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Phytomass accumulation and mineral composition of cowpea (*Vigna unguiculata*) under salt stress and phosphate fertilization

Francisco Vanies da Silva Sá[1]*, Miguel Ferreira Neto[1], Yuri Bezerra de Lima[1], Emanoela Pereira de Paiva[1], Ana Cláudia da Silva[1], Nildo da Silva Dias[1,5], Francisco Marto de Souza[2], Alberto Soares de Melo[3,6], Romulo Carantino Lucena Moreira[4], Luderlândio de Andrade Silva[4]

[1]Federal Rural University of Semi-Arid, Center of Agricultural Sciences, Mossoró, 59.625-000, Rio Grande do Norte, Brazil
[2]Federal University of Paraíba, Center of Agricultural Sciences, Areia, 58397-000, Paraiba, Brazil
[3]State University of Paraíba, Department of Biological Science, Campina Grande, 58.109-970, Paraíba, Brazil
[4]Federal University of Campina Grande, Academic Unit of Agricultural Engineering, Campina Grande, 58.109-970, Paraíba, Brazil
[5]Fellow of CNPq Research Productivity, level 1D, Brazil
[6]Fellow of CNPq Reseach Productivity, level 2, Brazil

***Corresponding author: vanies_agronomia@hotmail.com**

Abstract

This study aimed to evaluate the effect of irrigation with saline water and phosphate fertilization on phytomass accumulation and tissue mineral composition of cowpea shoots. The research was carried out in a greenhouse utilizing randomized block design in a 5 x 3 factorial scheme consisting of five levels salinity of irrigation water (0.5 as control, 1.5, 2.5 3.5 and 4.5 dS m^{-1}) and three doses of $P_2O_5^-$ (60, 100 and 140% from a recommended dose of 60 kg $P_2O_5^-$ ha^{-1}), with 5 replicates. The plants were grown in pots up to flowering and then phytomass accumulation and mineral composition of cowpea (indicate cultivar name) shoot were measured. Irrigation with saline water from 0.8 dS m^{-1} reduced phytomass accumulation and mineral composition of cowpea plants, making the plants to be considered moderately tolerant up to EC 2.5 dS m^{-1}. The 40% increase in recommended phosphorus dose promotes high iron and copper accumulations and, less sodium accumulation into tissues of cowpea shoot as influenced by saline stress. 60% reduction of the recommended dose of phosphate fertilizer increased the manganese and zinc accumulation of tissues of cowpea shoot under salt stress.

Key words: *Vigna unguiculata* L. Walp., irrigation, salinity, mineral nutrition.

Introduction

Cowpea has significant economic and social importance for farmers in the Brazilian semi-arid region, because it is a source of income and food for most of the population (Calvet et al., 2013). In Northeast Brazil, the cowpea crop occupies an area of approximately 1.2 million hectares (Silva et al., 2010). In a semi-arid conditions its yield is increased, when irrigated in a planned and efficient way, since water greatly influences agricultural production (Carvalho et al., 2015).

The use of moderately saline water in irrigation may be feasible for cowpea cultivation, when water salinity does not exceed 3.3 dS m^{-1}, and the electrical conductivity level is consistent with most of the waters in the semi-arid region (Ayers and Westcot, 1999). However, the use of highly-saline water for irrigation, associated with the climatic conditions of the semi-arid region, reduces the production of the crop, since its physiology and biochemistry are damaged (Santos et al., 2009; Sousa et al., 2014). For instance, reduction in absorption, transport and assimilation of nutrients, led to reduction in photosynthesis and consequently in growth (Munns and Tester, 2008; Oliveira et al., 2017; Sá et al., 2017).

Soil salinity occurs due to the accumulation of soluble salts, particularly Na^+ and Cl^- ions (Mesquita et al., 2015). Excess of salts in soil solution reduces the osmotic potential of the soil and, consequently, hinders water absorption by the plant. In addition, the plant can absorb the excess of Na^+ and Cl^- and accumulate high concentrations of these ions in the chloroplast to the point of causing toxic effects, interfering with the absorption, assimilation and transport of nutrients, with plasma membrane functions and with disorders in metabolic processes, such as synthesis of proteins, respiration and photosynthesis. Additionally, high Na/K ratio reduces the synthesis of proteins, inactivating several enzymes (Munns and Tester, 2008; Santos et al., 2009; Taiz et al., 2015).

On the other hand, besides the notable effects of salinity on plants under semi-arid conditions, where most soils have low contents of phosphorus, it is necessary to apply phosphate fertilizers to optimize production (Santos et al., 2008). In addition, phosphorous limitations at early vegetative cycles lead to often irreversible restrictions on root and shoot development, even if phosphorus supply is increased to adequate levels in other growth stages (Sousa et al., 2012).

Phosphorus is responsible for the synthesis of energy, allowing greater development of the root system, favoring the search for water and nutrients and can contribute minimizing the effects of salt stress (Castro et al., 2016; Sá et al., 2017).

Therefore, this study aimed to evaluate the effect of saline water irrigation associated with phosphate fertilization on the accumulation of phytomass and nutrients in the shoots of cowpea plants.

Results and Discussion

The interaction between levels of irrigation water salinity and phosphorus doses had significant effect on the contents of sodium, iron, manganese, zinc and copper in the shoots ($p < 0.05$). The other variables were significantly affected ($p < 0.05$) only by the levels of irrigation water salinity (Table 2).

Increase in irrigation water salinity above 0.8 dS m^{-1} reduced phytomass accumulation in cowpea, in which from 3.3 dS m^{-1}, the plants were considered as susceptible to salinity according to the classification of Fageria et al. (2010), with phytomass production below 60% (Figure 1). This result differs from the postulate of Ayers and Westcott (1999) that the salinity threshold for cowpea is 3.3 dS m^{-1}, since in the present study severe effects were already observed at this level.

Sodium content in cowpea shoots showed a quadratic behavior as a function of the salinity levels, and the highest sodium contents were found at salinity levels of 3.02, 2.97 and 3.04 dS m^{-1} in plants fertilized with 60, 100 and 140% of the recommended $P_2O_5^-$ dose, respectively (Figure 2A). It should be pointed out that the lowest sodium contents were found when plants were fertilized with 140% of phosphorus recommendation, but this result did not influence phytomass accumulation. Compared with the results of phytomass accumulation, the reductions in sodium accumulation from 3.0 dS m^{-1} are consistent with the drastic reductions in phytomass, indicating toxicity by the excess of sodium salts in plant tissue. The results obtained in this study demonstrated that there were no mechanisms of exclusion of toxic ions (Na^+) after absorption, resulting in accumulation in the shoots over time (Bosco et al., 2009).

The excess of salts in the irrigation water compromised phytomass accumulation and nutrient contents, causing linear reductions in the contents of nitrogen, phosphorus, calcium and magnesium on the order of 8.82, 2.41, 18.67 and 6.08 mg $plant^{-1}$ per dS m^{-1}, respectively (Figures 2B, D, E and F). High salinity, particularly related to sodium, can preclude the absorption of elements that are essential to plant growth, leading to nutritional imbalance (Wiladino and Câmara, 2010; Sá et al., 2017). Reduction in the accumulation of these nutrients is related to the effects of salt stress on the reduction in nutrient absorption and translocation associated with the decrease in growth rate and, consequently, the phytomass (Sousa et al., 2012; Calvet et al., 2013; Sousa et al., 2014; Oliveira et al., 2017).

Potassium accumulation in the shoots of cowpea plants showed a quadratic behavior as a function of increasing irrigation water salinity, and the highest content (115.53 mg $plant^{-1}$) was found at salinity of 0.97 dS m^{-1}. From this point of salinity and higher on, potassium accumulation was decreased by 75% between the highest (4.5 dS m^{-1}) and lowest (0.5 dS m^{-1}) salinity levels (Figure 2C). The effect of NaCl on K contents has also been attributed to the antagonism between these cations and the consequent competitive inhibition of Na on the absorption of K, since they compete for the same means of cellular absorption (carrier proteins) (Fernandes et al., 2002; Taiz et al., 2015). The reduction in K content under salt stress is an additional complicating factor for plant growth because of the osmotic damages, cellular ion imbalance and, consequently, disorders in enzymatic activation and synthesis of proteins (Saqib et al., 2005; Munns & Tester, 2008; Lima et al., 2015).

The accumulation of the micronutrients was significantly affected by the interaction between phosphorus doses and levels of irrigation water salinity (Figures 3A, B, C and D). The highest phosphorus dose (140% of the $P_2O_5^-$ recommended dose) positively influenced the accumulation of iron and copper, which showed higher values up to salinity level of 3.5 dS m^{-1}, compared to the other phosphorus doses, denoting the possibility of synergism between these nutrients in plants under salt stress (Figures 3A and D). It is important to point out that plants subjected to A3 (140% of phosphorus recommendation) also showed the lowest accumulation of sodium in the shoots, which may have contributed to the higher accumulation of iron and copper (Figure 2A).

The opposite was occurred for accumulation of. The manganese and zinc contents in cowpea plants fertilized with 60% of $P_2O_5^-$ dose were higher than plants under 100 and 140% of $P_2O_5^-$ recommended dose, at salinity levels of 1.5 to 3.5 dS m^{-1}, denoting the possibility of antagonism between phosphorus and these nutrients when plants are under salt stress (Figures 3B and C). Lower zinc contents in plants under higher phosphorus doses are related to the antagonistic action between these nutrients, commonly reported in the literature (Façanha et al., 2008). The reduction of manganese contents in glycophytes is associated with the composition of the saline medium, high soil pH and antagonism of the excess calcium (Sousa et al., 2007). This can be related to the nature of single superphosphate because, as phosphorus dose increases, the dose of calcium also increases using this fertilizer.

In general, increase in water salinity from 0.8 to 4.5 dS m^{-1} reduced the dry matter accumulation in the shoots of cowpea plants. Additionally, Na accumulation increased their shoot tissue. These phenomena also coincided with reductions in the accumulation of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu. It is important to note that plants subjected to salt stress fertilized with 140% of P recommendation had lower accumulation of Na and higher accumulation of Fe and Cu in comparison to other fertilization treatments.

Table 1. Physical and chemical characteristics of the soil collected in the 0-30 cm layer and the bovine manure used in cowpea cultivation.

Soil														
Clay		Sand		Silt		DS		DP		Porosity		Textural Class		
------------------%-----------------						g cm^{-3}		g cm^{-3}		-----%----				
10.0		89.0		1.0		1.57		2.51		37.45		Free Sand		
EC 1:2.5		pH	P	K^+	Ca^{+2}		Mg^{+2}	Na^+	Al^{3+}	H^++Al^{3+}	SB	T	OM	ESP
dSm^{-1}		H_20	mg dm^{-3}	-----------------------------$cmol_cdm^{-3}$---------------------------									g kg^{-3}	%
0.16		6.72	1.20	0.20	1.40		0.50	0.05	0.00	0.70	2.15	2.85	13.23	1.75
Bovine manure														
N	P	K	Ca	Mg	Na	Zn	Cu	Fe	Mn	pH	C.O.	CTC	C/N	EC
-----------------------g Kg^{-1}------------------						--------mg Kg^{-1}------				H_2O	%	Cmolc dm^{-3}		mS/cm
14.85	3.25	1.16	16.11	3.07	0.66	65	15	3.77	121	6.53	10.70	34.24	7.21	2.56

P, K^+, Na^+: extracted by Mehlich 1; Al^{3+}, Ca^{2+}, Mg^{2+}: extracted by 1.0 mol L^{-1} KCl; DS: Soil bulk density; DP: Soil particle density; EC: Electrical conductivity; SB: Sum of bases; T: Cation exchange capacity; OM: Walkley-Black wet digestion; ESP: Exchangeable sodium percentage.

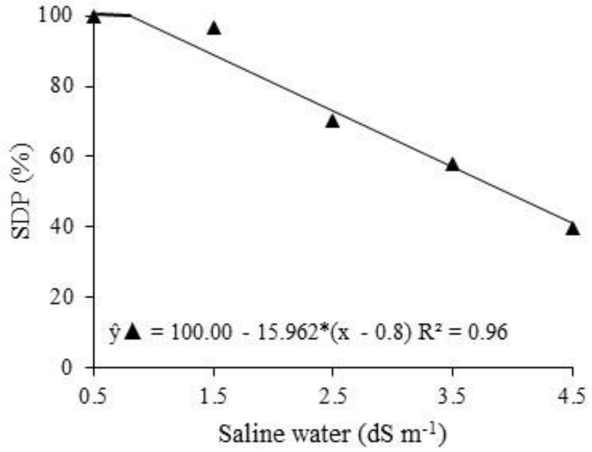


Fig 1. Shoot dry phytomass (SDP) of cowpea, cv. 'Paulistinha', under different levels of water salinity at 49 days after sowing. * = significant at 0.05 probability ($p < 0.05$).

Materials and Methods

Localization, experimental procedure and treatments

The study was conducted in a greenhouse of the Department of Environmental and Technological Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró-RN, Brazil, from September to December 2015. The municipality of Mossoró-RN is located in the semi-arid region of northeastern Brazil (5° 11' S; 37° 20' W; 18 m of altitude).

The experiment was carried out in randomized blocks, in 5 x 3 factorial scheme, corresponding to five levels of irrigation water salinity (S1 = 0.5 (control); S2 = 1.5; S3 = 2.5 S4 = 3.5; S5 = 4.5 dS m^{-1}) and three doses of P_2O_5 [A1 = 60%; A2 = 100% (60 kg P_2O_5 ha^{-1}) and A3 = 140% of the dose recommended by Cavalcanti (2008)], with 5 replicates, in a total of 75 experimental plots.

Phosphorus doses were calculated based on the initial phosphorus content in the arable layer of a Latosolic Red Yellow Argisol (EMBRAPA, 2013), under fallow, from the UFERSA's experimental farm. Soil samples were collected and analyzed at the Laboratory of Soil, Water and Plant Analysis – LASAP of the UFERSA, following the methodology of EMBRAPA (2009) (Table 1).

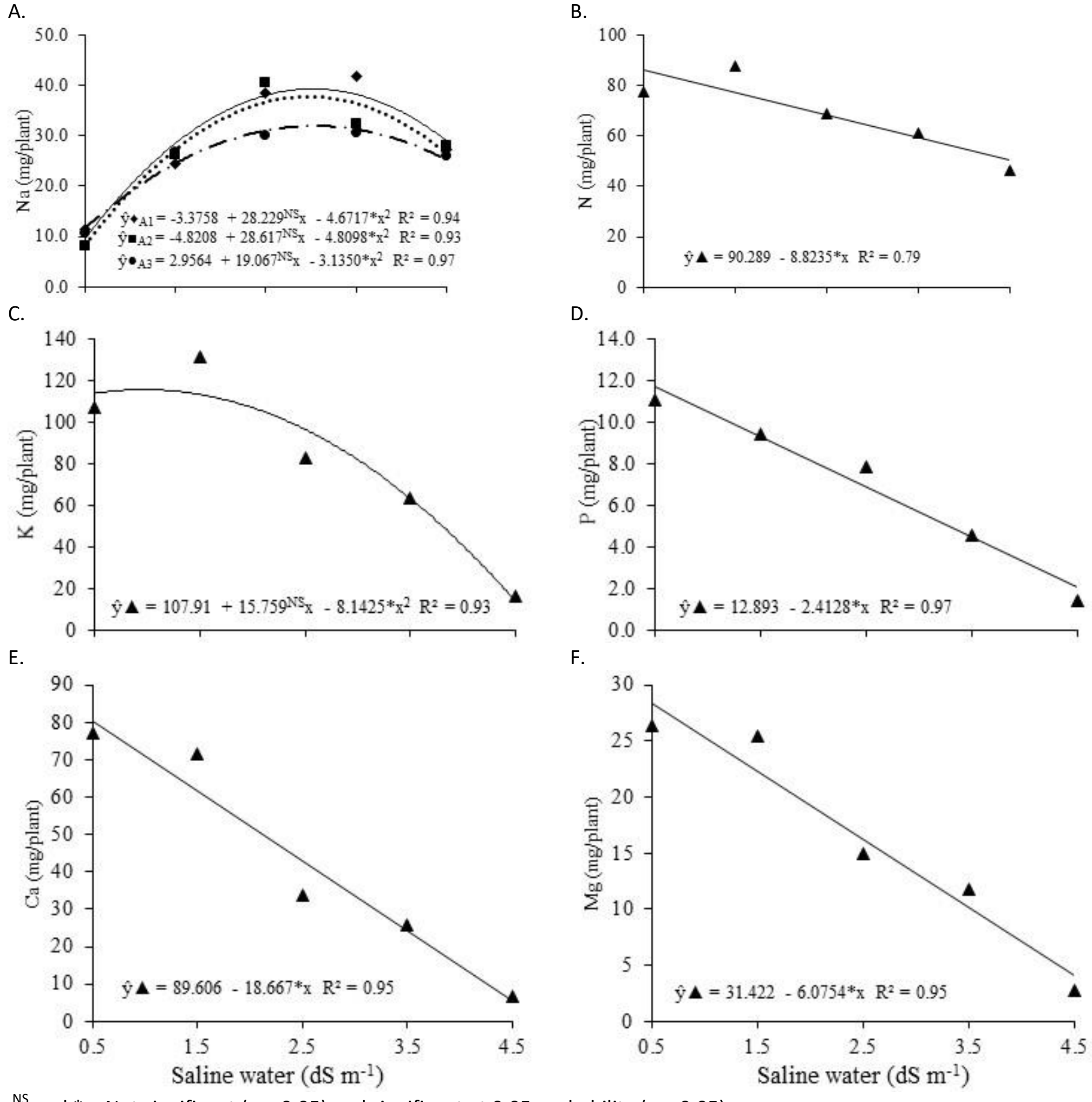
Management of the experiment

Fertilization was based on soil analysis and on the technical bulletin of fertilization recommendation for the Pernambuco state (Cavalcanti, 2008). The recommendation for cowpea is 60 kg ha^{-1} of P_2O_5, 20 kg ha^{-1} of K_2O and 50 kg ha^{-1} of N for one production cycle. The doses of P_2O_5 (A_1 = 36; A_2 = 60 and A_3 = 84 kg ha^{-1}) were calculated based on the fertilization recommendation, applied and incorporated in the form of single superphosphate (A_1 = 0.7; A_2 = 1.17 and A_3 = 1.64 g pot^{-1} of P_2O_5). Planting was carried out after 20 days (incubation period), aiming at stability and adsorption of phosphorus. Since the soil material had sandy texture, nitrogen and potassium fertilizers were applied as top-dressing; N was distributed proportionally at 14, 21 and 27 days after sowing, whereas K was applied at 28 and 35 days after sowing. The soil material was put in pots with capacity for 8 dm^3, of which 7 dm^3 were filled with soil and 0.5 dm^3 with bovine manure (Table 1), aiming to increase its water retention and negative charges, plus 0.5 dm^3 of crushed stone at the bottom, to facilitate drainage. The lysimeters were filled in the following order: screen; crushed stone; 2 dm^3 of soil; and the mixture of soil (5 dm^3): manure (0.5 dm^3): dose of $P_2O_5^-$, established for each treatment. After soil preparation, one irrigation was applied, leaving the soil close to its maximum water retention capacity.

Table 2. Summary of analysis of variance for shoot dry phytomass (SDP) and contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in the shoots of cowpea (cv. 'Paulistinha') subjected to levels of irrigation water salinity and phosphate fertilization at 49 days after sowing.

SV	DF	Significância do teste 'F'										
		SDP	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Block	4	ns	ns	ns	*	ns	*	ns	*	*	*	ns
Sal	4	*	*	*	*	*	*	*	*	*	*	*
Fertilizing	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sal x Fertilizing	8	ns	ns	ns	ns	ns	ns	*	*	*	*	*
CV		16,51	20,01	33,14	27,89	34,00	25,15	31,81	32,31	29,70	29,26	22,75

* - Significant at 0.05 probability; ns - Not significant; SV = Sources of variation; DF = degree of freedom; Sal - Salinity levels; CV – Coefficient of variation.



^{NS} and * = Not significant ($p > 0.05$) and significant at 0.05 probability ($p < 0.05$).

Fig 2. Contents of sodium, Na (A), nitrogen, N (B), potassium, K (C), phosphorus, P (D), calcium, Ca (E) and magnesium, Mg (F) in the shoots of cowpea plants (cv. 'Paulistinha') subjected to levels of irrigation water salinity and phosphate fertilization (♦A_1 = 60%, ■A_2 = 100% and ●A_3 = 140% of $P_2O_5^-$ recommendation) at 49 days after sowing.

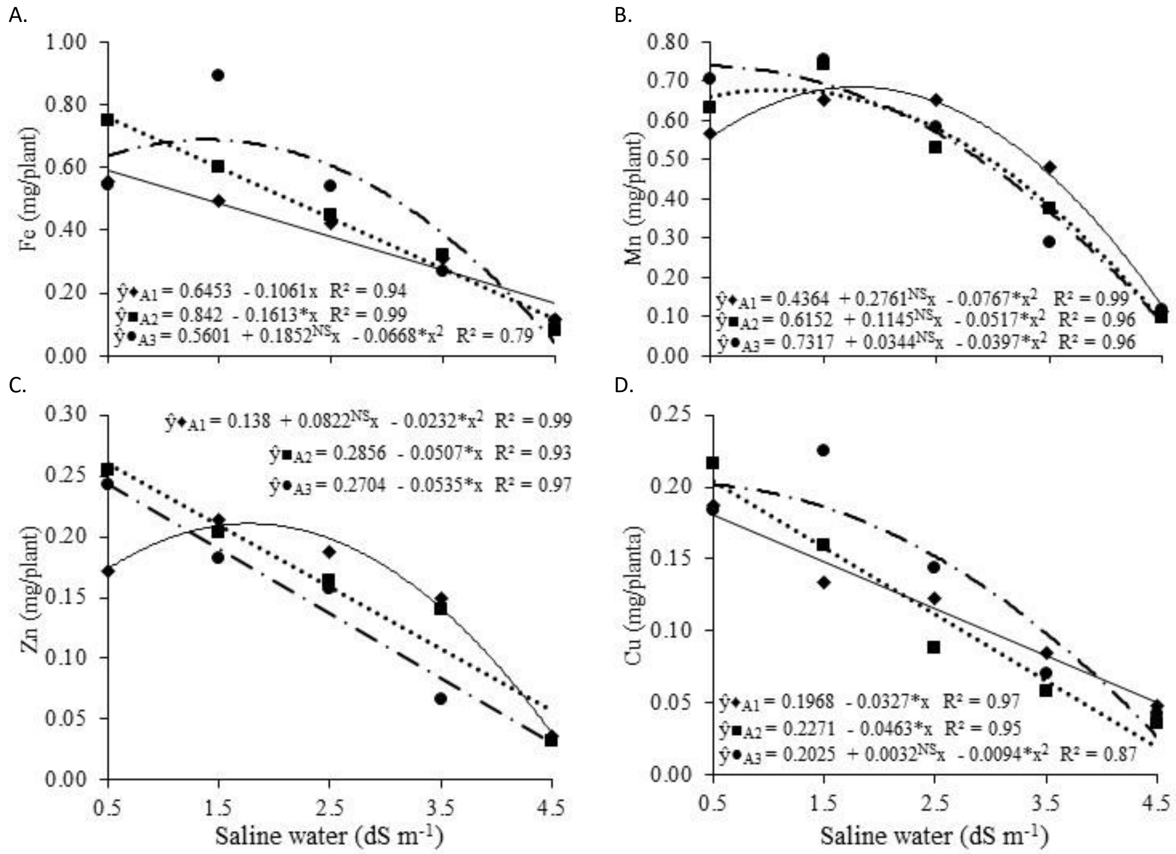


Fig 3. Accumulation of iron, Fe (A), manganese, Mn (B), zinc, Zn (C) and copper, Cu (D) in the shoots of cowpea plants, cv. 'Paulistinha', under different levels of water salinity and phosphate fertilization (♦A_1 = 60%, ■A_2 = 100% and ●A_3 = 140% of $P_2O_5^-$ recommendation) at 49 days after sowing. ^{NS} and * = Not significant ($p > 0.05$) and significant at 0.05 probability ($p < 0.05$).

Subsequent irrigations were conducted once a day to bring the soil to near its maximum water retention capacity, based on the drainage lysimetry method (Rhoades et al., 1992), and the applied water depth was added with a leaching fraction (LF) of 0.20 of the volume accumulated every seven days. The water volume applied per pot (Vw) was obtained by the difference between the previous water depth applied (Vprev) minus the average drainage (d), divided by the number of pots (n), as shown in Eq. 1:

$$Vw = \frac{Vprev - (D/n)}{1-LF} \qquad (1)$$

Saline solutions with different electrical conductivities were prepared by the addition of salts of sodium chloride (NaCl), which comprises 70% of the salt ions in sources of water used for irrigation in small properties of Northeast Brazil (Medeiros et al., 2003).

Irrigation solutions with different levels of salinity were prepared considering the relationship between water electrical conductivity (EC_w) and salt concentration (10*meq L^{-1} = 1 dS m^{-1} of EC_w), according to Rhoades et al. (1992), valid for the EC_w range from 0.1 to 5.0 dS m^{-1}, which encompasses the levels tested. The solutions were prepared using the potable water available (EC_w = 0.53 dS m^{-1}) and adding the salts as needed. The salt was weighed according to the treatment, and water was added until the desired level of electrical conductivity (EC) was reached. EC_w levels were verified using a portable conductivity meter, adjusted to temperature of 25 ºC. After preparation, the saline solutions were stored in 150-L plastic containers, one for each EC_w level, properly protected to avoid evaporation, entry of rainwater and contamination by materials that could compromise their quality.

Establishment and traits measured

After irrigation, the cowpea cv. 'Paulistinha' was sown on October 14, 2015, 20 days after application of the phosphorus dose, using 10 seeds per pot. Fifteen days after sowing, with total emergence of the seedlings, thinning was performed to leave only two plants per pot.

At 49 days after sowing, during the transition from the vegetative to the reproductive stage (V8/R1), plant shoots were collected to determine shoot dry phytomass (SDP). The material was dried in a forced-air oven at 65 ºC until constant weight. After that, the material was weighed on analytical scale and the values were expressed in % in relation to the control treatment (0.5 dS m^{-1}). Then, SDP was ground to determine the contents of nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu), following the methodology proposed by EMBRAPA (2009).

Statistical analysis

The obtained data were subjected to the analysis of variance by F test at 0.05 probability level and, in the cases of significance, linear or quadratic polynomial regression analyses were carried out at 0.05 probability level for irrigation water salinity, based on equation significance and highest R^2. In the case of individual significance of phosphate fertilization, Tukey's test was applied at 0.05 probability level, using the statistical program SISVAR® (Ferreira, 2011).

Conclusion

Irrigation with saline water from 0.8 dS m^{-1} reduced the accumulation of phytomass and nutrients in cowpea plants, and the crop was classified as moderately tolerant up to the electrical conductivity of 2.5 dS m^{-1}. The 40% increase in recommended dose of phosphorus promotes high iron and copper accumulations and, less sodium accumulation into tissues of cowpea shoot as influenced by under saline stress. Reduction of phosphate fertilization up to 60% of recommended dose increased the manganese and zinc accumulation of tissues of cowpea shoot under salt stress.

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