



# **Analysis of the *Atriplex* Subjected to *Claroideoglossum etunicatum* and to the Desalinator Reject**

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## **Authors' contributions**

This work was carried out in collaboration among all authors. Author CFM performed the experiment as a master's thesis, performed the statistical analysis and wrote the first draft of the manuscript. Authors EWFG, JPO, JGF and ASM managed the analysis of the study. Author ASM designed the study and wrote the protocol. All authors have read and approved the final manuscript.

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## **ABSTRACT**

The objective of this work was to analyze the minerals extracted from the soil and absorbed by *Atriplex nummularia* Lind. submitted to *Claroideoglossum etunicatum* and to the desalinator reject. The experiment was conducted in a greenhouse at the Agronomic Institute of Pernambuco - IPA, Recife, Pernambuco, Brazil. The experimental design was of randomized blocks with the treatments constituted in a factorial scheme, in five levels of salinity: AC: 2.87 mS / cm; T1: 11.54 mS / cm; T2: 12.04 mS / cm; T3: 13,13 and T4: 14,16 mS / cm, associated with the presence and absence of AMF, presence and absence of nutrient solution and autoclaved and non-autoclaved soil. 8.0 ml of Hoagland & Arnon complete nutrient solution was added every fortnight. After five months, the contents of the elements absorbed by the plant and present in the soil were evaluated.

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It was observed that in non-autoclaved soil *Atriplex* absorbed higher nutrient content. Furthermore, the best treatment was the T4 of EC of 14.16 mS/cm + AMF + Hoagland & Arnon solution. Therefore, the high sodium content absorbed (22%) by *Atriplex* evidences the potential of its use in phytoextraction programs in soils affected by salts.

**Keywords:** Mineral nutrition; saline soils; salinity tolerant plants; salt grass.

## 1. INTRODUCTION

Excess salts and sodium are one of the main factors responsible for soil degradation, causing negative impacts on agricultural production and the sustainability of ecosystems, especially in arid and semi-arid regions [1,2].

Moreover, as mechanisms for providing nutrients for seedling production there is the use of microorganisms. The mutualistic symbiosis between certain soil fungi and plant roots is called mycorrhiza. Among the various types of mycorrhiza, the arbuscular mycorrhiza that occur in most forest species stand out, and it is notable for its nutritional benefits, greater resistance to abiotic stress factors and greater tolerance to salinity [3,4].

The knowledge of the mineral contents in *Atriplex nummularia* can provide subsidies for a management program in saline areas, whose objectives are the extraction of salts and/or the use of this plant as forage [5,6].

Thus, the objective of this work was to analyze the minerals present in the soil and absorbed by *Atriplex nummularia* Lind. submitted to *Claroideoglossum etunicatum* and to the desalinator reject.

## 2. MATERIALS AND METHODS

### 2.1 Conducting the Experiment and Materials Used

The experiment was conducted in a greenhouse at the headquarters of Agronomic Institute of Pernambuco (IPA), Recife, Pernambuco, Brazil.

The soil used was obtained from the IPA Experimental Station, in the city of São Bento do Una, Pernambuco, air dried, dewormed, homogenized and sieved in a 2 mm mesh. Then part of the soil was weighed (8 kg) and used naturally, and the other part, after weighing, was autoclaved at 120°C for 1 hour. Finally, the soils were transferred to 80 polyethylene vessels.

In addition, a soil sample was collected and analyzed in the IPA Soil Fertility Laboratory, according to Tables 1 and 2.

The reject used came from the desalinator implanted in the city of Riacho das Almas, Pernambuco with the following characteristics: Electrical conductivity = 11.54 mS / cm at 25°C, Ca + 2 = 403 mg / L, Mg + 2 = 393.09 mg / L, Na + = 200 mg / L and K + = 40 mg / L, RAS = 23.67, pH = 7.9, Classification for irrigation = C4S4 (Very high salinity water and high sodium concentration; under these conditions it is not suitable for irrigation).

The water (absolute control) used in the experiment contains the following characteristics: Electrical conductivity = 2.87 mS / cm at 25°C, Ca + 2 = 10.21 mg / L, Mg + 2 = 9.05 mg / L, NA+ = 10.4 mg / L and K+ = 11.6 mg / L, RAS = 0.57, pH = 6.4, Classification for irrigation = C2S1 (Average salinity water, for irrigation whenever there is a moderate degree of irrigation).

*Atriplex nummularia* seedlings with 120 days of age were used, multiplied by means of vegetative propagation by cutting for 30 days. After rooting, the best seedlings were selected to be transplanted into the vessel.

The arbuscular mycorrhizal fungus, *Claroideoglossum etunicatum*, was obtained from the AMF Inoculum Bank of the IPA Soil Microbiology Laboratory, where they are kept under refrigeration at ± 4°C. During transplanting, each treatment received 50 g of soil-inoculum with 50 spores.

The nutrient solution used was that of [7], in which 8 ml per vessel was applied every 15 days for 5 months.

### 2.2 Treatments Used

The experimental design was a randomized block design consisting of a factorial scheme of 5

**Table 1. Chemical characteristics of the soil used in the experiment, with precision of 0.1 mg**

P mg/dm <sup>3</sup>	pH (H <sub>2</sub> O)	cmolc/dm <sup>3</sup>					
		Ca	Mg	Na	K	Al	H
350	7.8	16.0	3.9	3.0	0.7	0.0	0.0

**Table 2. Physical characteristics of the soil used in the experiment, with precision of 0.1 g**

Dap	Dr	Coarse sand	Sand	Silte	Clay	Texture
(g/cm <sup>3</sup> )		4	21	56	19	Franco-silty
1.3	2.6					

*Dap: Apparent density; Dr: Real density*

irrigation levels (AC: water – EC = 2.87 mS/cm; T1: reject – EC= 11.54 mS/cm; T2: reject plus 7g NaCl – EC= 12.04 mS/cm; T3: reject plus 14g NaCl – EC=13.13 and T4: reject plus 21g NaCl – EC= 14.16 mS/cm), associated to 2 levels of AMF (presence and absence), 2 levels of nutrient solution (presence and absence), and 2 soil levels (autoclaved and non-autoclaved). A 5x2x2x2 factorial with 2 replicates was used, totaling 80 experimental units.

### 2.3 Collection and Laboratory Analysis

After 5 months, the growth of the *A. nummularia* was monitored by measuring the height, then the aerial part and the root of the *Atriplex nummularia* were collected, separating them at the height of the plant colon and washed with distilled water. After this, all the material was packed in paper bags and dried in an air circulation oven at 60°C for 72 hours.

Then, the material was ground in a Wiley-type mill equipped with a 42 mm aperture sieve to determine the contents of the absorbed elements (P, K, Ca, Mg) by means of nitroperchloric digestion [8], the content of the elements absorbed (P, K, Ca, Mg, Na) and the total nitrogen were determined by the microkjeldhal method [8].

A soil sample was also collected for complete chemical analysis [9] and phosphorus determinations with Melich-1 solution and reading on the atomic absorption spectrophotometer.

### 2.4 Statistical Analysis

The obtained data were analyzed statistically comparing the means between the treatments through the analysis of variance - Anova and the

test of Tukey to 5% of probability using the software Statistica version 10.

## 3. RESULTS AND DISCUSSION

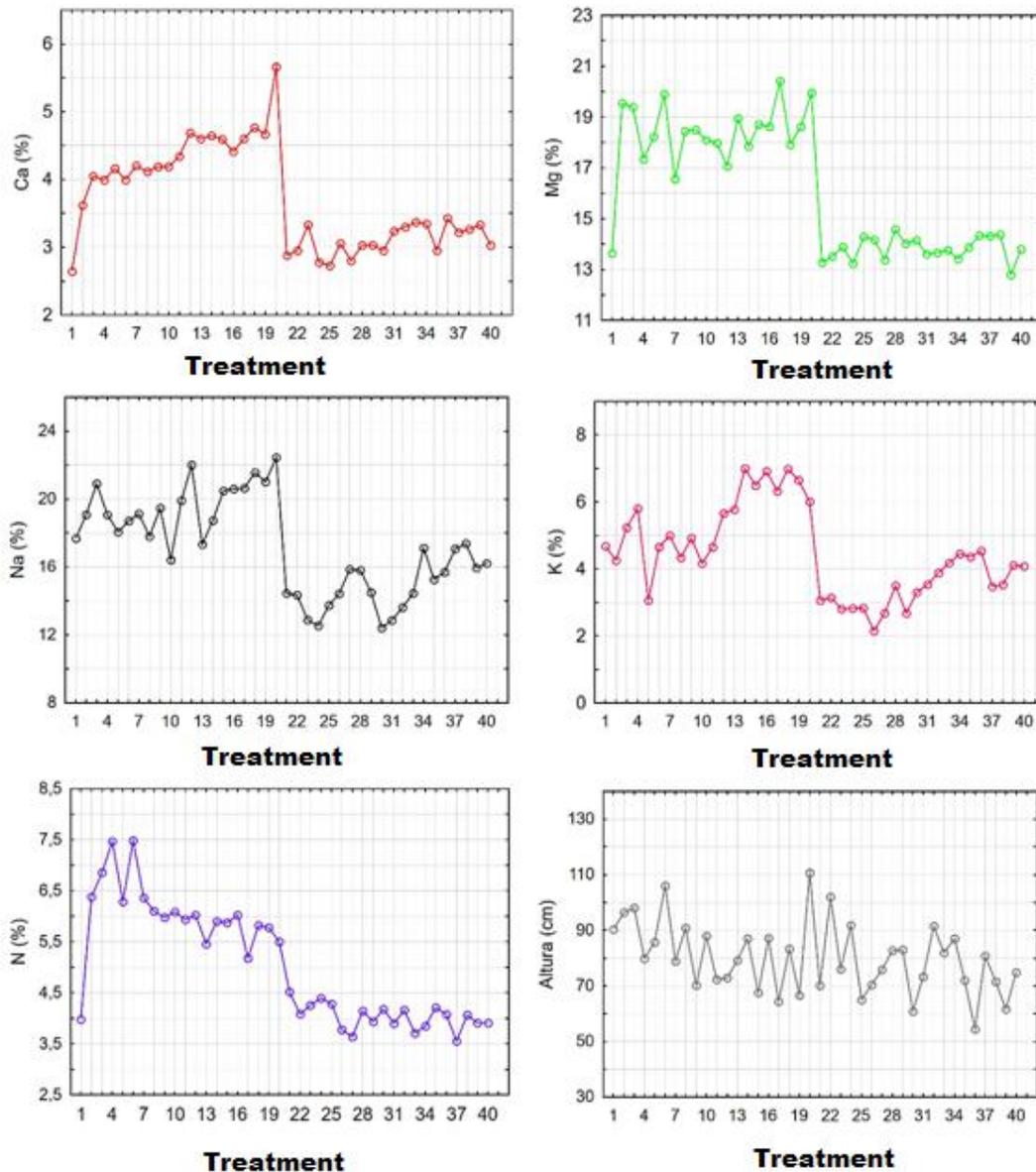
The results obtained in the laboratory determinations regarding the absorbed nutrients N, Ca, Na, Mg and K are presented in Fig. 1.

In Fig. 1 it can be observed, for the calcium element, that the best absorption was for the treatment T4 (reject + 21 g NaCl) + AMF + nutrient solution with 5.6%. In relation to the other treatments, the treatment with fungus plus nutrient solution stood out in the absorption of this element, being T2 (reject + 7 g NaCl) = 4.4% and T3 (reject + 14 g NaCl) = 4.7%.

The contents of the elements absorbed by *Atriplex* with mycorrhizal inoculation, according to Brito et al. [10], in research with peach palm with mycorrhizal fungus followed the decreasing order P> K> Mg> Ca.

For the nitrogen, it was observed that the best treatment was the T1 (reject) with nutrient solution, corresponding to 7%. Among the other variables, it was observed that the treatment with nutrient solution stood out in the absorption of this element, presenting T4 = 5.8% (Fig. 1).

Table 3 shows that the T4 treatment (reject plus 21 g NaCl) + nutrient solution + AMF was the most significant in relation to the amount of sodium absorbed, with 22%. In addition, among all treatments, the AMF + nutrient solution association was the most expressive for absorbed sodium, with AC (water) = 19.08%, T2 (reject plus 7 g NaCl) = 22%, T3 (reject plus 14g NaCl) = 21% and T4 = 22%; only T1 (reject) was more significant for treatment with nutrient solution (19%).



**Fig. 1. Elements absorbed by *Atriplex nummularia* depending on the treatments and height**  
 Legend: Treatments (AC=1; AC+AMF=2; AC+SHA=3; AC+AMF+SHA=4; T1=5; T1+AMF=6; T1+SHA=7; T1+AMF=8; T2=9; T2+AMF=10; T2+SHA=11; T2+AMF=12; T3=13; AC+AMF=14; T3+SHA=15; T3+AMF=16; T4=17; T4+AMF=18; T4+SHA=19; T4+FMA=20; \*AC= 21; \*AC+AMF=22; \*AC+SHA=23; \*AC+AMF+SHA= 24 \*T1=25; \*T1+AMF=26; \*T1+SHA=27; \*T1+AMF=28; \*T2=29; \*T2+AMF=30; \*T2+SHA=31; \*T2+AMF=32; \*T3=33; \*AC+AMF=34; \*T3+SHA=35; \*T3+AMF=36; \*T4=37; \*T4+AMF=38; \*T4+SHA=39; \*T4+AMF=40).  
 AC= water; T1= reject; T2= reject + 7gNaCl; T3= reject + 14g NaCl; T4= reject + 14gNaCl.  
 AMF= Arbuscular Mycorrhizal Fungus (*Claroideoglossum etunicatum*).  
 SHA= Nutrient solution of Hoagland and Arnon (1950)

The best treatment for potassium absorption (Table 3) consisted of T3 + AMF treatment with 7.0%. For magnesium (Table 3), it was observed that the best absorption corresponded to the T4

treatment with 17%. Among the other factors, the best absorption for this element consisted of treatments in the absence of fungus and nutrient solution: T2 = 18%, T3 = 19% and T4 = 20%.

In relation to the height of the aerial part of the plant, it was observed that the T4 + AMF + solution of Hoagland and Arnon indicated the highest growth with 110 cm.

The mutual association between arbuscular mycorrhizal fungi can provide the host plant with greater resistance to the effects of saline stress and to water deficit [11]. Thus, as *Atriplex nummularia* is a salinity-tolerant plant, the association of AMF may contribute to resistance to the deleterious effects of salinity. They showed in their results the presence of sodium in greater quantity in the aerial part than in relation to the root of all the treatments used, which reinforces the hyperaccumulation of sodium in the aerial part of *Atriplex nummularia*.

In Fig. 2, the analytical results obtained for non-autoclaved soil, the nutrient content increases as the electrical conductivity of the treatments increases. In this soil, it was observed that the chemical attributes presented higher levels for

the phosphorus with 377 mg / dm<sup>3</sup>, followed by sodium with 54.20 cmol.kg<sup>-1</sup>, calcium with 50.97 cmol.kg<sup>-1</sup>, and magnesium 18.84 cmol.kg<sup>-1</sup> and, finally, potassium with 0.75 cmol.kg<sup>-1</sup>. All these results were for the treatment T4 (reject + 21 g NaCl) with nutrient solution and AMF, except for the potassium whose highest content was in the treatment T2 (reject + 7gNaCl) with nutrient solution and AMF.

The soil elements were determined before the experiment with (Ca= 16 cmolc/dm<sup>3</sup>; Mg= 3.9 cmolc/dm<sup>3</sup>; Na= 3.0 cmolc/dm<sup>3</sup> and K= 0.7 cmolc/dm<sup>3</sup>), when compared with the final soil collection, presented increasing contents, except for potassium with values reaching 0.5 cmolc / dm<sup>3</sup>.

It was demonstrated by Melo [12] that in an experiment on the growth of *Atriplex nummularia* subjected to EC concentrations from 0 to 40 dSm<sup>-1</sup>, that EC: 0 (Control) showed the best plant growth.

**Table 3. Result of the tukey test at 5% in relation to the elements absorbed by atriplex in non-autoclaved soil**

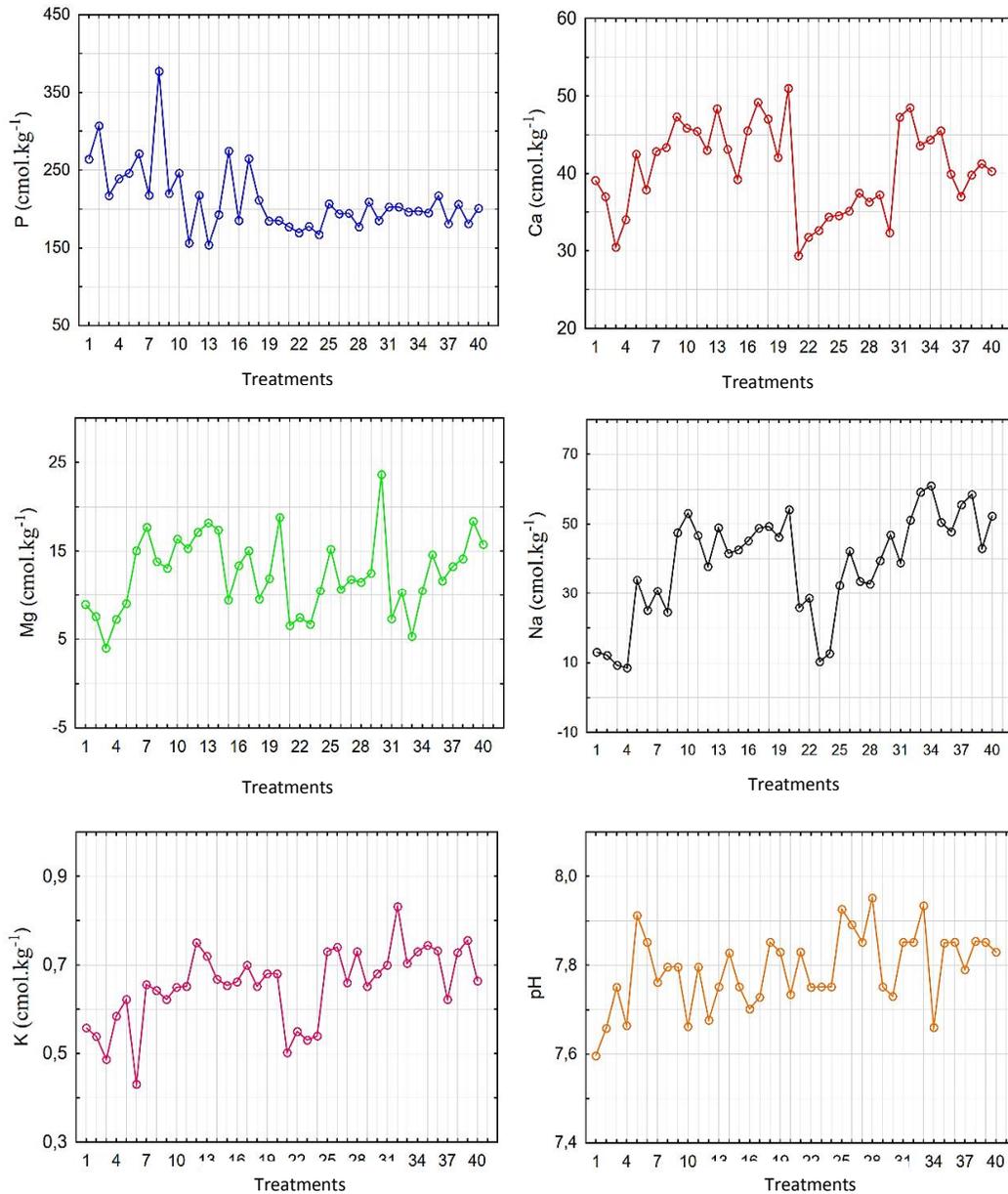
Absorbed nutrients	
<b>Treatments</b>	<b>Potassium (%)</b>
T3 + AMF	7.00 a
T4 + AMF	6.98 ab
T3 + AMF + SH	6.92 ab
T4 + SH	6.66 abc
T3	6.50 abcd
T4	6.32 abcdf
T4 + AMF + SN	6.00 abcdf
<b>Treatments</b>	<b>Magnesium (%)</b>
T4	20.4 a
T4 + AMF + SH	19.9 ab
T1 + AMF	19.8 ab
AC + AMF	19.5 abc
AC + SHA	19.3 abcd
<b>Treatments</b>	<b>Sodium (%)</b>
T4 + AMF + SHA	22.4 a
T2 + AMF + SHA	22.0 ab
T4 + AMF	21.6 abc
T4 + SH	21.0 abcd
T3 + AMF + SHA	20.9 abcd
<b>Treatments</b>	<b>Height (cm)</b>
T4 + AMF + SHA	110 a
T1 + AMF	106 ab
AC + AMF*	102 abc
AC + SHA	98 abcd

(\*) Autoclaved soil; AC = water; T1 = reject; T2 = reject + 7g NaCl; T3 = reject + 14g NaCl; T4 = reject + 20g NaCl, Different letters indicate significant difference at the 5% probability level.

AC = water; T1 = reject; T2 = reject + 7g NaCl; T3 = reject + 14g NaCl; T4 = reject + 20g NaCl.

AMF = Arbuscular Mycorrhizal Fungus (*Claroideoglomus etunicatum*).

SHA = Solution of Hoagland and Arnon (1950)



**Fig. 2. Nutrients P, Ca, Mg, Na and K and pH of non-autoclaved and autoclaved soil**

**Legend:** Treatments (AC=1; TA+AMF=2; AC+SHA=3; AC+AMF+SHA=4; T1=5; T1+AMF=6; T1+SHA=7; T1+AMF=8; T2=9; T2+AMF=10; T2+SHA=11; T2+AMF=12; T3=13; AC+AMF=14; T3+SHA=15; T3+AMF=16; T4=17; T4+AMF=18; T4+SHA=19; T4+AMF=20; \*AC= 21; \*AC+AMF=22; \*AC+SHA=23; \*AC+AMF+SHA= 24 \*T1=25; \*T1+AMF=26; \*T1+SHA=27; \*T1+AMF=28; \*T2=29; \*T2+AMF=30; \*T2+SHA=31; \*T2+AMF=32; \*T3=33; \*AC+AMF=34; \*T3+SHA=35; \*T3+AMF=36; \*T4=37; \*T4+AMF=38; \*T4+SHA=39; \*T4+AMF=40).  
 AC= water; T1= reject; T2= reject + 7gNaCl; T3= reject + 14g NaCl; T4= reject + 14gNaCl  
 AMF = Arbuscular Mycorrhizal Fungus (*Claroideoglomus etunicatum*).  
 SHA = Solution of Hoagland and Arnon (1950)

There was a slight decrease in soil pH of most treatments, which can be attributed to the ability of *Atriplex nummularia* to absorb sodium.

Reductions in pH values with *Atriplex nummularia* cultivar with EC: 63.45  $\mu\text{S cm}^{-1}$  were observed by Cunha et al. [13].

#### 4. CONCLUSION

The high absorption of Na and Mg by *Atriplex nummularia* demonstrates the potential of using this plant in phytoextraction programs in salt affected soils. *Atriplex nummularia* was not negatively influenced by irrigation with the reject and NaCl treatments. In addition, *Atriplex* behaved as a hyperaccumulating sodium plant, absorbing large amounts of the element with 22%. It was also observed that the treatments with *Claroideoglossum etunicatum* more nutrient solution and only with AMF were the most significant for plant height. In relation to the non-autoclaved soil, *Atriplex nummularia* absorbed more nutrients than in the autoclaved soil. Therefore, the best treatment consisted of T4 (rejection + 21 g NaCl) + AMF (*Claroideoglossum etunicatum*) + SHA (Hoagland and Arnon nutritive solution).

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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