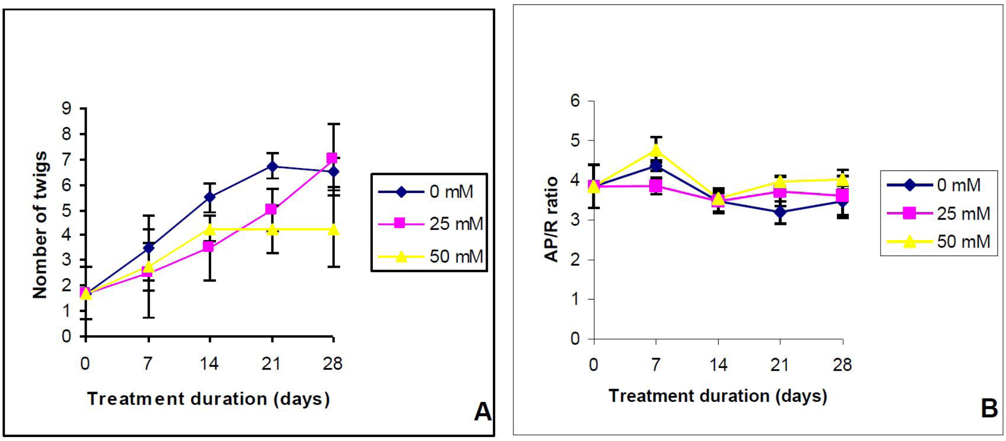



**Figure 1.** Variation of twig number (A) and AP/R ratio (B) during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.

under saline stress. The presence of NaCl in the culture medium did not exert any significant effect on the AP/R ratio. The latter remained constant throughout the experiment in both the absence and presence of NaCl (Figure 1B), confirming that the salt did not modify the allowance of the photosynthetic assimilates to the two parts of the plant.

*3.2. Growth*

*3.2.1. Root growth*

The length of the most developed root was increased linearly throughout the culture duration. In the presence of NaCl (25 and 50 mM), root elongation was initially maintained as in the control medium for up to two weeks of culture but then began to slow down (Figure 2A).

*3.2.2. Ponderal growth*

During four weeks of cultivation, the biomass of the whole plant, cultivated in the control medium, increased linearly but with a slowdown in the fourth week. The linearity of the growth curve was maintained at 25 mM NaCl, but at a slower rate than the control rate, except for the fourth week in which the biomasses became similar. With 25 and 50 mM NaCl, the growth curves merged during the first two weeks, after which the biomass was plateaued with 50 mM NaCl with growth stopping (Figure 2B). The same root system behavior reflected the evolution of this growth profile for the entire plant (Figure 3A) and the AP (Figure 3B). For all species, the salinity level of the medium above a certain threshold reduced the biomass. Nevertheless, the degree of growth inhibition depends on the genus, species, variety and development stage of the plant and plant part (Sané et al., 2021).

The mean of accumulation for DW by AP and roots was 3.5 and 10.3 mg per day, respectively, over the control medium. It was remained the same in the presence of 25 mM NaCl, throughout the course of treatment. The same initial velocity was maintained with 50 mM NaCl, but only during the first two weeks of culture, after which biomass production stopped.

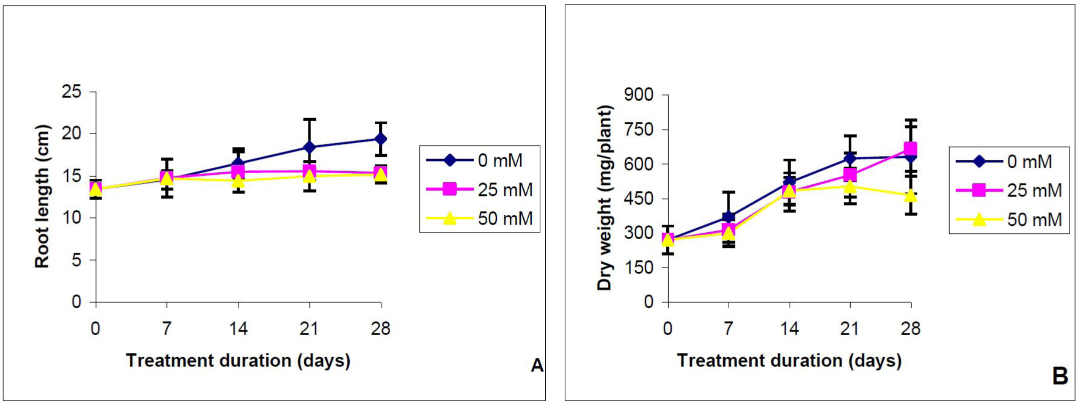



**Figure 2.** Variations in root length (A) and whole plant biomass production (dry matter) during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.

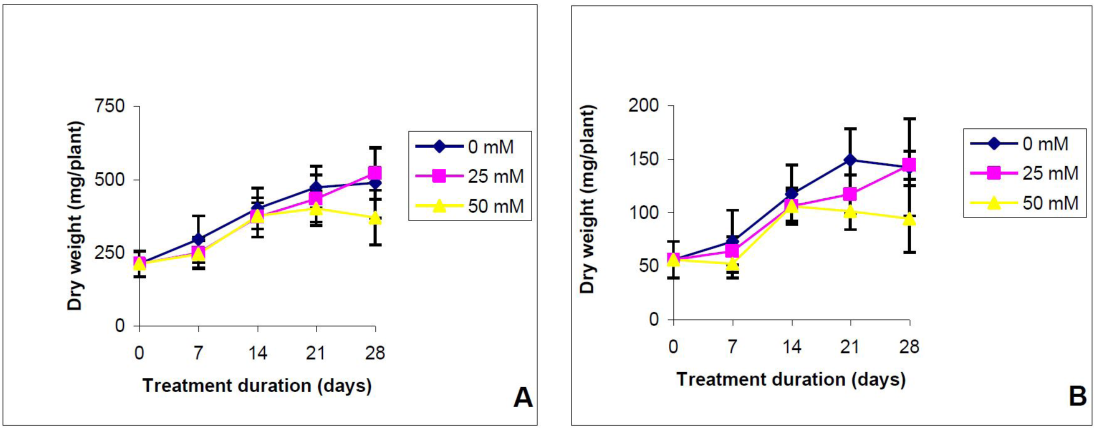



**Figure 3.** Variation of biomass production (dry matter) of roots (A) and aerial parts (B) during the treatment period in *Pinus pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.

The activity of organ growth was estimated by calculating RAG at 7-day intervals. Table 1 shows very irregular roots RAG and AP over time. RAG values were particularly high in the second week. In general, salt reduced the RAG of two types of organs. In particular, with 50 mM NaCl, the RAG of the roots became negative, indicating a biomass loss (necroses of part of the root system) from the first week of culture. With this concentration, the RAG of the roots was affected increasingly more than AP.

*3.2.3. Hydration*

Figure 4A-4B present the WC of the two plant organs. Before the application of NaCl, the WC of the roots and the AP were about 11 and 4.5 ml/g DW, respectively. Over time, the hydration of the roots, such as AP, was decreased in a progressive way, independently of the salt concentration in the medium, indicating root lignification (Franco et al., 2011).

*3.3. Transport of ions*

*3.3.1. Quantities of absorbed ions*

In the absence of NaCl, the quantity of $K^+$ in the roots was increased gradually during the four weeks of culture with a mean velocity of accumulation of 0.041 mg per day. With 25 mM NaCl, the quantity of $K^+$ also was increased progressively, but from the second week of culture. Thereafter, the $K^+$ accumulation rate was maintained at the same level as the control, but the accumulated $K^+$ quantities always remained lower than those of the control, due to the initial shift. In the presence of 50 mM NaCl, the quantities of potassium slowly were evolved during the culture period, indicating the presence of an energy source in $K^+$ (Figure 5A). In AP, the quantity of $K^+$ was increased linearly in the control medium, as with 25 mM NaCl, with a slight deceleration in the fourth week. This linear increase continued with 50 mM NaCl, but only during the first two weeks, and at the end of it no additional absorption of $K^+$ was recorded (Figure 5B) (Botella et al., 1997).

In the control medium, as with 25 mM NaCl, the root calcium showed a gradual increase over four weeks of culture, while the accumulated quantities were increased in the presence of 50 mM NaCl during the second week and then stabilized (Figure 5C). In AP, the accumulated quantities of calcium were increased according to time, in both the absence and presence of 25 mM NaCl, while this increase was only persisted during the first two weeks of culture with 50 mM NaCl (Figure 5D).

In the control medium, the roots, like the AP, initially contained small quantities of $Na^+$ and $Cl^-$. In the presence

**Table 1.** Relative average growth (per week) in roots (R) and aerial parts (AP) during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest.

| NaCl (mM) | 0 mM | | 25 mM | | 50 mM | |
|---|---|---|---|---|---|---|
| TD (days) | AP | R | AP | R | AP | R |
| **7** | 0.252±0.03 | 0.329±0.05 | 0.126±0.01 | 0.161±0.02 | -0.084±0.01 | 0.147±0.04 |
| **14** | 0.462±0.08 | 0.308±0.06 | 0.490±0.03 | 0.392±0.09 | 0.686±0.05 | 0.420±0.07 |
| **21** | 0.196±0.04 | 0.161±0.02 | 0.098±0.01 | 0.154±0.02 | -0.049±0.01 | 0.036±0.001 |
| **28** | 0.028±0.01 | 0.028±0.00 | 0.210±0.03 | 0.182± 0.04 | -0.077±0.02 | -0.084±0.01 |

TD: Treatment duration (days); values are means ± SD of three independent determinations.

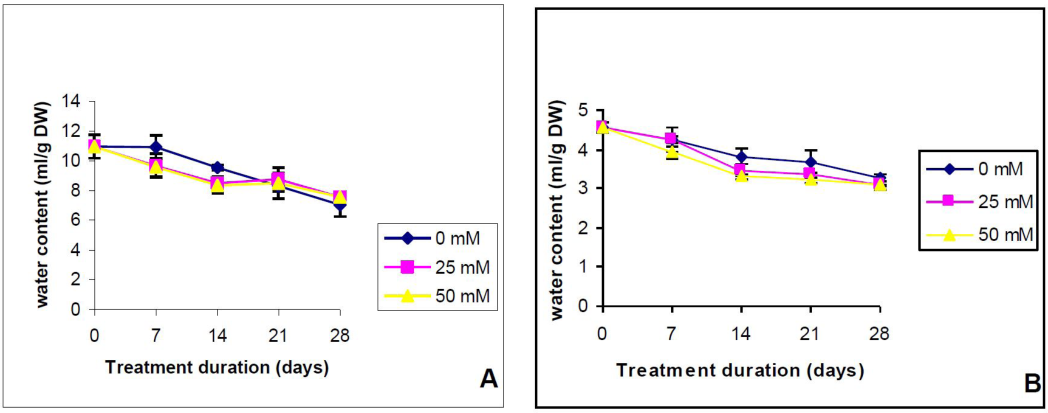



**Figure 4.** Variation of water content in roots (A) and aerial parts (B) during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.

of 25 mM NaCl, the amounts of $Na^+$ in the roots and AP were increased proportionally over time, without any sign of saturation, but the amounts absorbed were higher in the roots (Figure 6A) than in AP (Figure 6B). The $Na^+$ absorption velocity was 0.010 mg daily in the roots and 0.014 mg daily in AP. With 50 mM NaCl, the quantities of $Na^+$ accumulated in the roots were of the same order of magnitude as those with 25 mM NaCl. However, they were strongly increased in AP after two weeks of treatment.

The kinetics of chloride absorption according to time was slightly different from that of $Na^+$. At first, all the $Cl^-$ quantities present in the roots, and even more in AP, were particularly high in the control medium but were stable from the 7$^{th}$ to 28$^{th}$ day of culture. In the presence of salt (25 and 50 mM) the quantities of $Cl^-$ present in the roots were increased rapidly during the first two weeks, but the speed of accumulation in these organs was slowed during the following two weeks. The quantities accumulated in the roots were slightly higher with 50 mM of them with 25 mM (Figure 6C). In AP, the transport of chloride overtime was more uniform and was not significantly dependent on the NaCl concentration in the culture substrate (Figure 6D). (Ref)

*3.3.2. Rates of net uptake (J)*

Table 2 shows that in control plants, the net uptake rate of $K^+$ was predominant compared to that of $Ca^{2+}$ and $Na^+$ throughout the culture period. Moreover, it was reduced gradually over time, in the absence and presence of NaCl. In general, the net uptake rate of these three cations was higher during the first two weeks of culture compared to the following two weeks. The presence of NaCl (25 and 50 mM) generally reduced the net uptake rate of $K^+$ and $Ca^{2+}$ thus explaining the root accumulation defect of these two elements (Davenport et al., 1997; Lima et al., 2020).

The net uptake rate of $Na^+$ was usually lower than that of $K^+$, despite the prevalence of sodium in the culture medium ($Na^+$ = 25 or 50 mM; $K^+$ = 1.5 mM), suggesting that the stone pine has a strong K/Na selectivity of absorption. Accordingly, Safavi and Khajehpour (2008), and Apse and Blumwald (2007) reported that the $Na^+$ entry restriction of was attributable to the existence of an active system for the efflux of this cation, located on the plasmalemma of

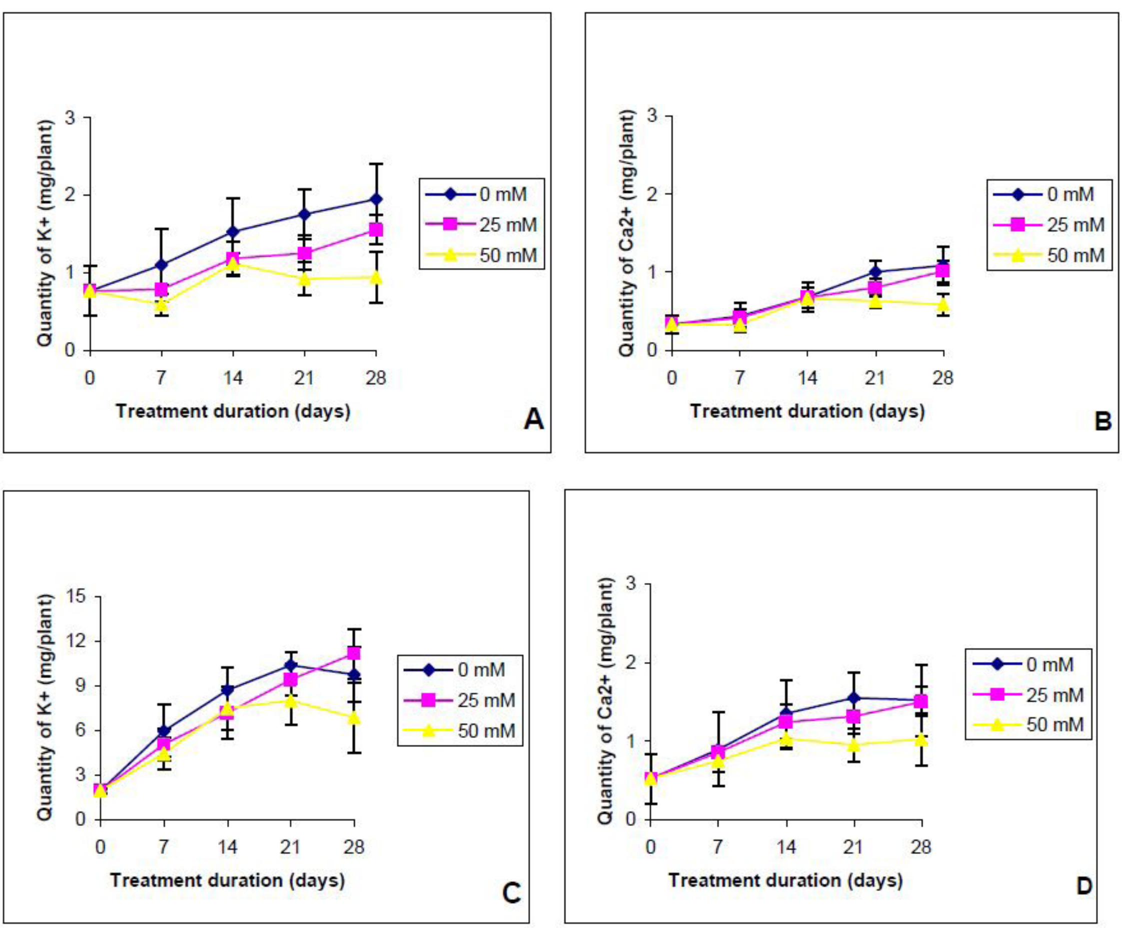



**Figure 5.** Variation of $K^+$ and $Ca^{2+}$ quantities in roots (A) and (B) and in aerial parts (C) and (D), respectively, during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.

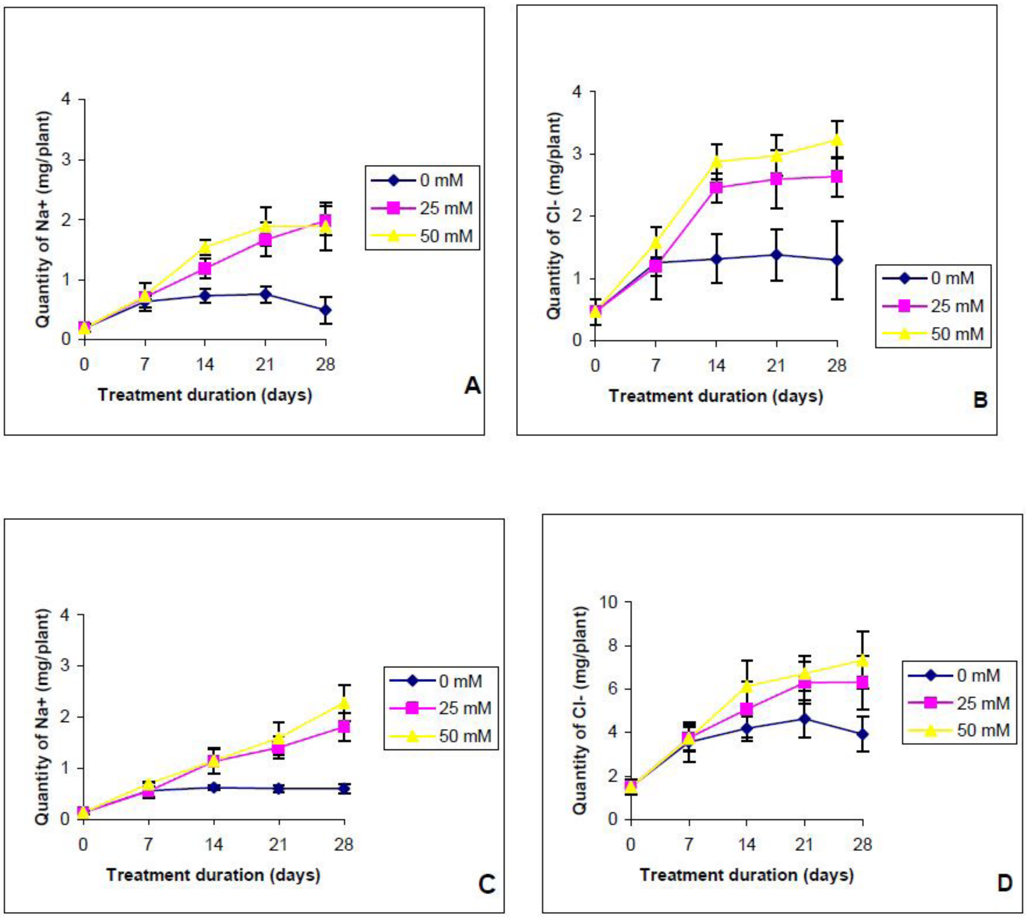



**Figure 6.** Variation of $Na^+$ and $Cl^-$ quantities in roots (A) and (B) and in aerial parts (C) and (D), respectively, during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.

**Table 2.** Variation of the $K^+$, $Ca^{2+}$, $Na^+$ and $Cl^-$ net uptake ($J_{AB}$) during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest. The results are expressed in mg/g DW of the root system/week. Negative values indicate nutrient loss.

| $J_{AB}$ | $K^+$ | | | $Ca^{2+}$ | | | $Na^+$ | | | $Cl^-$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TD (days)** | **0** | **25** | **50** | **0** | **25** | **50** | **0** | **25** | **50** | **0** | **25** | **50** |
| **7** | 61±5 | 46±3 | 39±6 | 7±1 | 7±1 | 4±1 | 9±2 | 15±2 | 20±4 | 28±3 | 54±8 | 76±11 |
| **14** | 34±6 | 30±3 | 23±1 | 7±1 | 8±1 | 6±1 | 2±0.5 | 13±3 | 17±2 | 7±1 | 27±4 | 42±20 |
| **21** | 15±2 | 21±4 | 8±1 | 4±1 | 2±0 | 2±0 | 0±0 | 7±1 | 14±3 | 3±0.5 | 14±2 | 10±2 |
| **28** | 9±1 | 13±2 | -11±2 | 1±0 | 3±1 | 3±1 | -2±0 | 5±1 | 8±2 | -6±1 | 1±0 | 13±2 |

TD: Treatment duration (days); values are means ± SD of three independent determinations.

cortical root cells. The uptake of $Cl^-$ was always higher than that of $Na^+$ but was reduced over time (Ballesteros et al., 1997; Bayuelo-Jiménez et al., 2003; Hasegawa, 2013).

### 3.3.3. *Rate of net transport in the AP ($J_s$)*

The depressive effect of salinity on plant growth can have two main-and non-exclusive causes: difficulties of water and nutrient supply, and the toxicity of excessive ion accumulation in the plant (Xiong and Zhu, 2002). Table 3 shows that the rate of net $K^+$ transport in AP was decreased gradually over time, for both the control plants and those cultivated in the presence of NaCl. Moreover, the salt reduced-$K^+$ transmission in AP. The net transport rate of $Ca^{2+}$ in AP was definitely lower than that of $K^+$ and was less sensitive to salinity (Caines and Shennan, 1999). That of $Na^+$ was also very low compared to $K^+$, although this cation was definitely represented in the medium less than $Na^+$. This result demonstrated the ability of the juvenile stone pine to restrict $Na^+$ transport in its photosynthetic organs, a characteristic attributed to the strong selectivity of xylem secretion in favour of $K^+$ (Yadav et al., 2011). The $Cl^-$ transport flows were higher than those of $Na^+$ and tended to decrease over time.

*3.4. Ionic Contents (IC)*

The IC indicated the enrichment of roots and AP tissues as this parameter integrates the velocity of ion absorption and organ growth. In the control medium, the $K^+$ contents of the roots were increased slightly and uniformly throughout the culture. However, in the salted medium, they dropped slightly and moderately with 25 mM NaCl compared to 50 mM NaCl (Figure 7A). In AP, the $K^+$ contents were strongly increased and doubled during the first week. Thereafter, they exhibited remarkable stability, with the level of $K^+$ accumulation remaining completely independent of the salt concentration in the medium. This suggests that there was a strict relation between the

**Table 3.** $K^+$, $Ca^{2+}$, $Na^+$ and $Cl^-$ net transport in aerial parts ($J_{AP}$) during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest. The results are expressed in mg/g DW of the root system/week. Negative values indicate nutrient loss.

| $J_{AP}$ | $K^+$ | | | $Ca^{2+}$ | | | $Na^+$ | | | $Cl^-$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TD (days)** | **0** | **25** | **50** | **0** | **25** | **50** | **0** | **25** | **50** | **0** | **25** | **50** |
| **7** | 52±9 | 41±5 | 26±2 | 5±1 | 5±1 | 4±1 | 7±1 | 7±2 | 11±1 | 32±4 | 37±3 | 46±6 |
| **14** | 29±2 | 25±4 | 17±2 | 7±1 | 5±1 | 5±1 | 1±0 | 7±1 | 6±1 | 7±1 | 16±3 | 32±6 |
| **21** | 13±2 | 18±3 | 5±1 | 2±0 | 1±0 | -1±0 | 0±0 | 2±0 | 8±1 | 3±1 | 11±2 | 6±2 |
| **28** | 6±0.5 | 13±3 | -12±1 | 0±0 | 1±0 | 1±0 | 0±0 | 3±0.5 | 7±1 | -5±1 | 0±0 | 10±3 |

TD: Treatment duration (days); values are means ± SD of three independent determinations.

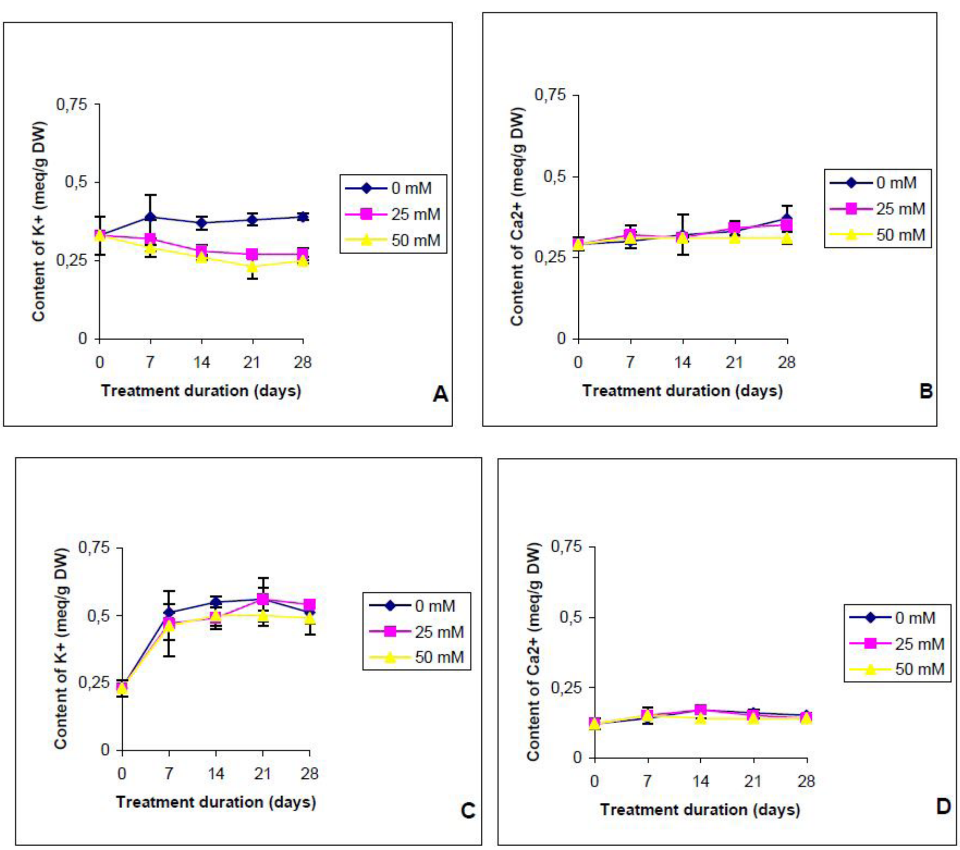



**Figure 7.** Variation of $K^+$ and $Ca^{2+}$ contents in roots (A) and (B) and in aerial parts (C) and (D), respectively, during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.

$K^+$ quantities transported in AP and the DW quantities produced by these parts (Figure 7C).

In the control medium, as with 25 mM NaCl, the roots were enriched in $Ca^{2+}$ over time, while the calcium contents of the roots remained constant throughout the culture with 50 mM NaCl. In both, the absence and presence of 25 mM NaCl (Figure 7B), the AP was enriched with calcium during the first two weeks, after which its $Ca^{2+}$ content decreased. With 50 mM NaCl, the calcium content decreased early after the first week of treatment (Figure 7D).

When the plants were cultivated in the control medium, the $Na^+$ and $Cl^-$ content peaked at the end of the first week, and gradually decreased to the minimum on the 28th day of culture. This fall was attributed to the reduction in the flows of absorption over time. With salt media, the first week of culture was marked by fast enrichment of plants (roots and AP) in sodium and chloride, after which the accumulation of two ions decreased but was generally higher with 25 mM than with 50 mM NaCl. In addition, AP was enriched to a lesser extent in $Na^+$ and $Cl^-$ from the roots (Figure 8A and 8C), because the transport flows were restricted in AP (5.3). Moreover, $Na^+$ accumulation in AP was always less than that of $Cl^-$ (Figure 8B and 8D).

### *3.5. K/Na selectivity of accumulation*

For all plants, and especially for cereals, the maintenance of good tissue K/Na selectivity can be regarded as a criterion for salinity tolerance. The relation between K/Na selectivity and salinity tolerance was the subject of several studies (Jeschke, 1984; Bizid et al., 1988; Gorham et al., 1990). Greenway (1962) showed that the tolerant barely cultivar has a greater K/Na selectivity than the sensitive one. In the same way, the tolerance of some clover cultivars is associated with a limitation in $Na^+$ transport into AP and the maintenance of high K/Na selectivity (Shannon and Noble, 1995). In *Setaria verticillata*, sensitivity to salt is probably related with the fall of this selectivity, even at the lowest NaCl concentrations (Ahmed et al., 2008). Figure 9. A and 9.B present the evolution over time of the ionic fraction $K^+/(K^++Na^+)$ to accumulate in roots and AP.

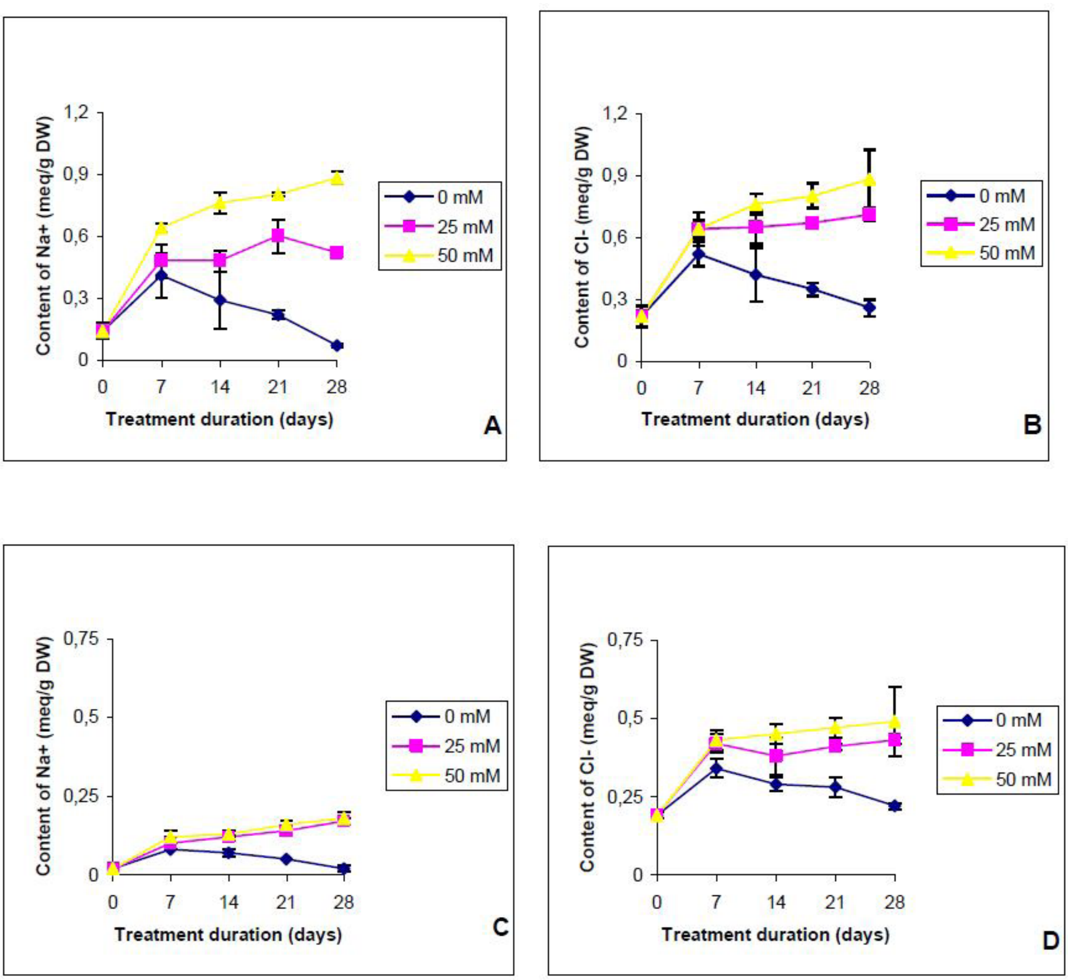
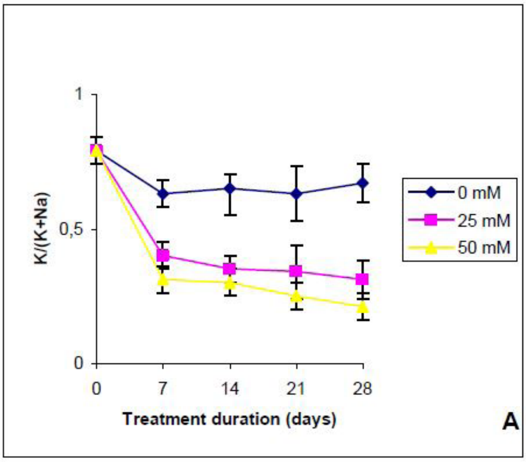



**Figure 8.** Variation of $Na^+$ and $Cl^-$ contents in roots (A) and (B) and in aerial parts (C) and (D), respectively, during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.



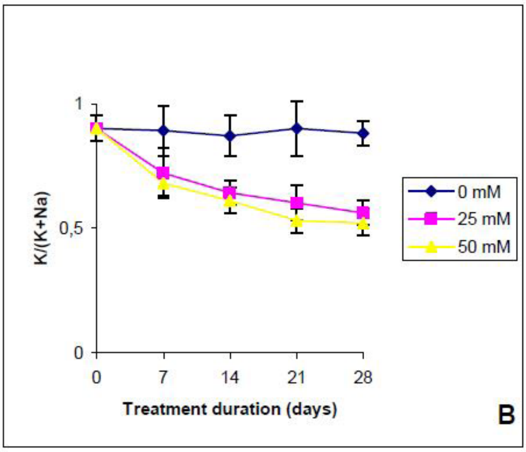




**Figure 9.** Variation of K/(K+Na) selectivity in roots (A) and in aerial parts (B) during the treatment period in *P. pinea* plants cultivated during 28 days on organic substrate, in absence or in the presence of NaCl (25 and 50 mM). The values are the mean of 10 seedlings per harvest; intervals of confidence calculated at the threshold of 0.05.

In the control medium, the ionic fraction was steady at 0.65 in the roots, throughout the culture. The addition of NaCl reduced it to 0.39 and 0.31 after the first week of culture at 25 and 50 mM, respectively, after which it continued to decrease but more slowly. In AP, the ionic fraction remained constant at about 0.90 overtime in the absence of NaCl but gradually decreased to 0.5 on day 28- of culture using both 25 and 50 mM NaCl. In all cases, it remained higher than that of the roots, indicating greater selectivity of AP compared to the root system in favour of potassium (Wang et al., 2020).

## 4. Conclusion

The stone pine cultivated for a month on a solid organic substrate was able to tolerate a NaCl concentration of 25 mM without reducing growth, compared to the non-saline control. However, when treated with 50 mM, seedling growth was stopped after two weeks of treatment. Compared to AP, the root growth activity was decreased further at applied NaCl concentrations. Seedlings restricted the transport of $Na^+$ ions in AP and were strongly selective in favour of $K^+$ ions. The presence of NaCl in the culture medium reduced the absorption and export flows of $K^+$ and $Na^+$ ions in AP. This was reflected in the accumulation rates of these two ions in the whole plant. Considering the relatively low growth of *P. pinea*, one of the interesting avenue for future study would be to evaluate the kinetics of growth and nutrient uptake of this pine species at more advanced stages and over longer treatment periods.

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