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# Leaching fraction impacts water use efficiency and nutrient losses in maize crop under salt stress<sup>1</sup>

Fração de lixiviação impacta uso eficiente da água e perdas de nutrientes em milho sob estresse salino

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#### HIGHLIGHTS:

Salinity reduces nutrient absorption regardless of the leaching fraction.

The leaching fraction determined by the Rhoades method reduces water use efficiency.

A leaching fraction of 0.15 according to soil water balance reduces nutrient losses.

**ABSTRACT:** Although leaching fraction (LF) is used to remove salts from the root zone under conditions of salinity, if miscalculated, it can decrease water use efficiency and lead to major losses of essential nutrients. This study evaluated the water use efficiency, leaf nutrient concentrations in maize plants, and nutrient losses as a function of two ways of determining the LF in maize crop grown in soil columns under salt stress. The experimental design used was completely randomized, with treatments arranged in split plots, with seven replicates. The plots were formed by two methods of determination of LF, and the subplots by four electrical conductivities of irrigation water – EC (0.5, 2.0, 4.0, and 6.0 dS m<sup>-1</sup>). The leaching fractions were established according to 1) the formula proposed by Rhoddes (RHO) and 2) by applying a LF of 0.15 calculated according to the soil water balance (SWB). The leaf concentrations of N, P, Ca, and Mg were higher in plants under SWB than under RHO method. The leaf concentrations of N, P, and K decreased with increased salinity, regardless of the LF. Adding a LF of 0.15 according to SWB resulted in decreased losses of nutrients and higher physical water productivity, as compared to the RHO. Thus, a more precise determination of the LF is needed to increase economic returns for maize cultivation in semi-arid regions when using brackish water for irrigation.

Key words: Zea mays L., salt stress, leaching of nutrients, water productivity

**RESUMO:** Embora a fração de lixiviação (FL) seja utilizada para remover sais da zona radicular em condições de salinidade, quando inadequadamente calculada, pode diminuir a eficiência do uso da água e levar a grandes perdas de nutrientes essenciais. No presente estudo avaliou-se o uso da água, as concentrações de nutrientes foliares em plantas de milho e as perdas de nutrientes em função de duas formas de determinação da FL em plantas de milho cultivadas em colunas de solo sob estresse salino. O delineamento experimental utilizado foi o inteiramente casualizado, com os tratamentos dispostos em parcelas subdivididas, com sete repetições. As parcelas foram formadas por duas formas de determinação da FL e as subparcelas por quatro condutividades elétricas da água de irrigação – CE (0,5; 2,0; 4,0 e 6,0 dS m<sup>-1</sup>). As FL foram determinadas de acordo com 1) a fórmula proposta por Rhoades (RHO) e<sup>a</sup>2) aplicando uma FL de 0,15 calculado de acordo com o balanço hídrico do solo (SWB). A concentração foliar de N, P, Ca e Mg foi maior nas plantas onde utilizou-se o SWB do que no método de RHO. A concentração foliar de N, P e K diminuiu de acordo com o aumento da salinidade da água, independentemente do método de determinação da FL. A adição de uma FL de 0,15 de acordo com o SWB resultou em diminuição das perdas de nutrientes e maior produtividade física da água, em relação ao RHO. Assim, uma determinação mais precisa da FL aumenta os retornos econômicos da cultura do milho no semiárido quando se utiliza água salina para irrigação.

Palavras-chave: Zea mays L., estresse salino, lixiviação de nutrientes, produtividade da água

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### Introduction

The scarcity of water in semi-arid regions imposes uncertainties about the viability of irrigated agriculture (Frizzone et al., 2021). At a time when freshwater is decreasing at an alarming pace, brackish water is a precious resource to be used for crop production. However, the use of these water sources can lead to soil salinization, if certain management practices are not properly implemented (Qiu et al., 2019). Among these techniques, the use of a leaching fraction (LF) stands out, as it makes it possible to remove salts from the root zone, reducing their impacts on crops (Gois et al., 2019).

Despite the positive effect of removing salts, the use of high LF can reduce water use efficiency and increase nutrient losses in plants irrigated with saline waters (Shenker et al., 2003; Farooq et al., 2015; Ferreira et al., 2020; Iqbal et al., 2020; Ribeiro et al., 2020), resulting in economic loss to the farmer and potential damage to the environment (Lacerda et al., 2016; 2018). Thus, it is important to evaluate which method of leaching fraction calculation is more efficient for each crop under given edaphoclimatic conditions.

In the Brazilian semi-arid region, it is common to use LF of 15% of the irrigation depth when saline water is used (Braz et al., 2019), and this value often does not consider the type of soil or electrical conductivity of irrigation water. On the other hand, the method proposed by Rhoades (1974), also used in areas with salinity problems, suggests the calculation of LF according to water salinity and crop salt tolerance. This method, however, results in high LF values, which can also result in considerable losses of essential nutrients by leaching, an aspect little considered in current research studies.

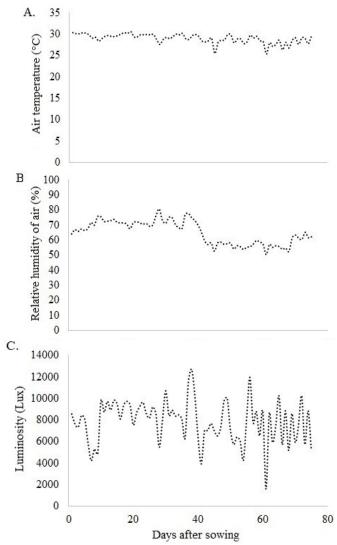
In this context, the present study tested the hypothesis that water use efficiency and the amount of nutrients leached from the soil are strongly influenced by the LF. Thus, the objective of the present study was to evaluate the water use efficiency, leaf nutrient concentrations in maize plants, and nutrient losses as a function of two methods of determining the LF in maize crop grown in soil columns under salt stress.

## MATERIAL AND METHODS

The experiment was set up in a greenhouse located in Fortaleza (3° 45' S; 38° 33' W, 19 m), Ceará, Brazil. During the experimental period, from November 2017 to February 2018, the average temperature inside the greenhouse was 29 °C, with an average thermal amplitude of 5 °C. The indoor environment of the greenhouse was monitored daily using a data logger (model HOBO° U12-012 Temp/RH/Light/Ext) as shown in Figure 1.

The experimental design used was completely randomized, with treatments arranged in split plots, with seven replicates. The plots were formed by two methods of calculation of the leaching fraction, and the subplots by four electrical conductivities of irrigation water –  $EC_w$  (0.5, 2.0, 4.0, and 6.0 dS m<sup>-1</sup>).

The EC $_{\rm w}$  levels were obtained by using NaCl, CaCl $_2$ .2H $_2$ O, and MgCl $_2$ .6H $_2$ O salts, in an equivalent proportion of 7:2:1, following the relationship between EC $_{\rm w}$  and salt concentrations



**Figure 1.** Air temperature (A), relative humidity of air (B), and luminosity (C) inside the greenhouse during the experimental period

(mmol $_c$  L $^{-1}$  = EC × 10). The leaching fractions (LF) were defined as follows: RHO - Application of leaching fractions calculated for each salinity level, according to the formula proposed by Rhoades (1974) (Eq. 1); SWB - Application of leaching fractions of 0.15 calculated from the soil water balance of the experimental plots.

$$LF = \frac{EC_{w}}{5EC_{-} - EC_{-}}$$
 (1)

where:

 $\mathrm{EC}_{_{\mathrm{w}}}$  - electrical conductivity of irrigation water (dS m-1); and,

 $EC_{se}$  - electrical conductivity of soil saturation extract that represents the salinity which allows a minimum yield of 90% (for maize, 2.5 dS m<sup>-1</sup>). LF values for  $EC_{w}$  of 0.4, 2.0, 4.0, and 6 dS m<sup>-1</sup> were 4, 19, 47, and 92%, respectively.

The soil columns were constructed using rigid PVC pipes with an internal diameter of 20 cm and length of 100 cm. The lower ends were closed and sealed with PVC caps. The inner wall of the pipes received a layer of glue with sand to prevent

the preferential flow of water between the soil and the pipe wall. The basal end of the column was filled with coarse sand (5 cm height) to facilitate drainage. To allow the collection of leachate, a tube was connected to 1000-mL plastic bottles positioned under the columns below ground level.

The columns were filled with an Ultisol, collected at the Pici Campus of the Federal University of Ceará, in Fortaleza, Ceará, Brazil. The columns were assembled following the same sequence of soil horizons as they are found in the field. However, since the thickness of the horizons exceeded 1 m (column length), three layers within the profile were defined to fill the columns. Thus, the following sequence was used: first layer, material collected from A horizon (0-20 cm); second layer, material collected in E and EB horizons (20-60 cm); third layer, material collected from textural B horizon (60-90 cm). Each column received approximately 42 kg of soil so that the density of the layers was 1.5 kg dm<sup>-3</sup>.

After soil collection, samples of each layer were used for the characterization of physical and chemical attributes according to the methods contained in Teixeira et al. (2017). The first two layers of the soil showed texture classified as loamy sand. The third and last layer were classified as sandy clay loam. In relation to the nutrients in the three layers, respectively from the surface to the deepest layer, the obtained values were: 1.24, 0.75, and 0.35 g kg<sup>-1</sup> of N; 33.0, 8.0, and 5.0 mg kg<sup>-1</sup> of P; 0.43, 0.24, and 0.90 cmol<sub>c</sub> kg<sup>-1</sup> of K; 1.90, 0.70, and 1.10 cmol<sub>c</sub> kg<sup>-1</sup> Ca; 0.30, 0.50, and 0.60 cmol<sub>c</sub> kg<sup>-1</sup> Mg. The mean EC<sub>se</sub> of the layers was 0.11 dS m<sup>-1</sup> and the pH varied between 6.3 and 5.7. For the exchangeable sodium percentage (ESP), the following values were observed: 4.42 (0-20 cm), 6.96 (20-60 cm), and 4.21% (60-90 cm).

The crop used in the experiment was the BRS 2020 hybrid maize (*Zea mays* L.). Each soil column received four seeds and, seven days after sowing, thinning was carried out, leaving only one plant per column. One day after thinning, saline water began to be applied according to each treatment. Each column received 5.8 g of urea, 8.3 g of single superphosphate, and 2 g of potassium chloride, respectively equivalent to 200 kg of N ha<sup>-1</sup>, 120 kg of P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 90 kg of K<sub>2</sub>O ha<sup>-1</sup>, as recommended by EMBRAPA (2006). The addition of nitrogen and potassium was split, with 15% at sowing, 25% supplied 20 days after sowing (DAS), 30% at 35 DAS, and the remaining 30% at 50 DAS. Phosphorus was applied all at once as a basal dose (EMBRAPA, 2006).

To determine crop water needs, one column of each treatment was used as a drainage lysimeter. Irrigation was performed every two days until 30 DAS. After this period, the irrigations were applied daily until the end of the experiment. Total water consumption was estimated by the difference between the water applied and the water drained in each irrigation.

At the end of the experiment, the shoots of the plant were subjected to drying in a forced-air circulation oven at 60 °C for three days. The values of physical productivity of irrigation water (PWP $_{IR}$ ) were estimated by the ratio between the total dry matter of shoots (in grams) and the total volume of water applied per plant (in liters), according to Frizzone et al. (2021).

Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> concentrations were measured directly in the leachate, using a flame photometer. NO<sub>3</sub><sup>-</sup> was determined in the presence of 5% salicylic acid and sodium hydroxide (2 N NaOH), according to Cataldo et al. (1975). Chloride was determined in the presence of a solution of mercury thiocyanate in absolute methanol plus iron nitrate at 20.2%, according to the method described by Gaines et al. (1984). For determination of HPO<sub>4</sub><sup>2-</sup>, 10 mL of the diluted ammonium molybdate solution and 100  $\mu$ L of the 10% ascorbic acid solution were added to the leachate samples, according to the blue molybdenum method (Braga & Defelipo, 1974). The accumulated totals of the chemical elements were estimated by multiplying the concentrations obtained by the respective leachate volumes over the 80 days of the experiment per soil column.

Samples of oven-dried leaves, collected at the end of the experiment (80 DAS), were used to determine the concentrations of chemical elements. Leaf N concentration was determined according to Baethgen & Alley (1989), with readings performed in triplicate in a spectrophotometer ( $\lambda = 650$  nm). Wet digestion with nitric-perchloric acid was used to determine the leaf concentrations of K, P, Ca, Mg, and Na. Ca and Mg concentrations were obtained by atomic absorption spectrophotometry (AOAC, 2012), and Na and K concentrations by flame photometry, and P was quantified according to Braga & Defelipo (1974). The leaf concentration of Cl was determined in aqueous extract, using the method proposed by Gaines et al. (1984).

The results obtained were subjected to analysis of variance by the F test and means of leaching fraction methods were compared by the Tukey test. Regression analysis was applied for electrical conductivities. The chemical attributes of leaf and leachate were also submitted to Pearson's correlation test. The statistical analysis was performed using SISVAR software version 5.6 (Ferreira, 2019), considering  $p \leq 0.05$ .

# RESULTS AND DISCUSSION

The analysis of variance for the total of chemical elements in the leachate indicated significant interaction between salinity  $\times$  LF determination method (p  $\leq$  0.01), except for the total P and NO<sub>3</sub>-/Cl<sup>-</sup> ratio (p  $\leq$  0.05) (Table 1). P was significantly affected by the leaching fraction determination method, while the NO<sub>3</sub>-/Cl<sup>-</sup> ratio was not affected by any factor.

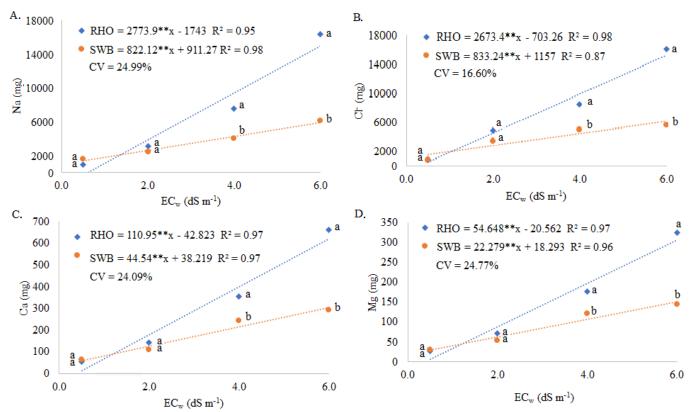
Figure 2 shows the amounts of chemical elements in the leachate. Elements which form the salts used for the saline composition of water showed exactly the same pattern of linear response to the  $\mathrm{EC}_{\mathrm{w}}$  increase. However, the intensity of the response depended on the method used to calculate the leaching fraction, and the angular coefficients of RHO method were higher than those of SWB method.

The greatest differences between the amounts of the elements in the leachate occurred for sodium and chloride, where the mean values obtained with RHO (Cl $^-$  = 7651.2 mg and Na = 6925.46 mg) were approximately twice those found with SWB (Cl $^-$  = 3760.2 mg and Na = 3480.39 mg). However, this difference was significant only when the EC $_{\rm w}$  was equal to or greater than 4 dS m $^{-1}$ . For the other elements, the difference

Table 1. Summary of analysis of variance with F calculated for the total sodium (Na<sup>+</sup>), chloride (Cl<sup>-</sup>), calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), phosphorus (P), potassium (K<sup>+</sup>) and NO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> in the leachate

Source		F calculated								
of variation	Na+	CI-	Ca <sup>2+</sup>	Mg <sup>2+</sup>	NO <sub>3</sub> -	K <sup>+</sup>	Р	NO <sub>3</sub> -/CI-		
MLF (A)	70.31**	188.12**	46.79**	47.33**	12.69**	38.33**	17.39**	0.68 <sup>ns</sup>		
ECw (B)	137.93**	103.82**	125.44**	118.38**	37.67**	57.55**	2.24 <sup>ns</sup>	13.78**		
$A \times B$	42.05**	55.35**	25.58**	23.39**	8.60**	21.17**	2.18 <sup>ns</sup>	0.92 <sup>ns</sup>		
CV₁ (%)	27.62	14.09	26.62	26.35	58.80	40.11	73.64	65.74		
CV <sub>2</sub> (%)	24.99	16.60	24.09	24.77	53.35	34.06	75.77	58.94		

SV - Source of variation; MLF - Method of determination of the leaching fraction;  $EC_w$  - Electrical conductivity of irrigation water; CV - coefficient of variation;  $^{ns}$  and \*\* - Not-significant and significant at  $p \le 0.01$  by the F test, respectively



RHO - Rhoades; SWB - Soil water balance; \*\*Significant at  $p \le 0.01$  by the F test, CV - Coefficient of variation. Lowercase letters compare means of methods of leaching fraction determination within the same EC<sub>w</sub> level, according to Tukey test ( $p \le 0.05$ )

**Figure 2.** Sodium (A), chloride (B), calcium (C), and magnesium (D) accumulated in the leachate of maize grown in soil column, as a function of electrical conductivity of irrigation water (EC, ) and method of leaching fraction determination

between the means of RHO and SWB was around 40% (Figure 2). It is worth pointing out that the purpose of applying the leaching fraction is precisely to remove salts from the root zone of plants. These salts are solubilized in water and transported within the soil profile. Thus, the higher volume of water used in the method proposed by Rhoades (RHO) contributed to the greater leaching of salts in the soil. The results show, however, that leaching does not depend only on the volume of water applied, since the physical-chemical characteristics of the elements also influence a greater or lower displacement through the soil layers.

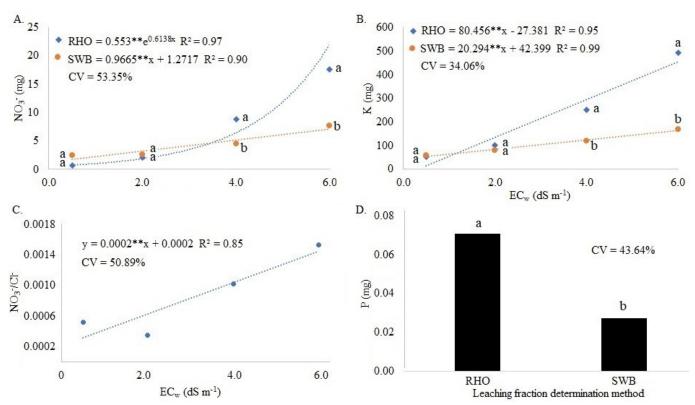
Increasing the volume of water used to leach ions that are toxic to plants (such as  $Na^+$  and  $Cl^-$ ) can also remove important nutrients for plants, such as N and K (Lacerda et al., 2016; 2018). The analysis of nitrate and potassium quantities leached during the experiment (Figures 3A and B) shows that LF also resulted in losses of nutrients.

The differences in NO $_3$  values between the LF determination methods were observed from EC $_{\rm w}$  of 4 dS m $^{-1}$  (Figure 3A). The increase in EC $_{\rm w}$  caused a greater difference in the accumulated

values of  $\mathrm{NO_3}^-$  in the leachate between LF determination methods, resulting in triple the amount of this element in RHO (22 mg) compared to the values found in SWB (7.1 mg), for  $\mathrm{EC_w}$  of 6 dS m<sup>-1</sup>. This result shows greater loss of N by leaching when using the Rhoades method (RHO), which tends to intensify as the electrical conductivity of irrigation water increases.

The nitrogen applied in the form of urea is amidic  $(NH_2)$ . However, this amidic nitrogen can be rapidly reduced to nitrate  $(NO_3^-)$  by the soil microbiota. This transformation increases the solubility of N and, for this reason the leaching of N in the form of  $NO_3^-$  is one of the main means of loss of this element (Ribeiro et al., 2020). These losses may be more pronounced when maize plants are under salt stress.

Several studies have pointed out that the lower utilization of N under these conditions is due to the lower capacity of maize to extract nutrients when subjected to salt stress and to the antagonistic effect caused by chlorine (Lacerda et al., 2016; Iqbal et al., 2020; Zhang et al., 2021). In environments with high concentrations of salts, chloride is the most abundant anion (Azevedo Neto & Tabosa, 2000; Zhang et al., 2019). This case



RHO - Rhoades; SWB - Soil water balance; \*\*Significant at  $p \le 0.01$  by the F test, CV - Coefficient of variation. Lowercase letters compare means of methods of leaching fraction determination within the same EC<sub>w</sub> level, according to Tukey test ( $p \le 0.05$ )

Figure 3. Nitrate (A) and potassium (B) accumulated in the leachate as a function of electrical conductivity of irrigation water  $(EC_w)$  and leaching fraction determination method,  $NO_3$ -/Cl- ratio (C) in the leachate as a function of  $EC_w$ , and phosphorus (D) accumulated in the leachate as a function of leaching fraction determination methods in maize grown in soil column

can be observed in Figure 2B, where the amount of chloride exceeds those of all elements analyzed in the leachate under high salinity.

Figure 3C reveals that the  $NO_3$ -/Cl ratio increases with increasing electrical conductivity of irrigation water. Thus, the increment in EC increased the leaching of N in the form of  $NO_3$ . Shenker et al. (2003) explained about the antagonistic effects between  $NO_3$  and Cl ions and found that they compete for the same absorption sites in plant cells. In addition to the antagonism observed in maize plants, Lacerda et al. (2018) found that excess chloride in the soil reduces the availability of  $NO_3$ .

The accumulated amounts of phosphorus in the leachate were low, with significant statistical difference ( $p \le 0.01$ ) only for LF determination methods (Figure 3D). On average, the RHO method showed 0.07 mg of P lost by leaching, which, despite being low when compared to the leaching of other elements, was still more than twice the amount of P found in the leachate originated using the SWB method (0.03 mg). P has strong adsorption to soil matrix, with consequent low mobility, in addition to the great possibility of precipitation in the form of phosphate. Because of this, P losses by leaching are considered low. This is one of the main reasons why phosphate fertilization is recommended to be applied all at once, as basal dose, in annual crops.

Potassium, in turn, has high solubility in water. This characteristic makes the element more susceptible to leaching, especially when using high leaching fractions in salt stress environments. The responses of this nutrient to each LF

determination method followed the linear regression pattern directly proportional to the increase in  $EC_w$ . As with  $NO_3^-$ , the statistical differences between the methods were only observed from  $EC_w$  of 4 dS  $m^{-1}$  (Figure 3A and B).

Both for the LF calculated by the formula proposed by Rhoades (RHO) and for the SWB method (+ 15% LF), K losses by leaching increased proportionally with the increase in the salinity of irrigation water. When using water with EC =  $0.5 \text{ dS m}^{-1}$  (32.7 mg), the losses were equivalent to only 10.5% of those observed when irrigation was performed with water of  $6.0 \text{ dS m}^{-1}$  (309.76 mg).

Mendes et al. (2016) evaluated K in two soils of different textures as a function of the volume of water applied and verified that, for both soils, the increase in water depth caused increase of K losses by leaching. However, it is important to point out that in the present study N and K, regardless of the method used to calculate the LF, showed higher losses proportional to the increase in the salinity of the water used to irrigate maize, confirming the effect of excess salts in reducing the capacity for water absorption and extraction of nutrients from the soil.

Lacerda et al. (2016) verified that maize plants under salt stress had better efficiency of use of inputs (water and nitrogen and potassium fertilizers) when fertilization was reduced according to the reduction in crop evapotranspiration. The same authors, in a complementary study, showed accumulation of  $NO_3^-$  and K in deeper layers of soil cultivated with maize (Lacerda et al., 2018), thus highlighting the transport (leaching) of these elements beyond the root zone.

The analysis of variance for leaf concentration of elements showed that, except for N, there was significant effect at  $p \le 0.05$  by the F test for the interaction between the LF determination method and electrical conductivity of irrigation water (Table 2). N concentration in leaf was affected by water salinity.

Leaf N concentration was influenced only by the increase in EC $_{\rm w}$  (p  $\leq$  0.05), and the mean N values were below 20 g kg $^{\rm -1}$  DM in all treatments (Figure 4A). It is important to highlight that there is a trend in the reduction of N content in maize plants with maturity (Ferreira et al., 2001). Ferreira et al. (2001) found that the N concentration decreases from 27.3 g kg $^{\rm -1}$  at 45 days to 20.0 g kg $^{\rm -1}$  at 63 days after emergence.

The response curve for leaf N concentration was quadratic, increasing initially as EC $_{\rm w}$  increased, reaching a maximum point (18.45 g of N kg $^{-1}$  of dry matter) at EC $_{\rm w}$  of 4 dS m $^{-1}$ . From 4 dS m $^{-1}$ , leaf N concentration decreased, reflecting salt stress in the plant. It is inferred that the low N concentrations in maize leaves under irrigation with low-salinity water were due to the plant development stage and the greater translocation of the

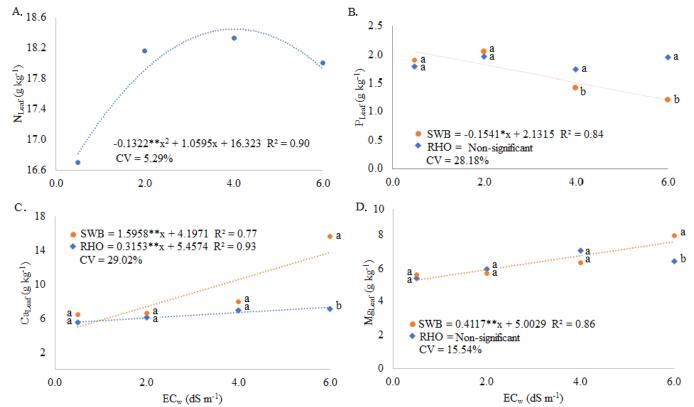
nutrient to the reproductive parts (ears), which are stronger sinks in control plants when compared to plants under salt stress (Ribeiro et al., 2020). However, when the plant was irrigated using water with EC above 4 dS m<sup>-1</sup>, the decrease in leaf N concentration was caused by metabolic stress and reduction in root absorption.

Leaf P concentration decreased inversely with the increase in EC<sub>w</sub> (Figure 4B), but only when using the SWB method (+15% LF). Leaf calcium and magnesium concentrations showed responses with an increasing pattern that was linear and directly proportional to the increasing in EC<sub>w</sub>, according to Figures 4C and D, with greater increments when using the SWB method. Although Ca and Mg are macronutrients, their high leaf concentrations are not related to better development of the plants for this specific case. The main source of these elements for plants was irrigation water, in the equivalent proportions of 20% CaCl<sub>2</sub>.2H<sub>2</sub>O and 10% MgCl<sub>2</sub>.6H<sub>2</sub>O. Despite that, when comparing the lines obtained by the responses of the plants to the LF determination methods, the slope of the

**Table 2.** Summary of analysis of variance with F calculated for the leaf concentrations of nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl), Na/K ratio, and N/Cl ratio

Source	F calculated										
of variation	N	P	Ca	Mg	Na	K	CI	Na/K	N/CI		
MLF (A)	0.68 <sup>ns</sup>	1.83 <sup>ns</sup>	9.41*	0.19 <sup>ns</sup>	101.18**	186.05**	7.65*	0.08 <sup>ns</sup>	1.17 <sup>ns</sup>		
ECw (B)	6.79**	2.07 <sup>ns</sup>	13.65**	7.36**	7.40**	46.40**	21.26**	19.37**	14.71**		
$A \times B$	1.70 <sup>ns</sup>	3.12*	9.32**	3.05*	17.60**	17.10**	13.48**	4.78**	6.89**		
CV <sub>1</sub> (%)	5.71	22.08	34.28	22.43	20.51	12.58	12.24	35.60	14.01		
CV <sub>2</sub> (%)	5.29	28.18	29.02	15.54	29.62	12.46	12.32	34.86	10.90		

SV - Source of variation; MLF - Method of determination of the leaching fraction;  $EC_w$  - Electrical conductivity of irrigation water; CV - Coefficient of variation; as, and - Not-significant and significant at  $p \le 0.01$  and  $p \le 0.05$  by the F test, respectively



RHO - Rhoades; SWB - Soil water balance; \*\* and \* - Significant at  $p \le 0.01$  and p < 0.05 by the F test, respectively, CV - Coefficient of variation. Lowercase letters compare means of methods of leaching fraction determination within the same  $EC_w$  level, according to Tukey test ( $p \le 0.05$ )

**Figure 4.** Leaf concentrations of nitrogen (A), phosphorus (B), calcium (C), and magnesium (D) in maize plants, as a function of electrical conductivity of irrigation water (EC<sub>w</sub>) and leaching fraction determination methods

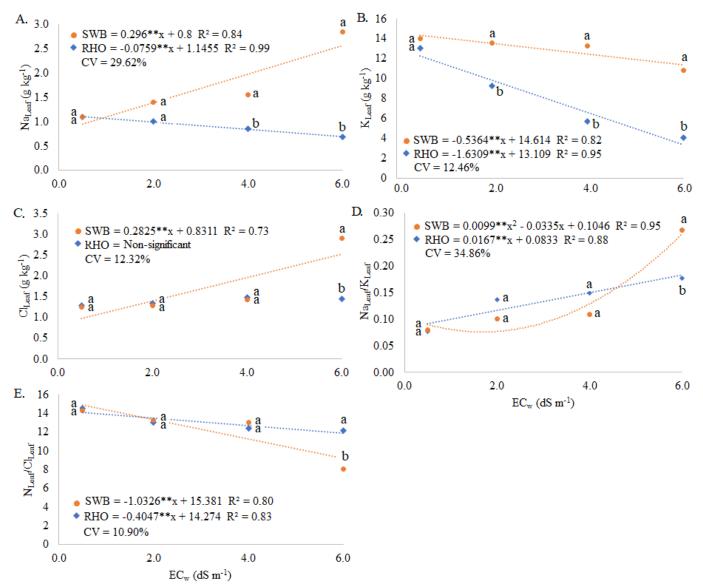
line corresponding to SWB was greater than the slope of that corresponding to RHO. Thus, the increase in Ca concentration in the maize leaf tended to be higher with SWB than with the RHO method for each unit increase in water salinity (Figure 4C).

The differences in leaf Ca concentration (Figure 4C) partly reflect the responses obtained in the leachate (Figure 2C). In the leachate, the highest concentrations of Ca were observed in plots where the RHO method was used, demonstrating that the greater volume of water used in the LF of this treatment (which reached up to 92% of the irrigation depth for EC $_{\rm w}$  = 6 dS m $^{-1}$ ) was able to remove part of this element from the root zone, when compared to the SWB treatment. Similar analysis can be made in the case of sodium, potassium, and chloride in this same treatment (Figure 5).

While an increase in sodium concentration in leaf as a function of  $EC_w$  was observed with SWB, the opposite occurred for RHO. The LF calculated by Rhoades method was able to maintain the mean concentration of Na below 0.94 g kg<sup>-1</sup> of DM. However, in the treatments where LF was determined by

the SWB method (+15% LF), the mean Na concentration was 88% higher (1.75 g kg $^{-1}$  of DM). Although the average sodium contents in the leaves of these treatments are considered low for a saline environment, they can interfere with plant metabolism. Significantly lower concentrations of Na in leaves were observed with RHO method only at high ECw levels (4 and 6 dS m $^{-1}$ ).

For leaf K, there was a linear reduction as a function of the increase in  $EC_w$  for both methods of LF determination. However, leaf K concentration was significantly lower (p  $\leq$  0.01) when the RHO method was used, compared to SWB. Therefore, the higher volume of water used in the LF by the RHO method removed not only ions that are potentially toxic to the plant, such as Na and Cl, but also essential elements such as K. Soil K losses clearly show a significant increase in K in the leachate resulting from the RHO method in comparison to the SWB method (Figure 3B). Pearson's correlation coefficient also showed that the increase of K in the leachate was inversely proportional to the concentration of this element in the maize leaf (r = -0.77). These significant differences in K loss in the



RHO - Rhoades; SWB - Soil water balance; \*\* Significant at  $p \le 0.01$  by the F test, CV - Coefficient of variation. Lowercase letters compare means of methods of leaching fraction determination within the same EC<sub>w</sub> level, according to Tukey test ( $p \le 0.05$ )

**Figure 5.** Leaf contents of sodium (A), potassium (B), chloride (C), Na/K ratio (D) and N/Cl ratio (E) in maize plants, as a function of electrical conductivity of irrigation water (EC<sub>w</sub>) and leaching fraction determination methods

leachate and reduction in leaf K concentration were more evident in the treatments with water salinity of 2.0 dS m<sup>-1</sup> and above (Figures 3B and 5B).

The mean leaf concentration of chloride in SWB (1.73 g kg<sup>-1</sup>) was higher than that observed in RHO (1.38 g kg<sup>-1</sup>), without statistical difference in Cl as a function of EC<sub>w</sub>. Through the SWB, there was linear increase in the element, directly proportional to the increase in EC<sub>w</sub> (Figure 5C). Chloride is an essential ion required in low amounts by plants. However, in a saline environment, its concentration in the shoots may exceed those of some macronutrients (Zhang et al., 2021). At high concentrations, in addition to the toxic effect of the element, it can inhibit nitrate absorption, limiting plant growth and production (Shenker et al., 2003; Zahra et al., 2020).

The response of the Na/K ratio to the RHO and SWB methods showed different patterns, and RHO was better represented by linear regression (R² = 95%) and SWB by quadratic regression (R² = 88%). The interaction between EC $_{\rm w}$  and the LF determination methods was significant at p  $\leq$  0.01, and the distances between the means of RHO and SBW for the Na/K ratio became more pronounced above the estimated EC $_{\rm w}$  of 4.77 dS m $^{-1}$ . From this point on, the SWB led to higher values of Na/K ratio in comparison to RHO (Figure 5D).

Azevedo Neto & Tabosa (2000) in a study with two maize cultivars, one sensitive and the other tolerant to salinity, showed in both cases the increase in the Na/K ratio in leaves as a function of the high salinity of the nutrient solution supplied to the plants. Farooq et al. (2015) stated that, for maize, sodium is the ion that most interferes with the absorption and transport of potassium, causing disturbances in stomatal modulations and causing water loss and necrosis in the plant. The competition between Na and K in a saline environment drastically reduces potassium contents in maize leaves and roots (Azevedo Neto & Tabosa, 2000) and reduces K content by up to 64% in the symplast of expanding tissue under salt stress (Farooq et al., 2015). The high Na/K ratio as well as the high concentration of total salts (such as chlorides) inactivate enzymes and inhibit protein synthesis (Almeida et al., 2017).

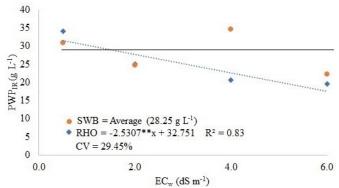
The leaf N/Cl ratio for both LF determination methods varied linearly and inversely proportional to the increase in EC<sub>w</sub> (Figure 5E). However, the reduction in N/Cl ratio tended to be higher in the SWB method (+15% LF), especially due to the greater accumulation of Cl in the leaves (Figure 5C), but significant differences between the two methods of calculation of LF were observed only at the highest level of ECw (6 dS m<sup>-1</sup>). According to Feijão et al. (2013), maize plants suffer from antagonistic effects between NO<sub>3</sub> and Cl<sup>-1</sup> ions on mineral nutrition. According to Dias et al. (2016), the lower nitrate concentration in the plant is due to the NO<sub>3</sub> /Cl<sup>-1</sup> interaction at the absorption sites (competitive inhibition) and/or the depolarization of the plasma membrane caused by sodium (non-competitive inhibition).

In general, the concentrations of chemical elements in maize leaves were higher for leaching fraction estimated by soil water balance (SWB) than for Rhoades method (RHO). The higher volumes of water used by RHO resulted in lower concentrations of Na, Cl, Ca, Mg, and K in the leaf tissue. High salinity also reduces the capacity of plants to extract nutrients

from the soil, notably those required in larger quantities, such as nitrogen and potassium (Shenker et al., 2003; Lacerda et al., 2016). These losses are greater as higher LF is used, and the benefits of reducing salts in the root zone are not necessarily reflected in plant growth and production (Freitas et al., 2018).

The statistical analysis of the physical productivity of irrigation water (PWP $_{\rm IR}$ ) showed significant interaction (p  $\leq$  0.05) between the LF determination methods and EC $_{\rm w}$ . In SWB, it was not possible to find a mathematical representation for the responses obtained, with an average value of 28.25 g L $^{-1}$  biomass. In RHO, there was linear decrease in water use efficiency as the EC $_{\rm w}$  increased (Figure 6), with a reduction of 2.53 g L $^{-1}$  (7.73%) for each unit increase in the salinity of irrigation water. Pearson's correlation coefficients indicate that higher PWP $_{\rm IR}$  is related to the lowest losses of K (-0.58), Ca (-0.55), Mg (-0.55) and NO $_{\rm 3}$  (-0.46) in the leachate and to the highest concentration of K (0.58) in the leaf.

For maize cultivation, the number of plants per hectare can vary between 40,000 and 70,000. If the lowest plant population (40,000) were multiplied by the average amount of water applied to produce one gram of biomass in each treatment, the difference between the methods would lead to water savings of 94 m³ ha⁻¹ in SWB compared to RHO. It should be noted that these values were calculated based on results obtained in a protected environment (greenhouse). Thus, for open field cultivation the values might be divergent.



RHO - Rhoades; SWB - Soil water balance; \*\*Significant at  $p \le 0.01$  by the F test, CV - Coefficient of variation

**Figure 6.** Physical productivity of irrigation water  $(PWP_{IR})$  in maize plants, as a function of electrical conductivity of irrigation water  $(EC_{w})$  and leaching fraction determination methods

### Conclusions

- 1. The increase in electrical conductivity of irrigation water decreased the absorption of nitrogen, phosphorus and potassium by the plant, regardless of the leaching fraction determination method.
- 2. The high volumes of water used in the leaching fraction determined by the Rhoades method contributed to the greater leaching of macronutrients and, consequently, lower concentrations of these in the leaf tissue of the plant, especially for  $EC_w$  equal to or higher than 4 dS m<sup>-1</sup>.
- 3. The leaching fraction of 0.15 according to soil water balance resulted in decreased losses of nutrients and higher water use efficiency, as compared to Rhoades method.

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