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Biomass production and salt extraction by species alone or associated in the revegetation strategy[1]

Produção de biomassa e extração de sais por espécies isoladamente ou associadas na estratégia de revegetação

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ABSTRACT - Anthropogenic actions have caused soil degradation and revegetation is a way to recover degraded soils. However, salt-affected soils require the use of suitable species to extract salts from the soil. This study investigated the growth of shrub and tree species in a soil degraded by salts, alone or associated with a phytoextraction salt species, evaluating plant biomass yield and extraction of elements by plants. A field experiment was assembled with eight treatments: 1) *Atriplex nummularia* Lindl., 2) *Mimosa caesalpiniifolia* Benth, 3) *Leucaena leucocephala* (Lam.) Wit, 4) *Azadirachta indica* A. Juss, 5) *A. nummularia* + *M. caesalpiniifolia*, 6) *A. nummularia* + *L. leucocephala* and 7) *A. nummularia* + *A. indica*; in randomized blocks with four replications. After 30 months of cultivation, plants were collected to measure biomass and concentrations of Na, K, Ca, Mg, Cl, P, and N. *A. indica* plants had the highest total biomass (10.75 kg plant^{-1}), about 15 times more than *M. caesalpiniifolia*, both in associated cultivation with *A. nummularia*. *A. nummularia* plants extracted more Na and Cl in the dry matter, maximum of 124.70 and 166.79 g plant^{-1}, respectively. The highest production of plant biomass (23.55 t ha^{-1}) and total extraction of elements (1438.51 kg ha^{-1}) were obtained by associated cultivation of *A. nummularia* and *A. indica*. Associated cultivation did not decrease the phytoextractor potential of Na and Cl by *A. nummularia* plants and it can be used to regenerate degraded areas.

Key words: Soil degradation. Phytoremediation. Soil quality. Semiarid.

RESUMO - Diversas ações humanas têm causado degradação de solos e a revegetação dessas áreas é uma alternativa de recuperação. Contudo, em solos afetados por sais, é fundamental escolher espécies adequadas para a extração de sais do solo. Neste estudo o objetivo foi avaliar o desenvolvimento e a resposta de espécies arbóreo-arbustivas cultivadas em solo degradado por sais, estabelecidas isoladamente ou associadas à espécie fitoextratora de sais, por meio da produção de biomassa e extração de elementos pelas plantas. Um experimento de campo foi instalado em Serra Talhada-PE com oito tratamentos: 1) *Atriplex nummularia* Lindl., 2) *Mimosa caesalpiniifolia* Benth, 3) *Leucaena leucocephala* (Lam.) de Wit, 4) *Azadirachta indica* A. Juss, 5) *A. nummularia* + *M. caesalpiniifolia*, 6) *A. nummularia* + *L. leucocephala* e 7) *A. nummularia* + *A. indica*; em blocos casualizados com quatro repetições. Após 30 meses de cultivo, as plantas foram coletadas e pesadas, analisando-se teores de Na, K, Ca, Mg, Cl, P e N. Plantas de *A. indica* produziram mais biomassa total (10,75 kg planta^{-1}), cerca de 15 vezes mais que *M. caesalpiniifolia*, ambas em cultivo associado com *A. nummularia*. Plantas de *A. nummularia* extraíram mais Na e Cl na matéria seca, com máximo de 124,70 e 166,79 g planta^{-1}, respectivamente. A mais alta produção de biomassa vegetal (23,55 t ha^{-1}) e extração total de elementos (1438,51 kg ha^{-1}) foi no cultivo associado de *A. nummularia* e *A. indica*. O cultivo associado não diminuiu o potencial fitoextrator de *A. nummularia*, e pode ser utilizado em áreas sob recuperação.

Palavras-chave: Solo degradado. Fitorremediação. Qualidade de solo. Semiárido.

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INTRODUCTION

Soil is an essential factor for agricultural production; nevertheless, it has undergone an increasing pressure of use, compromising its productive capacity and quality. Anthropogenic activities can cause soil degradation (MIKHA *et al.*, 2014), such as loss or reduction of biological or economic yield of agricultural land, pasture, and native forest or a combination of processes (ALVES; AZEVEDO; CÂNDIDO, 2017). Salinization is a common type of degradation in arid and semi-arid regions worldwide, triggered by climatic conditions that favor soil salinization, such as salt richness in the parent material and deficient drainage. In addition, inadequate management of irrigation aggravates this process.

Degraded soils lose the productive capacity thus restoration of soil quality is crucial, requiring alternatives for soil remediation (VAN HALL *et al.*, 2017). Revegetation is a low-cost procedure that improves soil physical, chemical, and biological attributes (NADAL-ROMERO *et al.*, 2016). However, a challenge for revegetation is the establishment of plants in degraded areas (RAMACHANDRAN; RADHAPRIYA, 2016). For that purpose, several plant species have been studied, such as the halophyte *Atriplex nummularia* Lindl., used mainly in environments under water and salt stress (SANTOS *et al*., 2013; SOUZA *et al*., 2011).

The use of plant species in degraded areas enables the litter maintenance and decomposition, returning part of the nutrients absorbed by plants to the soil, helping to improve soil fertility. The litter layer on degraded soils is essential for nutrient cycling between the plant and the soil, providing suitable conditions for soil restoration (MALUF *et al.*, 2015).

In addition to nutrient cycling, some plants are capable of absorbing and accumulating toxic elements in the shoots, such as Na (sodium) and Cl (chlorine). For instance, *A. nummularia* can be used in phytoextraction of Na and Cl from salt-affected soils (SANTOS *et al*., 2013). These plants favor the growth of other plants used for revegetation, more sensitive to salts, such as *Mimosa caesalpiniifolia* Benth. In addition to sensitivity to salts, *M. caesalpiniifolia* does not require high levels of soil fertility and moisture and develops well in highly degraded areas (CARVALHO, 2007). Other species that can be used in revegetation programs *are Leucaena leucocephala* (Lam.) de Wit, known to be drought tolerant (FREIRE; RODRIGUES, 2009) and *Azadirachta indica* A. Juss., which develops well in physically degraded soils and withstands long dry periods due to its rusticity (NUNES *et al*., 2012). Therefore, the use of associated cultivation between salt-tolerant plants adapted to the environment and other species enhance the beneficial effects of revegetation.

The association of plant species with distinct sensitivity levels to salinity may contribute to growth, biomass production, and extraction of salts from the soil under revegetation. We hypothesize that associated cultivation enables the use of less olerant species to salts in conjunction with species with high saltextraction capacity in the rehabilitation of salt-affected soils. However, the association with the other species alter the salt extraction capacity of *A. nummularia*.

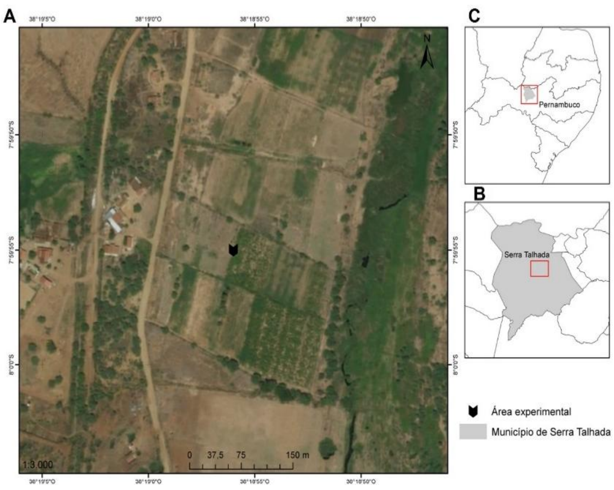
This study assessed plant growth and biomass yield of species adapted to the Brazilian semi-arid region alone and associated with *A. nummularia*, a phytoextractor plant, in a saline-sodic soil. We also estimated nutrient and Na extraction by different plant species in the stem, leaves, flowers, and fruit, evaluating the salt extraction potential of the plants cultivated alone and in association.

MATERIAL AND METHODS

Study site

The experiment was assembled under field conditions in November 2013 in the Irrigated Perimeter Cachoeira II (7°59'55.2084" S, 38°18'56.0448" W) in the municipality of Serra Talhada, Pernambuco State, Brazil (Figure 1). The climate is BShw type with mean annual temperature 25.9 °C and mean annual precipitation 642 mm (Köppen classification) concentrated between the months December and May with 85% of the annual rainfall (ALVARES *et al.*, 2013). The accumulated monthly precipitation (millimeters) between November 2013 and July 2016 is showed in Figure 2. Rainfall showed an irregular distribution during the experimental period.

Figure 1 - Experimental site: 4 P Lot – Irrigated Perimeter Cachoeira II (A); map of the municipality of Serra Talhada (B) and map of Pernambuco State (C), Brazil



The relief in the region is flat and the soil has a high proportion of fine sand and silt with dense layers in the subsurface, causing physical degradation of this soil. There is also accumulation of fluvial sediments in the area (Pernambuco Agroecological Zoning - ZAPE) and the soil is a Fluvic Neosol (SILVA *et al*., 2001).

Prior to experimentation (November/2013), soil samples were collected at 0-10, 10-30 and 30-60 cm deep in deformed structure to form a composite sample (Table 1).

The area was divided into four randomized blocks, with eight treatments each block, totaling 32 experimental plots, each plot of 8 x 8 m (64 m^2). The treatments comprised: 1) *Atriplex nummularia* Lindl., 2) *Mimosa caesalpiniifolia* Benth, 3) *Leucaena leucocephala* (Lam.) de Wit, 4) *Azadirachta indica* A. Juss, 5) *A. nummularia* + *M. caesalpiniifolia*, and 6) *A. nummularia* + *L. leucocephala* e 7) *A. nummularia* + *A. indica*.

The plant spacing was 2 x 2 m for species alone or associated (alternating plants), totaling 16 plants per plot and four plants in the useful area. The seedlings were obtained in organic substrate (soil/commercial organic compost, 1:1, about 1 dm^3 of substrate) and were transplanted at a height of about 20 cm to holes of 15 cm of diameter and 20 cm of depth. There was no inoculation in any of the legumes.

The seedlings were irrigated manually, applying water weekly for the first three months for seedling establishment. Some seedlings died in the first months and were then replaced by seedlings in the same conditions and following the same procedures. During the rainy periods, spontaneous vegetation was cleared by hoeing in a radius of 20 cm around each plant stem to avoid competition.

Plant sampling

The plants were grown up to 30 months after transplanting (Nov/2013 to Mai/2016), when they were collected and evaluated.

An entire plant was collected in the useful area of each plot for treatments with species alone, while two plants were collected in the plots with associated species, one from each species. The plants were cut at the base (10 cm from soil surface) and placed on a tarp to separate the parts to be analyzed. *A. nummularia* plants were divided into stem/branches and leaves; *M. caesalpiniifolia* in stems/branches, leaves, and inflorescences; and *L. leucocephala* and *A. indica* were

Figure 2 - Accumulated monthly rainfall (millimeters), between November 2013 and July 2016, in the municipality of Serra Talhada according to Serra Talhada Meteorological Station/Cachoeira dam (AGÊNCIA PERNAMBUCANA DE ÁGUAS E CLIMA, 2018)

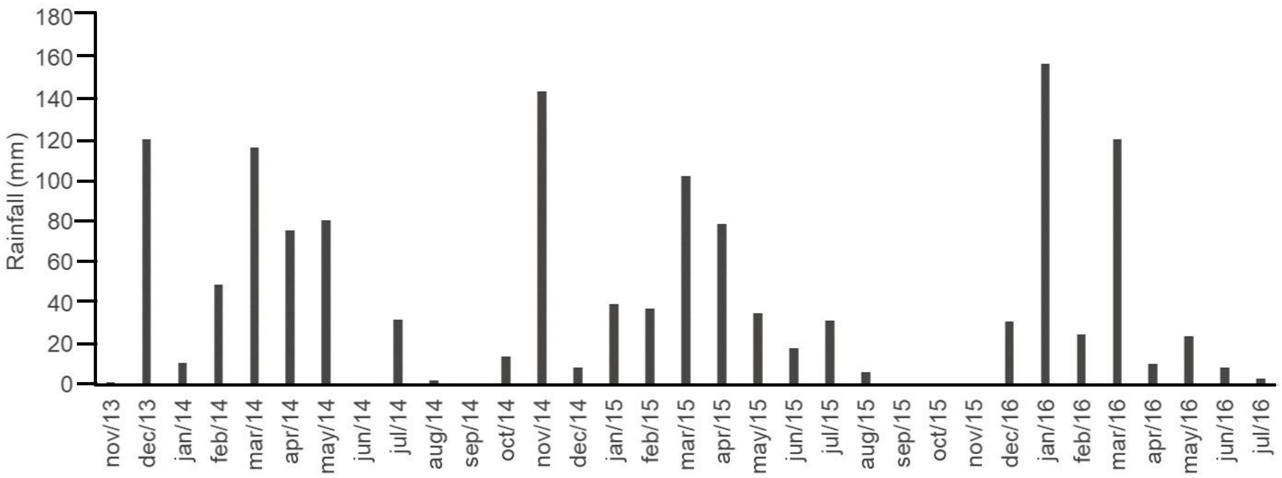


Table 1 - Soil physical and chemical characterization in the experimental area before experiment setup, in the layers 0-10, 10-30 and 30-60 cm deep

Layer	Sand	Silt	Clay	pH_{H2O}	EC1	Na^+	K^+	Ca^{2+}	Mg^{2+}	CEC2	ESP3
cm	------------ $g\ kg^{-1}$ -------------				$dS\ m^{-1}$	---------------------- $cmol_c\ kg^{-1}$ ----------------------					%
0 - 10	828	78	94	7.2	5.48	5.9	1.1	1.5	0.68	9.51	62
10 - 30	752	66	182	7.2	4.00	2.7	1.8	1.6	0.70	6.83	39
30 - 60	711	80	209	7.3	3.80	3.2	1.9	1.8	0.75	7.67	42

[1] Electrical conductivity of the saturation extract; [2] Cation exchange capacity (index cation method); [3] Exchangeable sodium percentage

fractionated in stem/branches, leaves, inflorescences, and fruit. All parts were weighed separately in the field. A subsample of each fraction was taken and weighed, wrapped in paper bags, and transported to the laboratory for drying.

Biomass production and element extraction by plants

Subsamples of the plant material collected were weighed (fresh biomass) and oven dried with forced air circulation at 65 ºC until constant weight (dry biomass). Moisture of the plant fractions (stem/branches, leaves, inflorescences, and fruit) was calculated based on the mass of fresh and dried subsamples to estimate the total dry plant biomass.

The plant fractions were ground in a Willey mill and submitted to nitric digestion by microwave heating, according to Horneck and Miller (1998). The concentrations of Na, K, Ca, Mg, and P were determined in the extracts: Na and K by flame emission photometry; Ca and Mg by atomic absorption spectrophotometry; and P by visible light spectrophotometry, with molybdenum-blue, at a wavelength of 725 nm. Nitrogen was determined by the Kjeldahl method (HORNECK; MILLER, 1998). Chloride was determined in aqueous extract by titration with silver nitrate. The results of dry biomass of plant fractions and the concentration of the elements in the plant tissues were used to calculate the contents extracted and to estimate extractions by each plant fraction.

Statistical analysis

The plants were evaluated separately, despite the consortium: 1) *Atriplex nummularia* alone; 2) *A. nummularia (A. nummularia + L. leucocephala*); 3) *A. nummularia (A. nummularia + A. indica*); 4) *A. nummularia* (*A. nummularia + M. caesalpiniifolia*); 5) *Leucaena leucocephala (L. leucocephala + A. nummularia)*; 6) *Azadirachta indica* (*A. indica + A. nummularia*); 7) *Mimosa caesalpiniifolia* (*M. caesalpiniifolia + A. nummularia*); 8) *L. leucocephala* alone; 9) *A. indica* alone; 10) *M. caesalpiniifolia* alone.

The plant results were previously tested for normal distribution and were subjected to the analysis of variance. Then, the data were compared by the Tukey test at 10% ($p < 0.10$) probability level (VIEIRA, 2006), using SISVAR 5.3 software.

RESULTS AND DISCUSSION

Plant biomass production

The highest stem biomass yield was observed in *A. indica* plants associated with *A. nummularia*, differing from *M. caesalpiniifolia* plants also associated to *A. nummularia*. The same behavior was observed in the total plant biomass. There was no difference for the leaf fraction between treatments. Among the plants with flowers, the highest biomass was obtained in *A. indica* plants alone or associated with *A. nummularia* and in *M. caesalpiniifolia* plants alone. *L. leucocephala* plants produced the highest fruit biomass, both alone or in association (Table 2).

There were no major differences in total dry biomass yield per plant, except between *M. caesalpiniifolia* with the lowest value and *A. indica* with the highest biomass yield per plant (Table 2), both under associated cultivation with *A. nummularia* plants. Plants of *M. caesalpiniifolia* in the associated cultivation did not adapt to the environment, showing the lowest vegetative growth. The other plants did not show yield differences in stem dry biomass and in total biomass. All plants studied produced more biomass in the stem fraction, because they are shrub s and trees with high stem growth. The flower and fruit fractions were expressed in g plant^{-1}, while the stem and leaves in kg plant^{-1}. Plants of *A. nummularia* had no flower or fruit.

Lower biomass yield by area was observed in treatments with *L. leucocephala* and *M. caesalpiniifolia* alone, while the highest yield was found in the associated cultivation of *A. nummularia* with *A. indica*. This occurred due to the smaller growth of *M. caesalpiniifolia*, resulting in lower total biomass yield. Conversely, *A. indica* had an excellent adaptation, generating high biomass yield.

Plants with high biomass yield allow for a more effective sustainable system, due to the incorporation of large amounts of organic matter (OM) into the soil (ÁVILA; ASSAD; LIMA, 2012). Nevertheless, few stems are incorporated into the soil, due to their diameter and lignification, hindering their decomposing and requiring a long time to be incorporated into the soil. The lower biomass yield of some treatments does not characterize a failure of soil recovery process, as stem yield was higher in some treatments with similar leaf yield among the species studied.

We calculated the total biomass yield of all plants per parts. For that, we calculated the number of plants per hectare considering the spacing between plants to estimate the total accumulated of dry biomass yield (Table 3).

The highest stem biomass yield was obtained for associated cultivation of *A. nummularia* and *A. indica* plants, followed by *A. nummularia* plants alone. The accumulated leaf biomass yield did not differ between treatments with the species studied. The highest flower biomass yield was observed in the treatments of *A. indica* and *M. caesalpiniifolia* alone and in associated cultivation of *A. indica* and *A. nummularia*. The total accumulated of dry biomass was higher in the treatment of *A. nummularia* associated with *A. indica*, differing from treatments with *M. caesalpiniifolia* and *L. leucocephala* alone.

Table 2 - Total dry biomass yield of plants and dry biomass by plant part of each species under treatments of species in alone or associated revegetation strategy in saline-sodic soil from the semi-arid region in Brazil at 30 months after transplanting

Treatment	Total dry biomass	Dry biomass by plant fraction			
		Stem	Leaf	Flower	Fruit
	---------------------------- kg plant^{-1}		----------------------------	---------------- g plant^{-1}	----------------
A. *nummularia*[1]	5.10 AB	4.15 AB	0.95 A	-	-
A. *nummularia*[2]	5.55 AB	4.28 AB	1.27 A	-	-
A. *nummularia*[3]	8.10 AB	6.67 AB	1.43 A	-	-
A. *nummularia*[4]	7.46 AB	5.88 AB	1.58 A	-	-
L. *leucocephala*[5]	2.90 AB	1.64 AB	0.52 A	5.93 B	736.95 A
A. *indica*[6]	10.75 A	7.39 A	3.22 A	32.74 A	135.41 B
M. *caesalpiniifolia*[7]	0.70 B	0.52 B	0.18 A	-	-
L. *leucocephala*[8]	2.73 AB	1.94 AB	0.29 A	6.45 B	501.29 A
A. *indica*[9]	5.22 AB	3.57 AB	1.52 A	34.55 A	124.65 B
M. *caesalpiniifolia*[10]	2.49 AB	1.58 AB	0.88 A	33.20 A	-
CV[11] (%)	32.18	55.34	42.12	58.87	37.57

[1] *Atriplex nummularia* alone; [2] *Atriplex nummularia* associated with *L. leucocephala*; [3] *Atriplex nummularia* associated with *A. indica*; [4] *Atriplex nummularia* associated with *M. caesalpiniifolia*; [5] *Leucaena leucocephala* associated with *A. nummularia*; [6] *Azadirachta indica* associated with *A. nummularia*; [7] *Mimosa caesalpiniifolia* associated with *A. nummularia*; [8] *Leucaena leucocephala* alone; [9] *Azadirachta indica* alone; [10] *Mimosa caesalpiniifolia* alone; [11] Coefficient of variation. Means followed by the same letter in the column do not differ statistically by the Tukey test at $p < 0.1$ probability level

The total biomass yield per plant part in associated cultivation of *A. nummularia* and *A. indica* exceeded that of *A. nummularia* or *A. indica* alone by 84.7 and 79.6%, respectively (Table 3), demonstrating the benefits for these two species cultivated in association. This is possibly due to the release of exudates by the roots that promoted greater microorganismal activity, releasing nutrients and aggregating soil particles, while increasing aeration for gas exchange in the root system (SALTON; TOMAZI, 2014).

This potential benefit should be highlighted, as the cultivation is carried out without irrigation or any inputs for plants in an environment of low biomass yield such as the Brazilian semi-arid region. Moreover, soil cover through revegetation tends to protect it against drying and raindrop impact, facilitating water passage in the soil profile and contributing to the natural leaching of salts.

Nutrient contents and accumulation in plant parts

The dry matter (DM) composition by fraction showed that *A. nummularia* plants had the highest concentrations of Na and Cl, both in the stems and leaves (Figures 3A and 3B). The other species had lower Na and Cl concentrations. The flowers of *L. leucocephala*, *A. indica*, and *M. caesalpiniifolia* did not differ in terms of Cl concentration (Figure 3C). Nevertheless, the fruit of *L. leucocephala* (alone and associated cultivation) had a much higher Cl concentration than fruit of *A. indica* (Figure 3D). Cl showed the highest concentration of all elements analyzed, reaching 69.41 g kg^{-1} in theleaves of *A. nummularia* associated with *L. leucocephala*.

The K concentration in the leaves varied widely among the plants. In the flowers, K concentration was higher in *A. indica* alone, followed by *A. indica* associated with *A. nummularia* (Figure 3C). However, K concentration in the fruit did not differ between *L. leucocephala* and *A. indica* (Figure 3D), which were the only plants with fruit in this study.

The highest Ca concentration in the stems was observed in *M. caesalpiniifolia* associated with *A. nummularia* (Figure 3A). However, Ca concentrations in the leaves were higher than in the stem, mainly in *A. indica* and *L. leucocephala* plants (Figure 3B). The Mg concentrations showed a difference only in the leaves and were higher in *A. nummularia* in the associated treatments and lower in *L. leucocephala* and *M. caesalpiniifolia* alone or in the associated revegetation strategy (Figure 3B).

The leaves of *L. leucocephala* (alone and associated) and *A. indica* (alone) had high N concentrations, while lower N concentrations were found in the leaves of *A. nummularia* (Figure 3B). In the flowers and fruit, higher N concentrations were also observed in *L. leucocephala*, both alone and in associated cultivation (Figures 3C and 3D). P concentrations in the plant stems showed a great variation. The highest P concentrations were found in *A. indica* in associated cultivation with *A. nummularia*, followed by *A. indica* and *M. caesalpiniifolia* alone (Figure 3A).

Table 3 - Dry biomass yield by plant parts and total accumulated dry biomass of plants grown alone and in associated cultivation at 30 months after transplanting

Treatment	Dry biomass by plant fraction				Total accumulated
	Stem	Leaf	Flower	Fruit	
	--------------- t ha^{-1} -------------		------------- kg ha^{-1} -------------		t ha^{-1}
A. *nummularia*[1]	10.38 AB	2.37 A	-	-	12.75 AB
A. *nummularia*/L. *leucocephala*[2]	7.40 B	2.24 A	7.41 B	1253.23 A	10.90 AB
A. *nummularia*/A. *indica*[3]	17.57 A	5.81 A	40.93 A	169.26 B	23.55 A
A. *nummularia*/M. *caesalpiniifolia*[4]	8.00 B	2.20 A	-	-	10.24 AB
L. *leucocephala*[5]	4.86 B	0.72 A	16.13 B	921.19 A	6.54 B
A. *indica*[6]	8.92 B	3.80 A	86.39 A	311.19 B	13.11 AB
M. *caesalpiniifolia*[7]	3.94 B	2.20 A	83.00 A	-	6.16 B
CV[8] (%)	70.46	51.46	80.88	38.08	73.40

1 Dry biomass yield by Atriplex nummularia alone; 2 Dry biomass yield by A. nummularia and Leucaena leucocephala associated; 3 Dry biomass yield by A. nummularia and Azadirachta indica associated; 4 Dry biomass yield by A. nummularia and Mimosa caesalpiniifolia associated; 5 Dry biomass yield by L. leucocephala alone; 6 Dry biomass yield by A. indica alone; 7 Dry biomass yield by M. caesalpiniifolia alone; 8 Coefficient of variation. Means followed by the same letter in the column do not differ statistically by the Tukey test at $p < 0.1$ probability level

M. caesalpiniifolia plants, both alone and in associated cultivation with *A. nummularia*, had low concentrations and contents of elements in the leaves (Figures 3, 4, and 5). However, this species loses all its leaves during the dry period of the year; thus, these plants can also contribute to OM input and nutrient cycling in the soil (FERNANDES *et al*., 2006). In contrast, *A. indica*, both alone and in associated cultivation with *A. nummularia*, had high values of total leaf contents for most elements evaluated, due to the high biomass yield (Figure 4). Despite the small number of leaf drops, this species significantly contributes to OM input and nutrient cycling to the soil (LEÓN; OSÓRIO, 2014).

We estimated the content extracted by plant fractions, considering element concentrations and biomass yield (Figure 4 - stems and leaves and Figure 5 - flowers and fruit).

The concentration of elements in plant tissues is different from the content accumulated by the plants, which depends directly on biomass yield of the plant. Some plants had higher concentrations of elements (Figure 3), which differed from high contents (Figure 4), due to lower growth and smaller biomass yield of the species.

The highest Na and Cl concentrations and contents in plant tissues were observed in *A. nummularia*, both alone and in associated cultivation. This highlights the phytoextractor potential of *A. nummularia* (SILVA *et al*., 2016; SOUZA *et al*., 2011). Plants in the associated revegetation strategy becomes an alternative for cultivation in areas where toxic elements are present. Tolerant species (phytoextractor) absorb the element at high proportions, allowing growth of less tolerant species.

Na and Cl extraction by the stems and leaves was greater in *A. nummularia* plants, alone and in associated cultivation (Figures 4A and 4B). The K content in the stems and leaves of the plants had great variability, with the highest value for stems observed in *A. indica* plants in associated cultivation (126.00 g plant^{-1}). The highest Ca content in the leaves was also found in *A. indica* plants in associated cultivation (Figure 4B). *A. indica* plants showed significant Mg contents in the stems and leaves, alone and in associated cultivation (Figures 4A and 4B). All elements were extracted at higher proportions by the stems and leaves of the plant species studied.

The flowers and fruit were only found in some plants and showed low biomass yield (Table 2), contributing little to the extraction of elements from the soil (Figures 5A and 5B). In the fruit, the highest K and N content extracted was observed in *L. leucocephala* plants, both alone and in associated cultivation (Figure 5B). There was no difference for the other elements evaluated in the flowers and fruit.

Total nutrient accumulation

Regarding element extraction by the whole plant, *A. nummularia* showed capacity of removing Cl and Na from soils, alone or in associated cultivation (Table 4). Na had little accumulation in the other species studied, which were not good Na extractors. In general, *L. leucocephala* and *M. caesalpiniifolia* were the species with the lowest extraction of elements in the DM. Although these species have symbiotic associations with atmospheric N-fixing micro-organisms, the plants were not inoculated and N was had less accumulation in these plant species, due to lower total biomass yield when compared to *A. indica*.

Figure 3 - Concentrations of elements in the stems (A), leaves (B), flowers (C), and fruit (D) in plants of [1] *Atriplex nummularia* alone, [2] *A. nummularia* associated with *L. leucocephala*, [3] *A. nummularia* associated with *A. indica*, [4] *A. nummularia* associated with *M. caesalpiniifolia*, [5] *Leucaena leucocephala* associated with *A. nummularia*; [6] *Azadirachta indica* associated with *A. nummularia*; [7] *Mimosa caesalpiniifolia* associated with *A. nummularia*; [8] *Leucaena leucocephala* alone; [9] *Azadirachta indica* alone; [10] *Mimosa caesalpiniifolia* alone

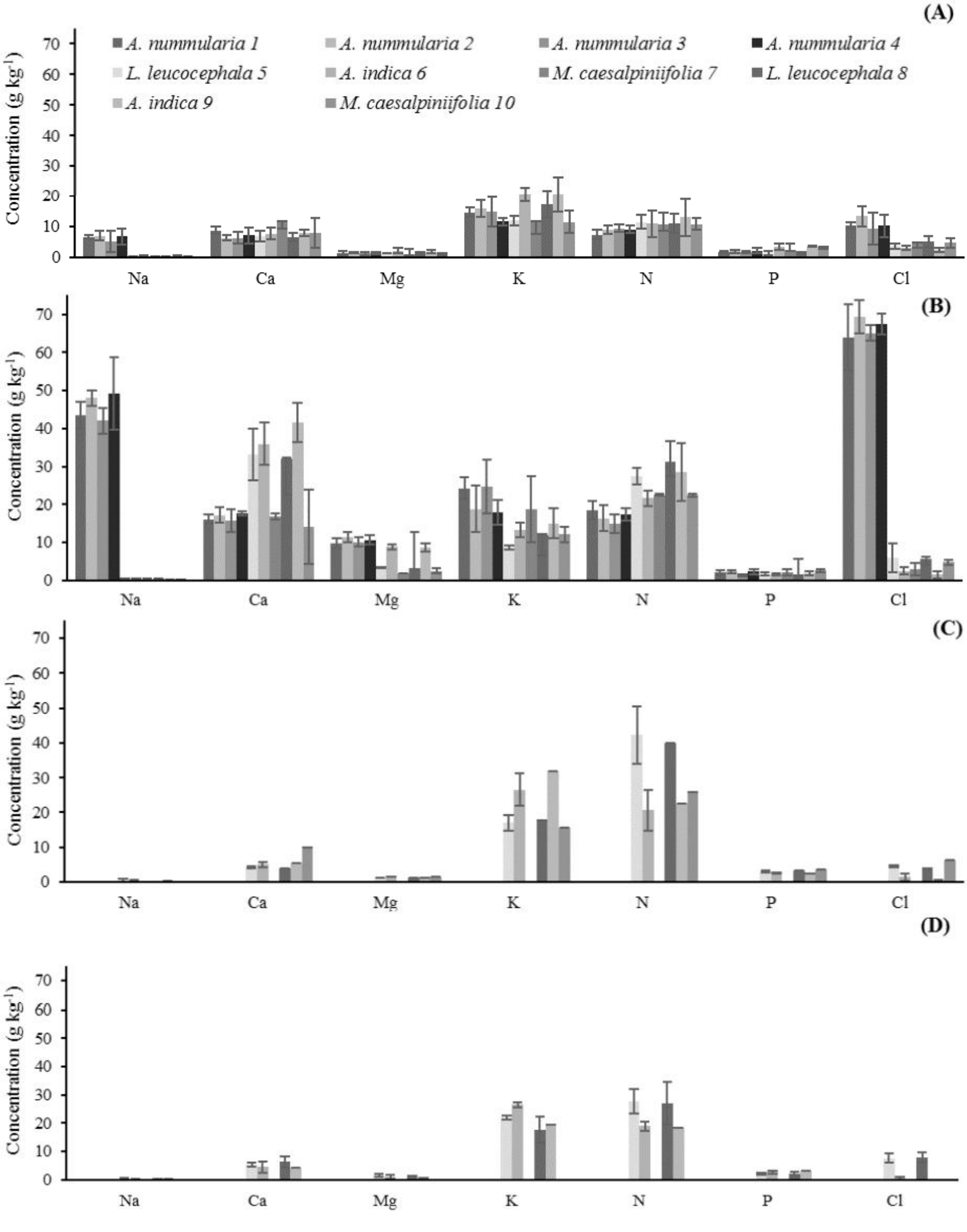


Figure 4 - Contents of elements in the stems (A) and leaves (B) of [1] *Atriplex nummularia* plants alone, [2] *A. nummularia* associated with *L. leucocephala*, [3] *A. nummularia* associated with *A. indica*, [4] *A. nummularia* associated with *M. caesalpiniifolia*, [5] *Leucaena leucocephala* associated with *A. nummularia*; [6] *Azadirachta indica* associated with *A. nummularia*; [7] *Mimosa caesalpiniifolia* associated with *A. nummularia*; [8] *Leucaena leucocephala* alone; [9] *Azadirachta indica* alone; [10] *Mimosa caesalpiniifolia* alone

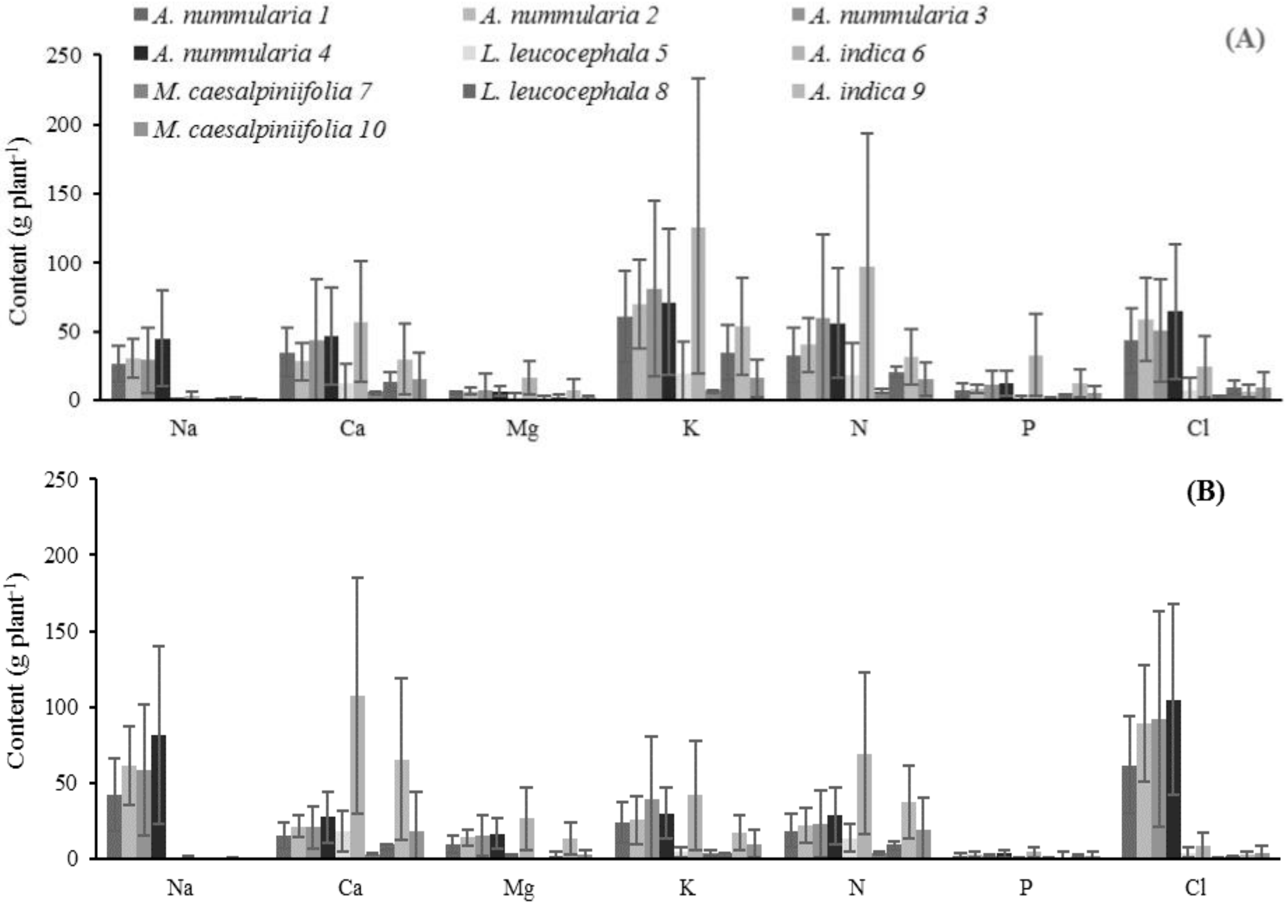
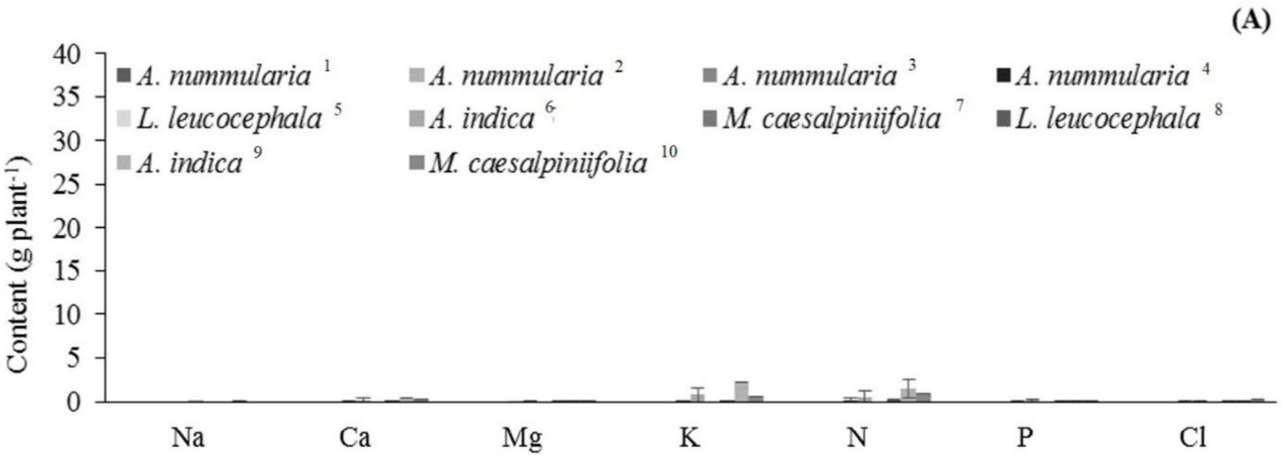
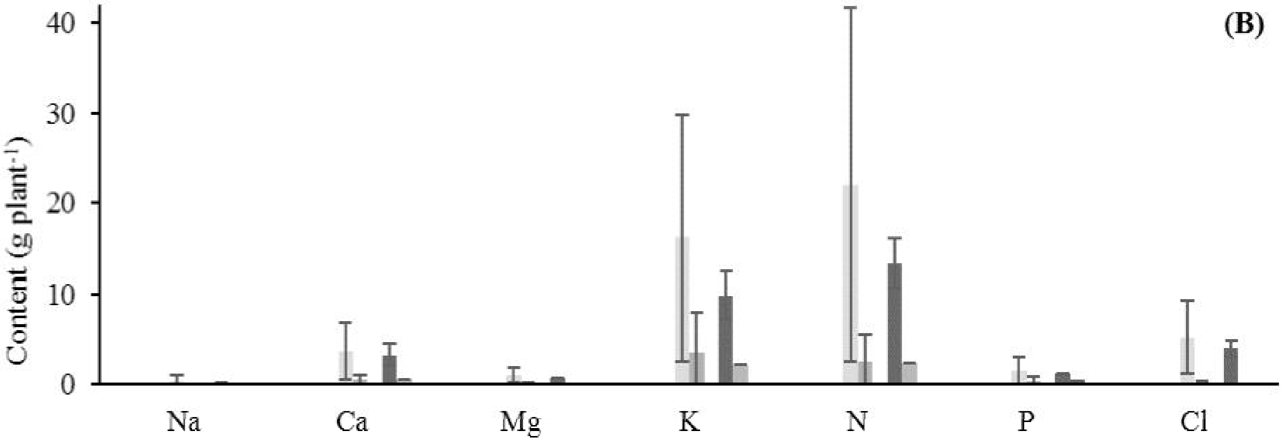


Figure 5 - Contents of elements in the flowers (A) and fruit (B) of [1] *Atriplex nummularia* plants alone, [2] *A. nummularia* associated with *L. leucocephala*, [3] *A. nummularia* associated with *A. indica*, [4] *A. nummularia* associated with *M. caesalpiniifolia*, [5] *Leucaena leucocephala* associated with *A. nummularia*; [6] *Azadirachta indica* associated with *A. nummularia*; [7] *Mimosa caesalpiniifolia* associated with *A. nummularia*; [8] *Leucaena leucocephala* alone; [9] *Azadirachta indica* alone; [10] *Mimosa caesalpiniifolia* alone



Continuation figure 5



Total element accumulation in the plants showed significant differences for *A. nummularia* and *A. indica* in relation to *L. leucocephala* and *M. caesalpiniifolia* (Figure 6). *A. indica* showed the highest total accumulation of elements, while *M. caesalpiniifolia* displayed the lowest accumulation in associated cultivation. *A. nummularia* plants also accumulated more elements in associated cultivation.

A. indica plants in associated cultivation showed the largest extractions of K, Ca, Mg, N, and P (Table 4), possibly due to the high yield of stem and total biomass (Table 2). This can be attributed to the adaptation of this species to regions of arid and semi-arid climate and to its tolerance to droughts and high temperatures (ARAÚJO; RODRIGUEZ; PAES, 2000). Associated cultivation of *A. indica* and *A. nummularia* possible promoted better conditions for growth and biomass yield for *A. indica* plants. Beneficial effects of using *A. nummularia* plants to regenerate salt-affected soils, such as Na and Cl absorption, should be further investigated. Greater extraction of elements should also result in richer litter and better litter decomposition, contributing thus nutrient absorption by the soil and improving soil soil quality (MALUF *et al.*, 2015). Nevertheless, litter decomposition can also promote the return of salts to the soil, which must be monitored.

In relation to the total elements extracted by plant part, associated cultivation of *A. nummularia* and *A. indica* promoted the greatest extraction of K, Ca, Mg, N, and P. In addition, this treatment showed the greatest extractions of Na and Cl by plants. This demonstrates the potential of this consortium for phytoextraction of salts from the soil by pruning and removing the shoots of *A. nummularia* plants. On the other hand, can be characterized as a nutrient source, due to the rich litter these plants produce, mainly because of leaf biomass concentration in *A. indica*. This is essentially important for nutrient return to the soil, constituting an important path of the biogeochemical cycling of elements (SEGURA *et al*., 2017).

The total extraction of the elements studied per plant part was also estimated, considering the spacing used between the plants (Table 5). treatments with *A. nummularia* plants had greater the total extraction of Na and Cl, while Na extraction was lower in the treatments of *L. leucocephala*, *A. indica*, and *M. caesalpiniifolia* alone.

The highest K extraction was observed in associated cultivation between *A. nummularia* and *A. indica*, with a total of 363.37 kg ha^{-1} and this associated cultivation also showed a significant extraction capacity of Ca, Mg, P, and N (Table 5). The highest Mg extraction per hectare was also obtained by associated cultivation between *A. nummularia* and *A. indica* (81.06 kg ha^{-1}), while the lowest extractions occurred in *L. leucocephala* and *M. caesalpiniifolia* alone (Table 5).

Among the elements evaluated, *A. nummularia* plants alone or in associated cultivation had the highest extractions of Na and Cl, which is the objective of most phytoremediation works in salt-affected soils. The other species tested (*L. leucocephala*, *A. indica* and *M. caesalpiniifolia*) did not have a high extraction capacity for these elements, because they are sensitive to these elements and are not phytoextractors.

Associated cultivation of *A. nummularia* and *A. indica* had the greatest extraction capacity of P and N (62.00 kg ha^{-1} of P and 314.03 kg ha^{-1} of N). *L. leucocephala* plants alone had the lowest P extraction and the treatment with *M. caesalpiniifolia* plants alone the lowest N extraction (Table 5). For almost all the elements evaluated, the association revegetation strategy of *A. nummularia* and *A. indica* was more efficient in the extractions, when compared to their species alone and with the other treatments. The estimated

Table 4 - Total extraction of elements per plant species alone or in associated cultivation at 30 months in a saline-sodic soil

Treatment	Na	K	Ca	Mg	N	P	Cl
	g plant^{-1}						
A. *nummularia*[1]	68.62 AB	84.46 C	50.34 C	14.06 AB	50.60 AB	9.15 AB	104.60 AB
A. *nummularia*[2]	124.70 A	111.38 B	61.97 BC	20.96 AB	76.50 AB	12.77 AB	166.79 A
A. *nummularia*[3]	87.33 AB	120.60 B	63.85 BC	22.07 AB	83.28 AB	12.23 AB	142.17 AB
A. *nummularia*[4]	93.20 AB	81.71 C	61.13 BC	22.49 AB	69.96 AB	13.75 AB	150.48 AB
L. *leucocephala*[5]	1.17 B	39.63 D	33.30 CD	4.75 B	54.14 AB	3.86 AB	16.08 B
A. *indica*[6]	3.58 B	170.09 A	164.62 A	42.78 A	167.94 A	37.37 A	32.90 AB
M. *caesalpiniifolia*[7]	0.28 B	10.50 E	8.94 D	0.74 B	9.50 B	1.69 B	2.84 B
L. leucocephala[8]	0.58 B	47.56 D	25.64 CD	3.95 B	42.47 B	4.46 AB	14.83 B
A. *indica*[9]	1.52 B	72.37 C	95.41 B	20.45 AB	36.17 B	14.44 AB	8.59 B
M. *caesalpiniifolia*[10]	0.39 B	25.64 DE	33.92 CD	4.16 B	34.90 B	7.17 AB	13.11 B
CV[11] (%)	23.24	58.47	41.04	70.38	72.14	11.29	48.22

[1] *Atriplex nummularia* alone; [2] *Atriplex nummularia* associated with *L. leucocephala*; [3] *Atriplex nummularia* associated with *A. indica*; [4] *Atriplex nummularia* associated with *M. caesalpiniifolia*; [5] *Leucaena leucocephala* associated with *A. nummularia*; [6] *Azadirachta indica* associated with *A. nummularia*; [7] *Mimosa caesalpiniifolia* associated with *A. nummularia*; [8] *Leucaena leucocephala* alone; [9] *Azadirachta indica* alone; [10] *Mimosa caesalpiniifolia* alone. [11] Coefficient of variation. Means followed by the same letter in the column do not differ statistically by the Tukey test at $p < 0.1$ probability level

Figure 6 - Total extraction of elements (Na, K, Ca, Mg, N, P, Cl) per species used in revegetation of a saline-sodic soil at 30 months. 1 *Atriplex nummularia* alone, 2 *A. nummularia* associated with *L. leucocephala*, 3 *A. nummularia* associated with *A. indica*, 4 *A. nummularia* associated with *M. caesalpiniifolia*, 5 *Leucaena leucocephala* associated with *A. nummularia*; 6 *Azadirachta indica* associated with *A. nummularia*; 7 *Mimosa caesalpiniifolia* associated with *A. nummularia*; 8 *Leucaena leucocephala* alone; 9 *Azadirachta indica* alone; 10 *Mimosa caesalpiniifolia* alone

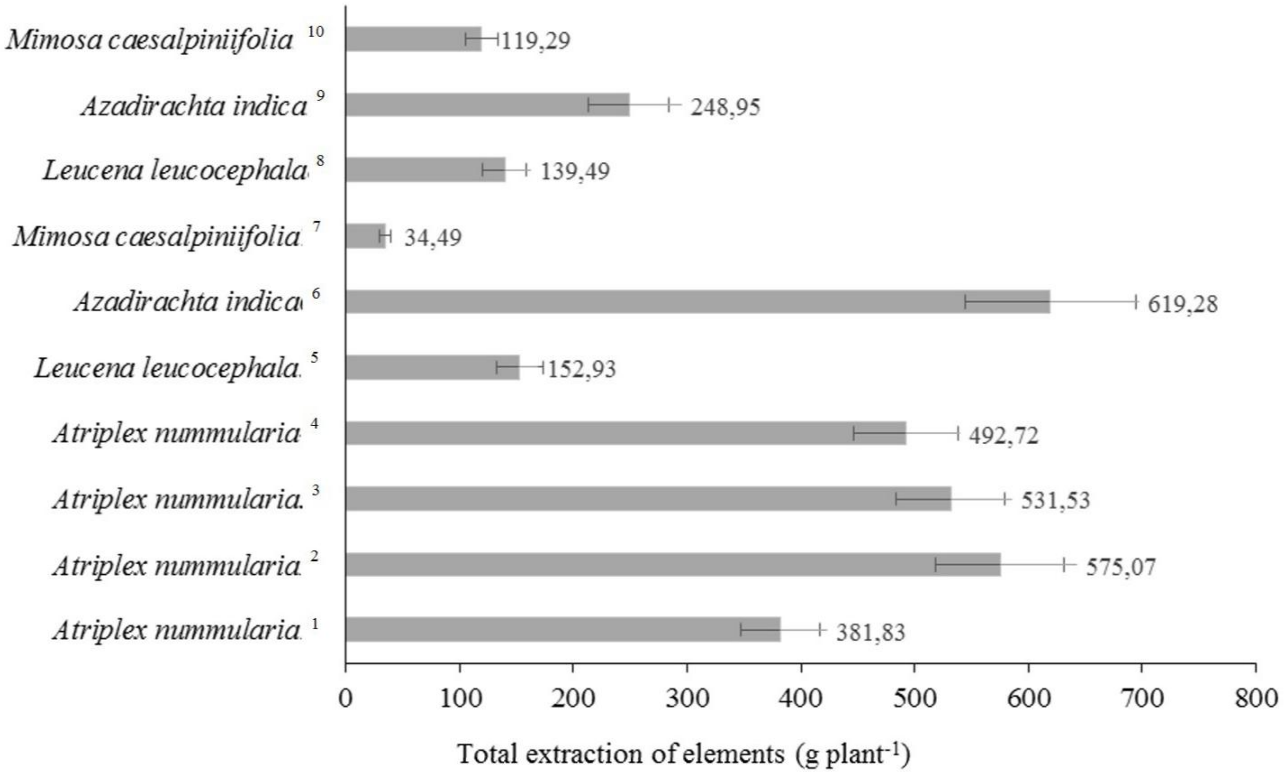


Table 5 - Total extraction of elements per plant part by species alone or in associated cultivation at 30 months of cultivation

Treatment	Na	K	Ca	Mg	N	P	Cl
	kg ha^{-1}						
A. *nummularia*[1]	171.54 A	211.15 AB	125.84 AB	35.15 AB	126.49 AB	22.86 AB	261.49 A
A. *nummularia*/ L. *leucocephala*[2]	157.33 A	188.76 AB	119.08 AB	32.14 AB	163.30 AB	20.79 AB	228.58 A
A. *nummularia*/ A. *indica*[3]	113.64 A	363.37 A	285.60 A	81.06 A	314.03 A	62.00 A	218.83 AB
A. *nummularia*/ M. *caesalpiniifolia*[4]	116.84 A	115.26 B	87.60 AB	29.05 AB	99.32 AB	19.31 AB	191.65 ABC
L. *leucocephala*[5]	1.45 B	118.90 B	64.09 AB	9.88 B	106.19 AB	11.16 B	37.09 BCD
A. *indica*6	3.80 B	180.92 AB	238.53 AB	51.13 AB	173.94 AB	36.10 AB	21.48 D
M. *caesalpiniifolia*[7]	0.99 B	64.10 B	84.82 AB	10.40 B	87.24 B	17.93 AB	32.77 CD
CV[8] (%)	40.85	27.41	37.56	55.49	72.66	26.17	37.28

[1] Plants of *Atriplex nummularia* alone; [2] Plants of *A. nummularia* and *Leucaena leucocephala* in associated cultivation; [3] Plants of *A. nummularia* and *Azadirachta indica* in associated cultivation; [4] Plants of *A. nummularia* and *Mimosa caesalpiniifolia* in associated cultivation; [5] Plants of *L. leucocephala* alone; [6] Plants of *A. indica* alone; [7] Plants of *M. caesalpiniifolia* alone; [8] Coefficient of variation Means followed by the same letter in the column do not differ statistically by Tukey test at $p < 0.1$ probability level

total of elements extracted by plants per hectare, alone or in associated cultivation, is shown in Figure 6.

The treatment of associated cultivation between *A. nummularia* and *A. indica* showed evident capacity of extracting elements, reaching almost 1,500 kg ha^{-1} of the total of Na, K, Ca, Mg, P, N, and Cl in the plant shoots (Figure 7). This was the only treatment that surpassed *A. nummularia* alone in the revegetation strategy. Once again, *M. caesalpiniifolia* alone was the treatment with the least extraction capacity of elements, followed by *L. leucocephala* also alone. In associated cultivation with *A. nummularia*, the treatments of these two species were equated by the extraction of *A. nummularia* plants.

Studies have reported sustainable maintenance in systems with higher plant biomass yield, due to the greater incorporation of OM into the soil (ÁVILA, ASSAD and LIMA, 2012). Part of the biomass accumulated in the stems can also be incorporated by fallen branches during dry periods, representing another OM source returning to the soil.

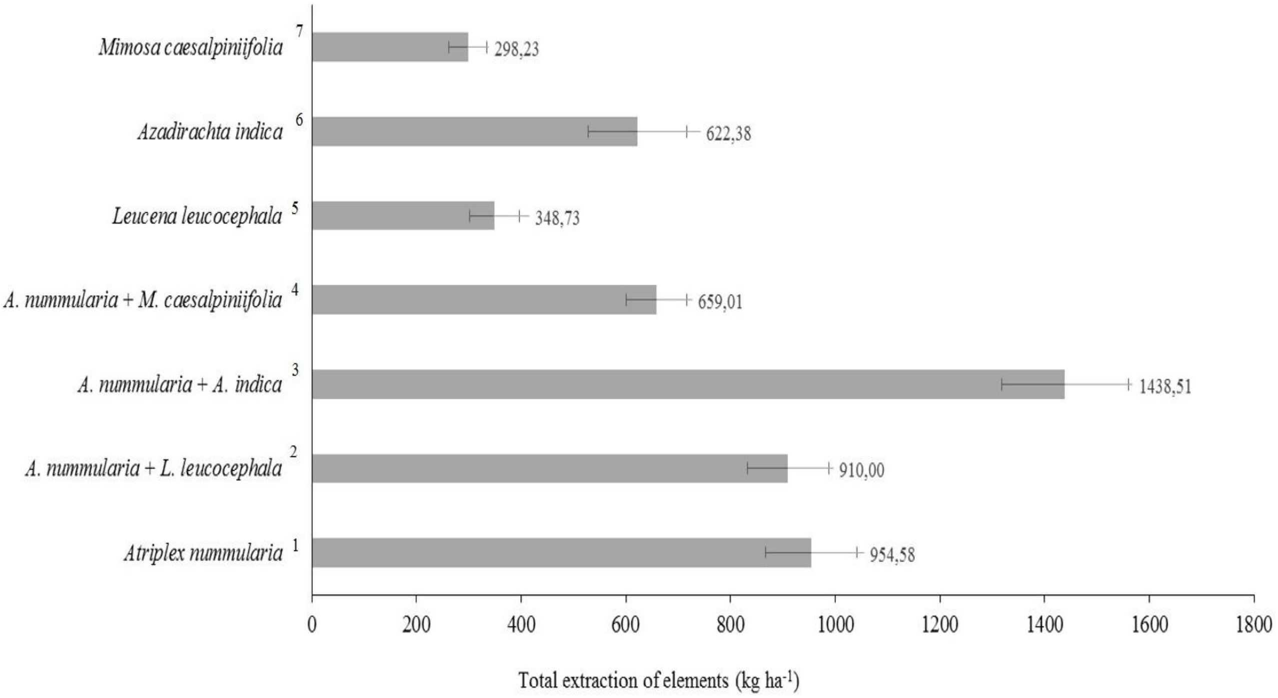
M. caesalpiniifolia plants, both alone and cultivated in association with *A. nummularia*, had low levels and contents of most evaluated elements in the leaves. However, due to the total loss of leaves in the dry season, these plants also contribute significantly to OM input into the soil and to nutrient cycling (FERNANDES *et al*., 2006). In contrast, *A. indica* plants both alone and in associated cultivation with *A. nummularia*, had high values of total leaf content for most of the evaluated elements, due to the large production of leaf biomass. Thus, even with a small leaf fall of this species, the contribution to organic matter and the return of the elements to the soil can be significant (LEÓN; OSÓRIO, 2014).

The highest concentration and content of Na and Cl in *A. nummularia* plants proves its phytoextractor potential (SILVA *et al*., 2016; SOUZA *et al*., 2011). Associated cultivation with *A. nummularia* plants becomes an alternative for areas where Na and Cl ions are present in high concentration in soil or irrigation water. The tolerant species (phytoextractor) absorbs the ions in high proportions (SILVA *et al*., 2016), enabling the growth of other less tolerant species. The extraction of Na by *A. nummularia* plants also contributes to the decrease in salinity and sodicity of the soils, as observed by Miranda *et al.* (2021) and Silva *et al.* (2016).

A. indica plants alone stood out in the concentrations of Mg and P, and they stood out with high values of K, Ca, Mg, P and N when associated with *A. nummularia* plants. This may be attributed due to the adaptation of this species to the Northeast region of Brazil because it is tolerant to long dry periods and high temperatures (ARAÚJO; RODRIGUEZ; PAES, 2000). The rapid growth of this plant with high total dry matter production may also have been benefited by associated cultivation with *A. nummularia*. On the other hand, the greater extraction of elements by *A. indica* plants would result in the release of litter richer in nutrients. According to Maluf *et al*. (2015), the decomposition of litter contributes to the return of part of nutrients absorbed by plants, influencing the improvement of soil fertility.

Regarding the total extracted of the elements by area, associated cultivation of *A. nummularia* and *A. indica* had the highest extraction of K, Ca, Mg, P and N, in addition

Figure 7 - Sum of elements (Na, K, Ca, Mg, N, P, Cl) extracted per area in biomass of plants used in species alone or associated revegetation strategy in a saline-sodic soil at 30 months. [1] *Atriplex nummularia* plants alone, [2] *A. nummularia* associated with *L. leucocephala*, [3] *A. nummularia* associated with *A. indica*, [4] *A. nummularia* associated with *M. caesalpiniifolia*, [5] *Leucaena leucocephala* plants alone; [6] *Azadirachta indica* alone; [7] *Mimosa caesalpiniifolia* plants alone



to being among the treatments with the highest values of Na and Cl. It demonstrates the potential of this association from the phytoextracting point of view, suggesting the pruning and removal of the aerial part of *A. nummularia* periodically. Additionally, it can be characterized as a source of nutrients by the litter rich in essential elements, mainly accumulated in *A. indica* leaf biomass.

Growing plants in degraded areas can promote significant improvements in soil quality by deepening the root system to deeper layers. Miranda *et al.* (2018) reported improvements in physical properties of a saline-sodic soil by *A. nummularia* cultivation, especially in subsurface. Despite the cultivation of plants had caused similar improvement as chemical and organic conditioners application, the authors highlighted the importance of phytoremediation as a more sustainable alternative, which can still provide complementary material for animal feed.

The combination of plants in associated systems should promote more positive effects on several soil properties, due to better distribution of roots throughout the soil profile. Studies assessing growing set of various species can be interesting, by increasing the biological activity in the area. It is also important to highlight that research of this nature must be maintained in the field for longer periods, enabling more significant effects and sustainability of the recovery.

CONCLUSIONS

1. *A. nummularia* may be used in associated cultivation for phytoextraction of salts from soils;
2. Associated revegetation strategy by *Atriplex nummularia* and *Azadirachta indica* is more efficient inthe extraction of K, Ca, Mg, P an N from the soil;
3. *Atriplex nummularia* may be used to increase Na and Cl extraction from soils in association with more sensitivity plants to salt;
4. *Azadirachta indica* has higher biomass production and *Mimosa caesalpiniifolia* lower under salinity conditions studied in Brazilian Semiarid.

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