

## Different planting densities improve the nutritional composition of cactus pear in semi-arid region

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### ABSTRACT

Crop densification can influence the nutritional quality of cactus pear. However, studies considering harvest timing and cultivation density in high-altitude regions are essential for optimizing management and supporting sustainable livestock farming. Thus, the aim of this study was to evaluate the chemical, mineral composition and mineral accumulation of cactus pear under different planting densities. A randomized block design was used, with four planting densities (30,000; 45,000; 60,000 and 75,000 plants.ha<sup>-1</sup>) and four replications, evaluated at 120, 240 and 360 days after planting (DAP). Field sample collections occurred every 120 days. The samples were processed for further evaluation of their chemical and mineral composition, as well as mineral accumulation in relation to degree days. Increasing planting densities reduced dry matter, crude protein, neutral and acid detergent fiber contents, while increasing hemicellulose, glucose, sucrose, and starch ( $p < 0.05$ ). A quadratic effect was observed for ether extract and pectin contents with increasing planting densities ( $p < 0.05$ ). At 360 DAP, the highest levels of dry matter, organic matter, carbon, ether extract, neutral and acid detergent fiber, hemicellulose, cellulose, lignin, total and non-fibrous carbohydrates, sucrose, starch, and pectin were observed ( $p < 0.05$ ). The highest mineral matter, sulfur, manganese, and zinc contents occurred at 120 and 240 DAP ( $p < 0.05$ ). Crude protein and iron levels peaked at 120 DAP, while glucose, potassium, calcium, magnesium, and sodium were highest at 240 DAP ( $p < 0.05$ ). The highest phosphorus levels were found at 120 and 360 DAP ( $p < 0.05$ ). Mineral accumulation varied according to planting density and accumulated degree days. Phosphorus, calcium, and magnesium showed specific peaks, while potassium and zinc followed rainfall patterns. Sodium increased progressively. Higher planting densities (75,000 plants.ha<sup>-1</sup>) increased carbohydrate reserves without altering mineral composition. The findings contribute to the knowledge of cactus pear agronomic management and reinforce the importance of strategies that promote sustainable production, aiming to ensure greater efficiency in the use of cultivable areas and forage production in semi-arid regions.

### 1. Introduction

Agriculture plays a key role in a country's food and economic sustainability. However, it faces increasing challenges, especially due to climate change, which has contributed to the reduction of fresh water

available for irrigation, which directly affects crop production, aiming to feed animals with forage in quantity and quality (Marengo et al., 2022). Furthermore, with the continued growth of the world's population, the demands for potable water have intensified, putting even more pressure on this available resource (Tzanakakis et al., 2020). Thus, the adoption

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of technologies aimed at increasing food production for livestock with reduced use of potable water and in a short period of time is highly viable in dryland regions.

Dryland regions cover 40 % of the world's land area, creating immense pressure for the efficient use of natural resources in these areas, as there is a need to increase production to provide food and effectively nourish livestock (Dubeux Junior et al., 2015). In this production context, optimizing water and soil use is essential, as soils in semi-arid regions are prone to degradation due to land use and management practices. They are also severely affected by climate change, agricultural activities, and land management strategies (Liu & Dai, 2020).

Among the plant species adapted to semi-arid conditions and possessing crassulacean acid metabolism (CAM), cactus pear exhibits the greatest resilience. This is evidenced by its morphophysiological and anatomical characteristics, which contribute to its adaptation (Cardoso et al., 2019), with yields ranging from 188.9 to 366.6 tons per hectare of natural matter (Pereira et al., 2022). In this context, cactus pear is an excellent strategy for cultivation in semi-arid regions, playing a crucial role in animal feed as a source of roughage due to its mineral content (248.2–266.5 g.kg<sup>-1</sup>; Mayer & Cushman, 2019), energy (30.3 Kcal.100 g<sup>-1</sup>; Santiago et al., 2018), carbohydrates (795.2 g.kg<sup>-1</sup> DM; Cunha et al., 2022), antioxidants (24.04 ± 0.13 µM ET.g<sup>-1</sup>; Nunes et al., 2022) and water (921 g.kg<sup>-1</sup> natural matter; T.S. Silva et al., 2021; T.G.P. Silva et al., 2021; K.B. Silva et al., 2021). Cactus pear has been widely studied as a roughage source for ruminant feeding in semi-arid regions, providing water and nutrients (Oliveira et al., 2021; T.S. Silva et al., 2021; T.G.P. Silva et al., 2021; K.B. Silva et al., 2021; Tegegne et al., 2007) and as a substitute for concentrates such as corn (Alves et al., 2023) and wheat bran (Gebretsadika et al., 2024) in ruminant diets. Its inclusion enhances dry matter intake and digestibility, improves carcass yield and meat quality, reduces animal water consumption, and lowers feed costs.

The mineral composition of cactus pear can vary according to factors such as environmental conditions, phenology, management practices, and cultivar or species (Dubeux Junior et al., 2021). Reis Filho et al. (2022) report that increased biomass in irrigated forage cactus cultivation systems may lead to the dilution of nutritional components, such as minerals, compromising nutritional quality due to water accumulation. According to T.S. Silva et al. (2021), T.G.P. Silva et al. (2021), K.B. Silva et al. (2021), levels of K (24.22–39.18 g.kg<sup>-1</sup> DM), Mg (6.89–7.39 g.kg<sup>-1</sup> DM), S (3.34–4.72 g.kg<sup>-1</sup> DM), Fe (93.4–128.24 mg.kg<sup>-1</sup> DM) and Zn (52.04–135.89 mg.kg<sup>-1</sup> DM) can be found. Despite scarce studies related to mineral composition and the dynamics of mineral accumulation in cactus pear, knowledge of the proportion of these nutrients generates subsidies for the formulation of feeds for ruminants (Magalhães et al., 2019).

The planting of cactus pear at different densities is a simple practice used to increase productivity in semi-arid regions, modify radiation interception and competition for water and nutrients, influencing crop growth, yield, and nutritional composition (Silva et al., 2024). Thus, several studies investigate the effect of crop density (Lemos et al., 2021), the effect of fertilization (Saraiva et al., 2022), and irrigation (Pereira et al., 2021) on the nutritional characteristics of cactus pear. Pereira et al. (2022), when evaluating the productivity and structural responses of the forage cactus 'Orelha de Elefante Mexicana' (OEM) (*Opuntia stricta* (Haw.) Haw.) at different planting densities (20,000, 25,000, 33,333, 50,000, and 100,000 plants.ha<sup>-1</sup>), found that the dry matter content of the cactus pear increased with the use of up to 61,428 plants.ha<sup>-1</sup>, decreasing at higher densities. However, higher densities favored greater forage production.

Considering the current scenario of research applied to the Brazilian semi-arid region, the basic perception is the scarcity of studies focused on the nutritional evaluation of forage cactus at different cultivation densities in relation to the days after planting in high-altitude regions. Therefore, understanding the various responses of the crop to growth and development becomes essential to assist in decision-making and

support farmers in choosing the best planting density based on the days after planting.

The creation of a database is crucial for defining appropriate management techniques and providing relevant support for the stability and sustainable development of regional livestock farming. Our hypothesis is that planting density increases the dry matter and neutral detergent fiber content and alters the mineral composition of the cactus pear after 360 days of planting.

The aim of this study was to evaluate the chemical composition, mineral content and accumulation dynamics in cactus pear at different planting densities.

## 2. Material and methods

### 2.1. Study area

The experiment was conducted at the Brazilian Agricultural Research Corporation– Embrapa Semi-arid in the caatinga experimental field in Petrolina, state of Pernambuco – Brazil (09°04'01.0" S, 40°19'32.1" W, 379 m altitude), with a hot semi-arid climate (Köppen, 1923). The experimental period from October 12, 2020, to October 12, 2021, lasted 360 days. During the experimental period, rainfall, evapotranspiration, temperature, wind speed, relative air humidity, and global radiation were registered daily by a weather station close to the experimental area (Fig. 1).

### 2.2. Soil

The soil in the experimental area was classified as Eutrophic Abrupt Plinthite Yellow Argisol (WRB/FAO, 2014), located on flat relief. Before planting, soil samples were taken in the experimental area at depths of 0–0.10 m; 0.10–0.20 m; 0.20–0.30 m, and 0.30–0.40 m for the characterization of the physical and chemical properties (Table 1). The electrical conductivity was determined using a conductivity meter (AZ, 6505) (AOAC, 2016) while the soil pH was read using a potentiometer (Digimed, dmpH-2) previously calibrated. The levels of phosphorus (P), potassium (K<sup>+</sup>), sodium (Na<sup>+</sup>), calcium (Ca<sup>2+</sup>), and magnesium (Mg<sup>2+</sup>) were quantified according to Holanda Filho et al. (2011). The levels of copper (Cu<sup>2+</sup>), iron (Fe<sup>2+</sup>), manganese (Mn<sup>2+</sup>), and zinc (Zn<sup>2+</sup>) were obtained as proposed by the Claessen methodology (1997). Exchangeable acidity (H + Al), sum of bases (SB), cation exchange capacity (CEC), and base saturation (V) were determined using the methodology of Coldebella et al. (2018).

Total porosity was obtained according to the Claessen methodology (1997) and the particle density according to the sand, silt, and clay fractions was quantified using the Embrapa methodology (1997).

### 2.3. Planting system

The cactus pear (*Opuntia stricta* Haw.) used was clone IPA-200016/ Mexican elephant ear planted in a total area of 2560 m<sup>2</sup>. Cactus pear was planted at a spacing of 2.00 m between rows, and 15.36 m<sup>2</sup> (4.8 × 3.2 m) was considered the useful area. Before planting, cladodes were harvested, and selected according to size and health conditions (medium and large cladodes were chosen). Four crop densities were analyzed 30,000; 45,000; 60,000 and 75,000 plants per hectare. Planting was done in double rows, the experimental units consisted of four double rows, of which two central rows are considered useful and the other two are the borders (Fig. 2).

To achieve the planting densities, double rows were used (utilizing both sides of the furrow), in a "V" style. For the planting density of 30 thousand plants.ha<sup>-1</sup>, three cladodes were distributed per meter, totaling six plants per linear meter, spaced 33 cm apart from one cladode to another. For the density of 45 thousand plants.ha<sup>-1</sup>, five cladodes were distributed on the left side of the furrow (20 cm between cladodes) and four cladodes on the right side of the furrow (25 cm

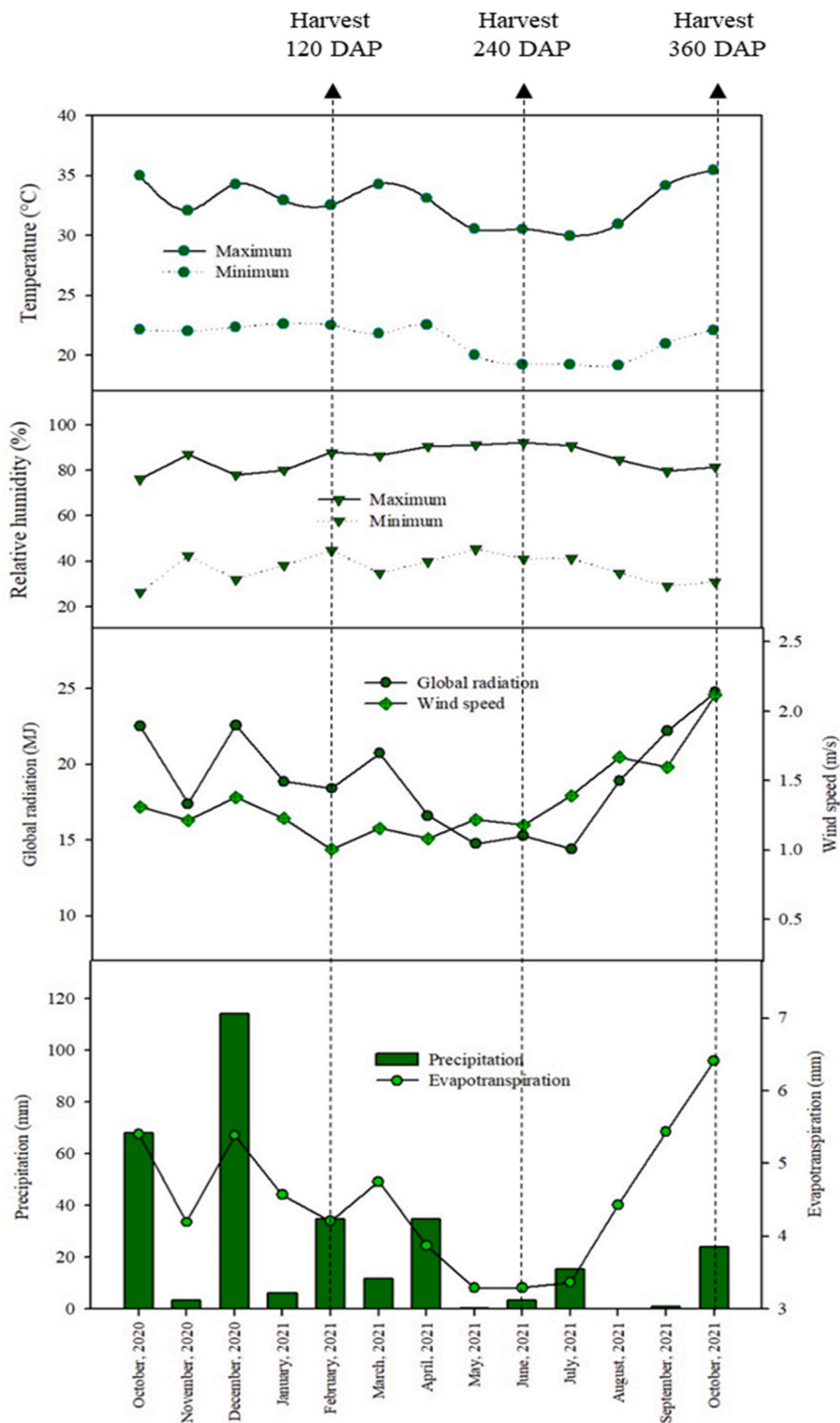


Fig. 1. Variation in environmental conditions during the experiment: air temperature (°C; A), relative humidity (%; B), global radiation (MJ; C) and wind speed (m/s), rainfall (mm; D) and evapotranspiration (mm; D).

between cladodes), totaling nine plants per meter. For the density of 60 thousand plants.ha<sup>-1</sup>, six cladodes were distributed per linear meter (16 cm between cladodes) on both sides of the furrow, totaling 12 plants per meter. For the density of 75 thousand plants.ha<sup>-1</sup>, seven cladodes

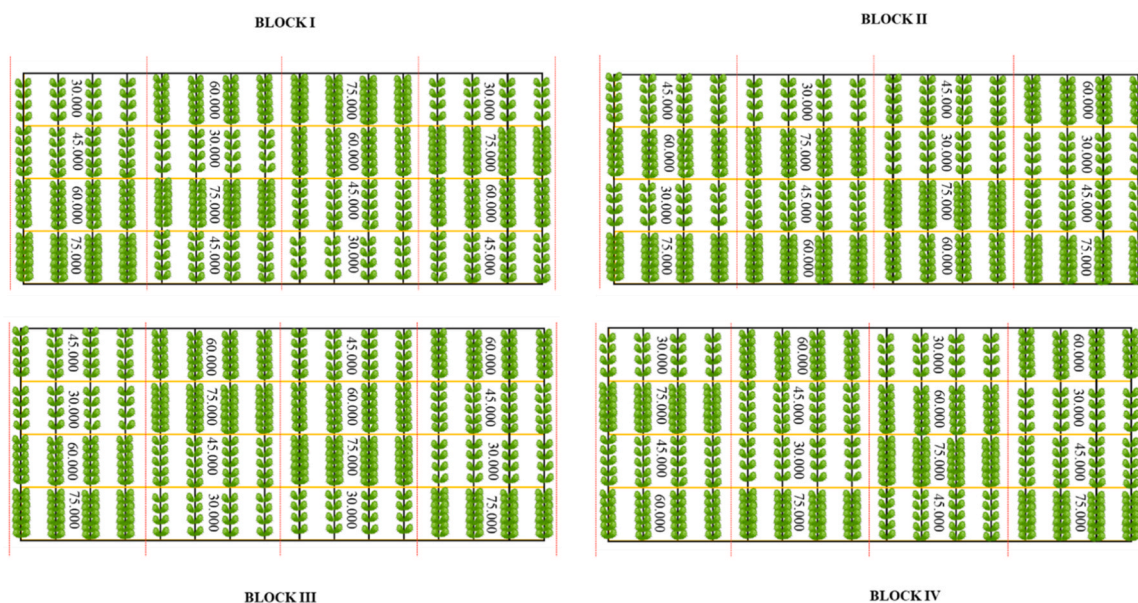
were distributed on the left side of the furrow (14 cm between cladodes) and eight cladodes on the right side of the furrow (12 cm between cladodes), totaling 15 plants per linear meter.

This was a 4 × 3 factorial randomized block experimental design.

**Table 1**  
Chemical and physical characterization of the soil in the experimental area.

| Chemical analysis |                        |           |                    |                     |                  |                  |                  |                                    |                 |                  |                  |      |        |      |      |       |
|-------------------|------------------------|-----------|--------------------|---------------------|------------------|------------------|------------------|------------------------------------|-----------------|------------------|------------------|------|--------|------|------|-------|
| Depth (m)         | EC dS cm <sup>-1</sup> | pH        | P                  | Cu <sup>2+</sup>    | Fe <sup>2+</sup> | Mn <sup>2+</sup> | Zn <sup>2+</sup> | K <sup>+</sup>                     | Na <sup>+</sup> | Ca <sup>2+</sup> | Mg <sup>2+</sup> | Al   | H + Al | SB   | CEC  | V %   |
|                   |                        |           |                    | mg dm <sup>-3</sup> |                  |                  |                  | cmol <sub>c</sub> dm <sup>-3</sup> |                 |                  |                  |      |        |      |      |       |
| 0–0.10            | 3.90                   | 7.10      | 18.00              | 0.77                | 23.56            | 13.98            | 2.33             | 0.16                               | 0.71            | 2.68             | 1.28             | 0.00 | 0.13   | 3.95 | 4.08 | 97.60 |
| 0.10–0.20         | 3.71                   | 7.15      | 18.72              | 0.75                | 23.52            | 23.04            | 2.46             | 0.16                               | 0.71            | 2.73             | 1.33             | 0.00 | 0.23   | 4.05 | 4.28 | 95.13 |
| 0.20–0.30         | 3.38                   | 6.20      | 3.53               | 1.13                | 28.65            | 20.44            | 0.98             | 0.11                               | 0.73            | 1.90             | 0.93             | 0.00 | 1.15   | 2.83 | 3.98 | 74.78 |
| 0.30–0.40         | 3.30                   | 6.23      | 3.82               | 1.17                | 29.42            | 15.99            | 0.99             | 0.12                               | 0.74            | 1.90             | 1.18             | 0.00 | 1.53   | 3.08 | 4.60 | 69.43 |
| Physical analysis |                        |           |                    |                     |                  |                  |                  |                                    |                 |                  |                  |      |        |      |      |       |
| Depth (m)         | Soil density           |           | Total porosity (%) |                     |                  |                  | Particle size    |                                    | Silt            |                  | Clay             |      |        |      |      |       |
|                   | Soil                   | Particles |                    |                     |                  |                  | Sand             | g kg <sup>-1</sup>                 |                 |                  |                  |      |        |      |      |       |
| 0–0.10            | 1.52                   | 2.56      | 40.80              |                     |                  |                  | 575.75           | 353.29                             | 70.96           |                  | 0                |      |        |      |      |       |
| 0.10–0.20         | 1.53                   | 2.54      | 39.74              |                     |                  |                  | 616.52           | 300.15                             | 83.33           |                  |                  |      |        |      |      |       |
| 0.20–0.30         | 1.51                   | 2.54      | 40.52              |                     |                  |                  | 568.04           | 312.51                             | 119.47          |                  |                  |      |        |      |      |       |
| 0.30–0.40         | 1.45                   | 2.55      | 43.08              |                     |                  |                  | 476.01           | 267.34                             | 256.68          |                  |                  |      |        |      |      |       |

EC = electrical conductivity; pH = hydrogenionic potential; P = phosphorus; Cu<sup>2+</sup> = copper; Zn<sup>2+</sup> = zinc; K<sup>+</sup> = potassium; Na<sup>+</sup> = sodium; Ca<sup>2+</sup> = calcium; Mg<sup>2+</sup> = magnesium; Al = aluminum; H + Al = Potential acidity; SB = Sum of Bases; CEC = Cation Exchange Capacity; V = Base Saturation.



**Fig. 2.** Graphical scheme of the experimental blocks and planting densities of cactus pear.

The treatments consisted of four planting densities (DEN; 30; 45; 60 and 75 thousand plants.ha<sup>-1</sup>) and three harvest periods (120; 240 and 360 days after planting – DAP). During the cultivation period, spontaneous plants were controlled by pesticides with the use of 1.5 % isoxaflutole. Plants that showed diseases such as soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*) were removed from the area and incinerated. To control cochineal (*Dactylopius coccus*), 1 % mineral oil was used.

## 2.4. Chemical composition

### 2.4.1. Sample preparation

Collections for chemical analysis were carried out every 120 days, after planting, by randomly sampling two plants between the rows in each plot. Samples were transported to the Animal Nutrition Laboratory at Embrapa Semi-arid, weighed for green matter determination, chopped with a knife, packed in duly identified aluminum containers (capacity of 1.5 kg), and then dried in a forced-air oven at 60 °C for 96 ± 12 h. After pre-drying, the air-dried samples were weighed, and processed in a knife mill (Marconi, MA 350/70/NYL, Piracicaba, SP,

Brazil) with a 1.0 mm sieve for chemical composition analysis. Approximately 100 g of processed samples were set aside for chemical analyses, anticipating potential repetitions. All analyses were performed in triplicate, except for neutral and acid detergent fibers, which were performed in quintuplicate.

### 2.4.2. Determination of dry matter, mineral matter, organic matter, crude protein, and ether extract

For chemical composition analysis, we used the methods described by the Association of Official Analytical Chemists (AOAC, 2016) were used for dry matter (DM) (method 967.03), mineral matter (MM) (method 942.05), organic matter (OM), and crude protein (CP) (method 988.05), while the method described by the American Oil Chemists Society (AOCS, 2017) were used for ether extract (EE).

Dry matter was quantified by weighing 2.0 g of the sample and drying it in a forced-air oven (105 °C ± 16 h). Mineral matter was determined sequentially after DM quantification, using the same samples, which were incinerated in a muffle furnace (550 °C ± 5 h) to determine ash content. The MM content was used to estimate organic

matter (OM = 100 – MM). Crude protein content was determined by quantifying the total nitrogen in the sample using the Kjeldahl method. For this, 2.0 g of the sample were digested in a digestion block (350 °C) with sulfuric acid (0.2 N), distilled with sodium hydroxide (0.2 N), and then titrated with hydrochloric acid (0.1 N). Crude protein (CP) was calculated by multiplying total nitrogen by 6.25 (assuming protein contains, on average, 16 % nitrogen). Ether extract was determined using a fat extractor (ANKOM TX-10, Macedon, NY, United States), where 1.0 g of the sample was extracted with petroleum ether.

#### 2.4.3. Determination of neutral and acid detergent fiber, lignin, cellulose, and hemicellulose

For fiber extraction, neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were carried out according to the methodology of Van Soest et al. (1991), with modifications proposed by Senger et al. (2008), using an autoclave at 110 °C for 40 minutes. One gram of the sample was placed in bags (5 cm × 5 cm, 100 g/m<sup>2</sup>) made of non-woven fabric (TNT). The bags were inserted into glass jars (12 samples per jar) and treated with a neutral or acid detergent solution, using 80 mL of detergent solution per sample. Stable thermophilic alpha-amylase (Termamyl – 2 ×) was added to glass jars in the proportion of 0.25–0.50 mL.g<sup>-1</sup> of sample. The jars were placed in a vertical autoclave (Primatec, CS-50, Botucatu-SP, Brazil), with a capacity for six jars per analysis batch.

After autoclaving, the jars were removed, and the bags were washed with boiling distilled water and soaked in acetone for 5 minutes. Subsequently, the bags were dried (105 °C ± 2 h) and weighed.

For the determination of acid detergent lignin (ADL), the ADF residues were washed with 72 % sulfuric acid, aiming at solubilizing the cellulose and obtaining the ADL (Van Soest et al. 1991), and the fractions of hemicellulose (HEM) and cellulose (CEL) were estimated by the equations: HEM = NDF – ADF and CEL = ADF – ADL, respectively.

#### 2.4.4. Determination of total carbohydrates, non-fibrous carbohydrates, and pectin

Total carbohydrates (CHO) were obtained using the equation proposed by Sniffen et al. (1992) in which, CHO = 100 – (CP + EE + MM), and subsequently estimated the non-fibrous carbohydrates (NFC) contents, corresponding to the difference between TC and NDF.

Pectin was extracted according to Zanella and Taranto (2015), by weighing 0.5 g air-dried samples and centrifuging them with acidified water (pH 2.0–2.5) with 1 M citric acid. After centrifugation, pectin was condensed with absolute ethyl alcohol and after filtering through filter paper, the pectin content was quantified.

#### 2.4.5. Determination of glucose, sucrose, and starch

Glucose (GLU), sucrose (SUC), and starch (STA) contents were obtained by the Lane-Eynon method as described by the Adolf Lutz Institute food analysis methods (IAL, 2008). Glucose determination was performed by reducing a known volume of alkaline copper reagent (Fehling) to cuprous oxide. Sucrose was determined by acid hydrolysis, resulting in two molecules of reducing sugars, one of glucose and one of fructose. Starch is quantified by energetic hydrolysis in a strongly acid medium (IAL, 2008).

### 2.5. Mineral composition

Mineral contents were determined according to Nogueira and Souza (2005), with the phosphorus (P) content quantified by molecular absorption spectrophotometry (Biometer, UV-2802, China), potassium (K), and sodium (Na) quantified by flame photometry, calcium (Ca) was evaluated by titration. Later, the Ca + Mg contents were quantified and through the subtraction between the Ca and Ca + Mg contents, the Mg content was obtained. Sulfur (S) was indirectly quantified through sulfate concentrations taking into account the molecular weight. The contents of B, Cu, Fe, Mn, and Zn were determined in an atomic

absorption spectrophotometer (Skycray Instrument, model AAS9000, China).

### 2.6. Mineral accumulation

Mineral accumulation was evaluated by regression analysis, between the mineral contents with the independent variable accumulated degree-days (ADD). After selecting the regression model, based on the coefficient of determination (R<sup>2</sup> greater than 0.85), the model that presented the best fit was derived for the calculation of accumulations, demonstrating the dynamics of the accumulation of minerals, later a descriptive evaluation was carried out.

### 2.7. Statistical analysis

The results were evaluated for normality (Shapiro-Wilk at 5 % probability) using the UNIVARIATE procedure (PROC UNIVARIATE) of the Software Statistical Analysis System University (SAS, 2015). After the analysis of normality, analysis of variance and regression at 5 % probability for type I error were run using the PROC REG procedure of SAS. As a criterion for selecting the regression models, the significance of the parameters estimated by the models and the values of the coefficient of determination were adopted. The following statistical model was used:  $Y = \mu + Bi + Tj + Dl + Tj * Dl + eijl$ , where: Y = observed value of the variable;  $\mu$  = overall mean; Bi = block effect; Tj = effect of different planting densities; Dl = effect of days after planting; Tj \* Dl = interaction between densities and days after planting; eijl = random error associated with each repetition.

To simplify data interpretation, the multivariate statistical procedure, principal component analysis (PCA), was adopted. Principal component analysis was applied using the R software (R Core Team, 2018), for which the treatments were plotted against the first two components (PC1 and PC2). Figures were constructed in SigmaPlot software version 2014.

## 3. Results

### 3.1. Chemical composition of cactus pear

There was no effect of planting densities on MM, OM, C, CEL, ADL, CHO, and NFC contents ( $p > 0.05$ ; Table 2). There was a decreasing of planting densities on DM content ( $p < 0.001$ ) in the cactus pear, with a reduction of 0.26 g per thousand plants.ha<sup>-1</sup> (Fig. 3A). There was a quadratic effect of planting densities on EE content ( $p < 0.001$ ; Table 2), with a minimum point of 14.58 g.kg<sup>-1</sup> DM for a population of 53,950 plants.ha<sup>-1</sup> (Fig. 3B). Planting densities reduced CP content ( $p < 0.001$ ; Table 2) with a reduction of 0.44 g CP per additional thousand plants.ha<sup>-1</sup> (Fig. 3C).

There was an effect of planting densities on the NDF content ( $p < 0.001$ ; Table 2), which was reduced with increasing planting densities, with a reduction of 4.19 g NDF per additional thousand plants.ha<sup>-1</sup> (Fig. 4A). The planting densities also altered the ADF content ( $p < 0.001$ ; Table 2), decreasing with increasing density (Fig. 4B). There was an increasing linear effect as a function of planting densities on HEM content ( $p < 0.001$ ; Table 2), with a 103 % increase for the density of 75 thousand plants.ha<sup>-1</sup> compared to the density of 30 thousand plants.ha<sup>-1</sup> (Fig. 4C).

Planting densities caused an increase in cactus pear glucose ( $p < 0.001$  Table 2). Contents increased by 92.10 g in plants grown at a density of 75 thousand plants.ha<sup>-1</sup> compared to cactus pear grown at a density of 30 thousand plants.ha<sup>-1</sup> (Fig. 5A). There was an increasing linear effect for the sucrose content ( $p < 0.001$ ; Table 2) with an increase of 2.81 g for every thousand plants.ha<sup>-1</sup> (Fig. 5B). The starch content also increased with the increasing planting densities ( $p < 0.001$ ; Table 2) showing an increase of 1.35 g per additional thousand plants.ha<sup>-1</sup> (Fig. 5C). There was a quadratic effect of planting densities

**Table 2**  
Chemical composition of cactus pear as a function of planting densities and days after planting.

| Variables<br>(g.kg DM) | Densities<br>(thousand plants ha <sup>-1</sup> ) |        |        |        | SEM   | Days after planting<br>(DAP) |                     |                     | SEM   | P-value |         |           |
|------------------------|--|--------|--------|--------|-------|------------------------------|---------------------|---------------------|-------|---------|---------|-----------|
|                        | 30   | 45     | 60     | 75     |       | 120                          | 240                 | 360                 |       | Den     | DAP     | Den * DAP |
| DM*                    | 214.21   | 118.46 | 114.49 | 110.32 | 3.89  | 105.65 <sup>b</sup>          | 84.42 <sup>c</sup>  | 159.35 <sup>a</sup> | 3.37  | 0.025   | < 0.001 | 0.942     |
| MM                     | 191.93   | 199.17 | 207.49 | 211.63 | 15.18 | 236.64 <sup>a</sup>          | 210.92 <sup>a</sup> | 160.10 <sup>b</sup> | 13.15 | 0.801   | 0.003   | 0.064     |
| OM                     | 808.60   | 800.82 | 792.50 | 788.38 | 15.18 | 763.35 <sup>b</sup>          | 789.07 <sup>b</sup> | 839.89 <sup>a</sup> | 13.15 | 0.801   | 0.003   | 0.064     |
| C                      | 468.71   | 464.51 | 459.69 | 457.30 | 8.81  | 442.78 <sup>b</sup>          | 457.70 <sup>b</sup> | 487.18 <sup>a</sup> | 7.63  | 0.801   | < 0.001 | 0.064     |
| EE                     | 17.52  | 14.87  | 14.87  | 16.78  | 0.63  | 13.92 <sup>b</sup>           | 14.39 <sup>b</sup>  | 19.73 <sup>a</sup>  | 0.54  | 0.001   | < 0.001 | 0.005     |
| CP                     | 95.11  | 91.85  | 77.94  | 77.41  | 0.67  | 97.79 <sup>a</sup>           | 101.73 <sup>b</sup> | 57.21 <sup>c</sup>  | 0.56  | < 0.001 | < 0.001 | < 0.001   |
| NDF                    | 313.64   | 317.25 | 320.97 | 283.76 | 8.55  | 289.53 <sup>b</sup>          | 299.60 <sup>b</sup> | 337.58 <sup>a</sup> | 7.41  | 0.010   | < 0.001 | < 0.001   |
| ADF                    | 253.01   | 237.73 | 209.06 | 160.29 | 8.54  | 146.57 <sup>c</sup>          | 220.10 <sup>b</sup> | 278.40 <sup>a</sup> | 7.40  | < 0.001 | < 0.001 | < 0.001   |
| HEM                    | 60.63  | 79.52  | 111.90 | 123.47 | 11.46 | 59.18 <sup>b</sup>           | 79.50 <sup>b</sup>  | 142.95 <sup>a</sup> | 9.93  | < 0.001 | < 0.001 | < 0.001   |
| CEL                    | 250.72   | 235.60 | 206.92 | 158.25 | 8.51  | 114.85 <sup>c</sup>          | 218.38 <sup>b</sup> | 275.39 <sup>a</sup> | 7.37  | < 0.001 | < 0.001 | < 0.001   |
| ADL                    | 2.28   | 2.13   | 2.14   | 2.03   | 0.10  | 1.47 <sup>b</sup>            | 1.48 <sup>b</sup>   | 1.86 <sup>a</sup>   | 0.09  | 0.419   | < 0.001 | < 0.001   |
| CHO                    | 695.42   | 694.09 | 699.68 | 694.18 | 15.10 | 651.64 <sup>b</sup>          | 672.94 <sup>b</sup> | 762.94 <sup>a</sup> | 13.08 | 0.993   | < 0.001 | 0.177     |
| NFC                    | 381.78   | 376.83 | 378.70 | 410.41 | 14.30 | 362.11 <sup>b</sup>          | 373.33 <sup>b</sup> | 425.35 <sup>a</sup> | 12.38 | 0.308   | < 0.001 | < 0.001   |
| GLU                    | 344.38   | 348.87 | 409.49 | 436.48 | 10.87 | 248.76 <sup>c</sup>          | 511.67 <sup>a</sup> | 393.98 <sup>b</sup> | 9.42  | < 0.001 | < 0.001 | < 0.001   |
| SUC                    | 210.05   | 239.81 | 282.06 | 336.93 | 8.07  | 219.82 <sup>c</sup>          | 271.90 <sup>b</sup> | 309.92 <sup>a</sup> | 6.99  | < 0.001 | < 0.001 | 0.334     |
| STA                    | 178.59   | 210.46 | 257.76 | 329.85 | 8.22  | 49.15 <sup>c</sup>           | 215.69 <sup>b</sup> | 467.66 <sup>a</sup> | 7.17  | < 0.001 | < 0.001 | < 0.001   |
| PEC                    | 116.95   | 113.48 | 135.06 | 148.28 | 1.24  | 108.04 <sup>c</sup>          | 127.75 <sup>b</sup> | 149.53 <sup>a</sup> | 1.29  | < 0.001 | < 0.001 | 0.001     |

Means followed by different letters on the same line differ statistically from each other by Tukey's test at 5 % probability. SEM = standard error of the mean; P-value = probability value; DEN = planting densities; DAP = days after planting; DEN \* DAP = interaction between planting densities and days after planting; DM = dry matter; MM = mineral matter; C = carbon; OM = organic matter; EE = ether extract; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; HEM = hemicellulose; CEL = cellulose; ADL = acid detergent lignin; CHO = total carbohydrates; NFC = non-fibrous carbohydrates; GLU = glucose; SUC = sucrose; STA = starch; PEC = pectin.

\* g.kg natural matter.

( $p < 0.001$ ) on the pectin (PEC) of cactus pear (Table 2) showing a minimum point of 143.48 g.kg<sup>-1</sup> PEC for a population of 68.59 thousand plants.ha<sup>-1</sup> (Fig. 5D).

There was an effect of DAP on DM content ( $p < 0.001$ ; Table 2), with an increase in DM content at 360 DAP compared to 120 and 240 DAP (Table 2). DAP reduced MM ( $p = 0.003$ ) and CP ( $p < 0.001$ ) content at 360 DAP (Table 2). There was an increase in MO ( $p = 0.003$ ), C ( $p = 0.003$ ), and EE ( $p < 0.001$ ) at 360 DAP corresponding to an increase of 10.02; 10.00, and 41.73 % respectively for OM, C, and EE compared to 120 DAP (Table 2).

An increase in NDF ( $p < 0.001$ ), ADF ( $p < 0.001$ ), HEM ( $p < 0.001$ ), CEL ( $p < 0.001$ ), and ADL ( $p < 0.001$ ) was found (Table 2) at 360 DAP. Total carbohydrates ( $p < 0.001$ ) and NFC ( $p < 0.001$ ) contents were influenced by DAP with an increase at 360 DAP compared to 120 and 240 DAP (Table 2). The glucose content of cactus pear was altered as a function of DAP with a higher value at 240 DAP ( $p < 0.001$ ; Table 2). Sucrose contents increased at 360 DAP with a 40.98 % increase compared to 120 DAP ( $p < 0.001$ ; Table 2). The starch content was influenced ( $p < 0.001$ ) with content 9.5 times higher at 360 DAP compared to the starch content in the plant at 120 DAP ( $p < 0.001$ ; Table 2). The PEC content was influenced ( $p < 0.001$ ) by DAP, with higher values at 360 DAP with 149.53 g.kg<sup>-1</sup> PEC (Table 2).

No interaction ( $p > 0.05$ ) was detected between planting densities and DAP on DM, MM, OM, C, EE, TC, and SUC (Table 2). There was an effect of the DEN × DAP interaction ( $p < 0.001$ ) on CP, NDF, ADF, HEM, CEL, ADL, NFC, GLU, STA, and PEC contents (Table 2).

### 3.2. Mineralogical characteristics of cactus pear

There was no effect of the DEN × DAP interaction on minerals found in cactus pear ( $p > 0.05$ ; Table 3). There was also no effect of planting densities on the minerals found in cactus pear, with mean values P = 0.99 g.kg<sup>-1</sup>; K = 3.64 g.kg<sup>-1</sup>; Ca = 37.29 g.kg<sup>-1</sup>; Mg = 10.45 g.kg<sup>-1</sup>; S = 1.56 g.kg<sup>-1</sup>; B = 44.14 mg.kg<sup>-1</sup>; Cu = 13.12 mg.kg<sup>-1</sup>; Fe = 102.09 mg.kg<sup>-1</sup>; Mn = 115.59 mg.kg<sup>-1</sup>; Zn = 50.69 mg.kg<sup>-1</sup>; and Na = 677.75 mg.kg<sup>-1</sup> ( $p > 0.05$ ; Table 3).

There was an isolated effect of DAP on P content ( $p < 0.001$ ; Table 3), with higher values at 120 and 360 DAP. DAP altered the concentration of K, Ca, Mg, and Na, showing higher values at 240 DAP,

and lower at 120 DAP ( $p < 0.001$ ; Table 3). The content of S, Mn, and Zn showed lower values at 360 DAP ( $p < 0.001$ ; Table 3). The Fe content was reduced as DAP advanced ( $p < 0.001$ ; Table 3). There was no effect of DAP on B and Cu contents with mean values of 44.14 and 13.12 mg.kg<sup>-1</sup>, respectively ( $p > 0.05$ ; Table 3).

Phosphorus accumulation was higher at the densities of 30 and 45 thousand plants.ha<sup>-1</sup> along the ADD (Fig. 6A). Regarding the accumulation of K, the model demonstrated that the accumulation pattern follows the behavior of the rainfall distribution (Fig. 6B). Calcium presented a curve in its accumulation, with a maximum point for all densities close to 1500 ADD (Fig. 6C). Magnesium showed a sigmoid curve for accumulation at the density of 30 thousand plants.ha<sup>-1</sup>, while the density of 60 thousand plants.ha<sup>-1</sup> showed an increasing accumulation (Fig. 6D).

At 1800 ADD, a reduction in S was verified in all studied densities (Fig. 7A). The accumulation of B showed no specific pattern of accumulation, with a tendency to increase accumulation with the passage of ADD at the densities of 60 thousand plants.ha<sup>-1</sup>, while the B at the density of 75 thousand plants.ha<sup>-1</sup> showed a reduction (Fig. 7B). The accumulation of Cu presented variations as a function of planting densities, while the densities of 45; 60, and 75 thousand plants.ha<sup>-1</sup> showed an increase in Cu accumulation close to 1300 ADD. The density of 30 thousand plants.ha<sup>-1</sup> showed a reduction in Cu accumulation from 1000 ADD (Fig. 7C). Iron showed higher accumulations up to 500 ADD (Fig. 7D).

The accumulation of Mn followed a stable pattern up to 1000 ADD; from 1500 ADD, it increased in the density of 60 thousand plants.ha<sup>-1</sup> but decreased in the other densities (Fig. 8A). Zinc Accumulation followed the rainfall distribution pattern, showing a greater accumulation at 1000 ADD (Fig. 8B). Sodium presented an increasing behavior, with the increase of ADD, however, the density of 30 thousand plants.ha<sup>-1</sup> stabilized at 1800 ADD (Fig. 8C).

### 3.3. Analysis of principal components

Principal component analysis for DM, MM, NDF, ADF, EE, CP, ADL, TC, GLU, SUC, STA, and PEC explained 72.30 % in the first two principal components (PC) (Fig. 9A). Dry matter (11.66 %), CP (13.15 %), TC (10.91 %), and STA (14.01 %) contents present greater contributions to

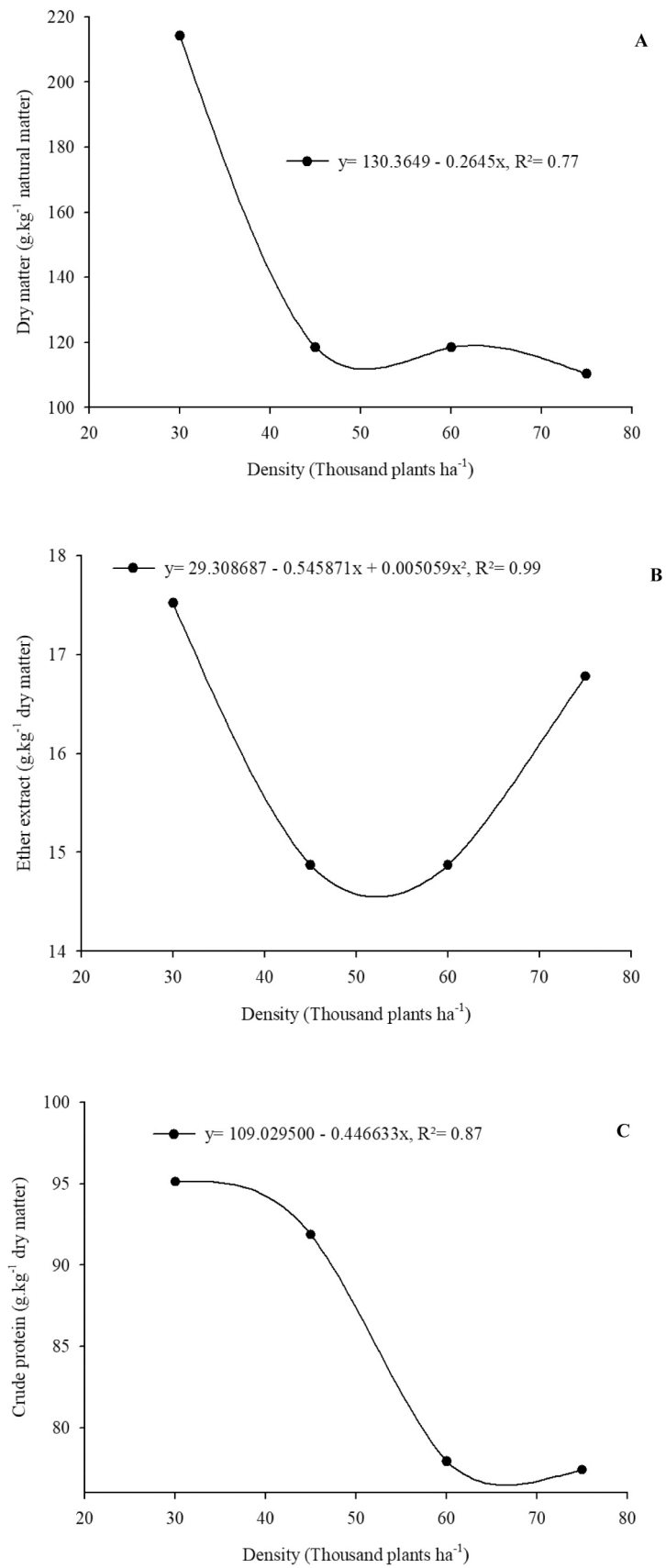


Fig. 3. Dry matter (A), ether extract (B) and crude protein (C) content of cactus pear under different planting densities.

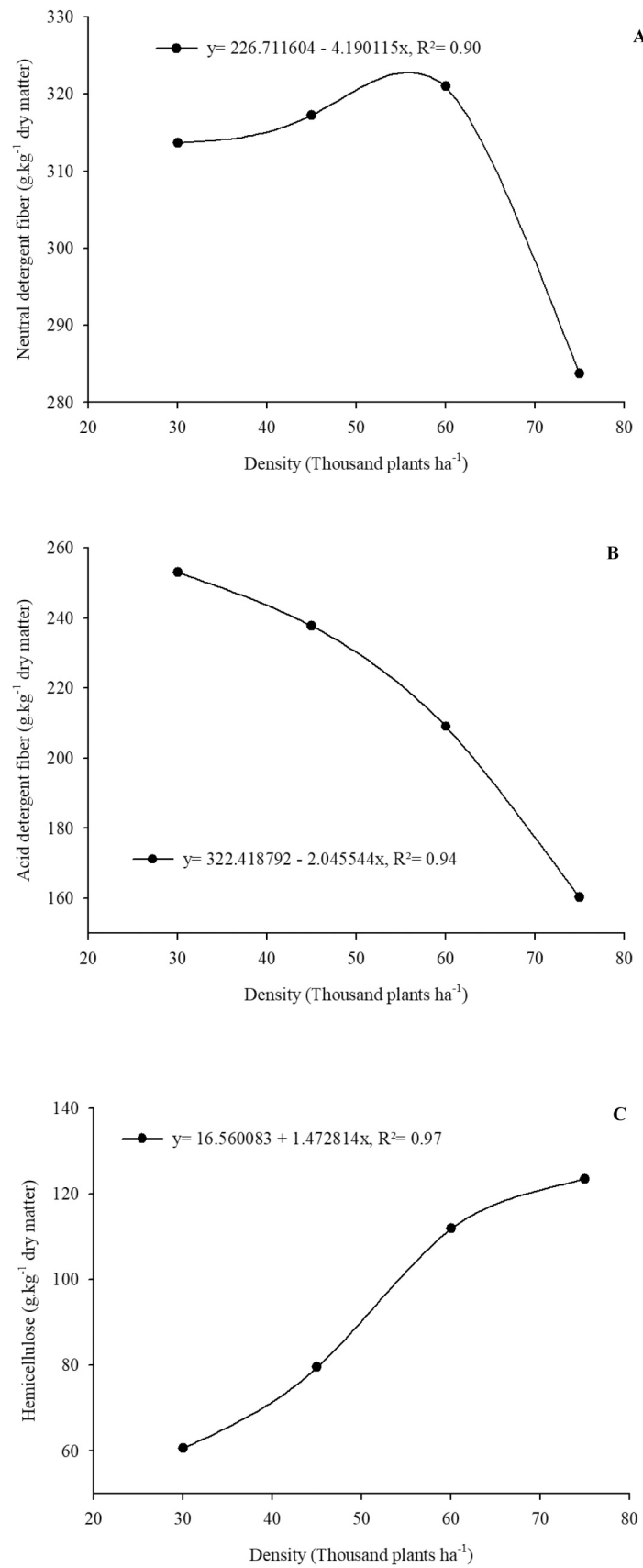


Fig. 4. Neutral detergent fiber (A), acid detergent fiber (B) and hemicellulose (C) content of cactus pear under different planting densities.

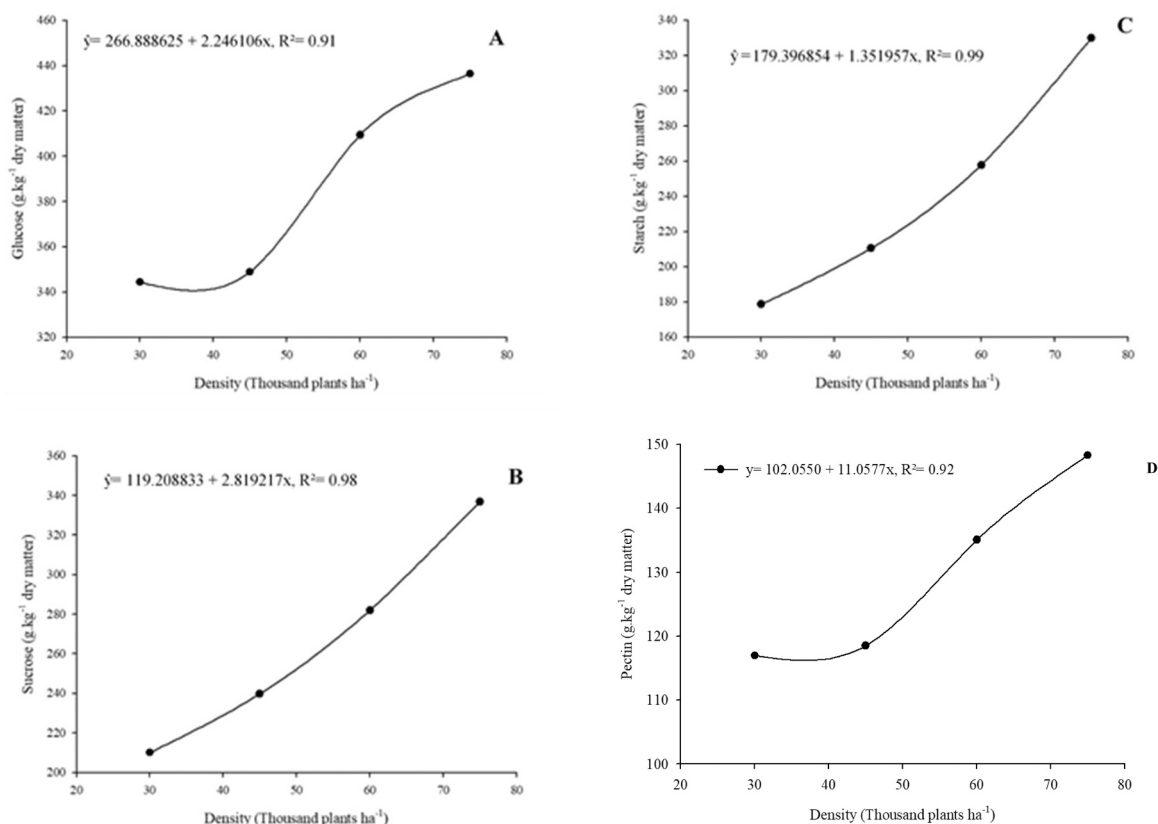


Fig. 5. Glucose (A), sucrose (B), starch (C) and pectin (D) content of cactus pear under different planting densities.

Table 3

Mineralogical characteristics of cactus pear as a function of planting densities and days after planting.

| Variables<br>(g.kg <sup>-1</sup> ) | Densities<br>(thousand plants ha <sup>-1</sup> ) |        |        |        | SEM   | Days after planting<br>(DAP) |                     |                     | SEM   | P-value |         |           |
|------------------------------------|--|--------|--------|--------|-------|------------------------------|---------------------|---------------------|-------|---------|---------|-----------|
|                                    | 30   | 45     | 60     | 75     |       | 120                          | 240                 | 360                 |       | DEN     | DAP     | DEN * DAP |
| P (g.kg <sup>-1</sup> )            | 0.99   | 1.03   | 0.98   | 0.97   | 0.02  | 1.05 <sup>a</sup>            | 0.93 <sup>b</sup>   | 1.00 <sup>a</sup>   | 0.01  | 0.168   | < 0.001 | 0.226     |
| K (g.kg <sup>-1</sup> )            | 3.63   | 3.71   | 3.56   | 3.66   | 0.06  | 3.21 <sup>c</sup>            | 4.04 <sup>a</sup>   | 3.67 <sup>b</sup>   | 0.05  | 0.420   | < 0.001 | 0.523     |
| Ca (g.kg <sup>-1</sup> )           | 37.73  | 36.19  | 38.53  | 36.73  | 1.57  | 25.10 <sup>c</sup>           | 46.92 <sup>a</sup>  | 36.36 <sup>b</sup>  | 1.36  | 0.533   | < 0.001 | 0.851     |
| Mg (g.kg <sup>-1</sup> )           | 9.79   | 10.66  | 10.79  | 10.58  | 0.29  | 7.09 <sup>c</sup>            | 13.12 <sup>a</sup>  | 11.15 <sup>b</sup>  | 0.25  | 0.080   | < 0.001 | 0.798     |
| S (g.kg <sup>-1</sup> )            | 1.46   | 1.65   | 1.56   | 1.57   | 0.07  | 11.70 <sup>a</sup>           | 1.82 <sup>a</sup>   | 1.16 <sup>b</sup>   | 0.06  | 0.413   | < 0.001 | 0.368     |
| B (mg.kg <sup>-1</sup> )           | 47.10  | 42.09  | 44.67  | 42.71  | 2.16  | 45.56                        | 43.50               | 43.36               | 1.98  | 0.358   | 0.650   | 0.086     |
| Cu (mg.kg <sup>-1</sup> )          | 13.48  | 12.47  | 12.21  | 14.32  | 1.14  | 12.72                        | 13.06               | 13.57               | 0.99  | 0.544   | 0.830   | 0.462     |
| Fe (mg.kg <sup>-1</sup> )          | 96.17  | 114.79 | 95.64  | 101.77 | 8.22  | 124.30 <sup>a</sup>          | 97.72 <sup>b</sup>  | 84.26 <sup>b</sup>  | 7.12  | 0.321   | < 0.001 | 0.241     |
| Mn (mg.kg <sup>-1</sup> )          | 105.27   | 114.52 | 121.67 | 120.91 | 7.61  | 125.01 <sup>a</sup>          | 124.44 <sup>a</sup> | 97.33 <sup>b</sup>  | 6.59  | 0.397   | 0.003   | 0.475     |
| Zn (mg.kg <sup>-1</sup> )          | 50.62  | 50.34  | 49.94  | 51.87  | 2.39  | 52.69 <sup>a</sup>           | 57.63 <sup>a</sup>  | 41.76 <sup>b</sup>  | 1.37  | 0.948   | < 0.001 | 0.164     |
| Na (mg.kg <sup>-1</sup> )          | 676.25   | 681.85 | 688.56 | 664.37 | 16.97 | 578.57 <sup>c</sup>          | 814.54 <sup>a</sup> | 640.15 <sup>b</sup> | 14.69 | 0.778   | < 0.001 | 0.427     |

Means followed by different letters on the same line do not differ from each other by Tukey's test at 5 % probability. SEM = standard error of the mean; P-value = probability value; DEN = planting densities; DAP = days after planting; DEN \* DAP = interaction between planting densities and days after planting. P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc; Na = sodium.

PC1 (Fig. 9B). Regarding PC2, GLU, SUC, and PEC were the variables that contributed most to the PC, with 23.56; 15.38, and 19.11 % respectively (Fig. 9C). In the distribution of the variables in the multivariable plan, SAC and PEC presented similar contributions for the first two components (Fig. 9D), so that carbohydrates (GLU, SUC, PEC, and AMI) were more representative and expressive at the density of 75 thousand plants.ha<sup>-1</sup> (Fig. 9E).

The distribution of the variables in the multivariate plane demonstrates that the vectors corresponding to GLU, SUC, STAR, and PEC are aligned, representing a strong positive correlation due to their angles being less than 45°. However, the opposing vectors represented by ADF and NDF in comparison to the vectors of GLU, SUC, STAR, and PEC confirm that they are inversely proportional, suggesting a negative

correlation (Fig. 9D). Meanwhile, the vectors corresponding to CP and MM have an angle smaller than 90° and are oriented perpendicularly, demonstrating that these variables are not related (Fig. 9E).

Principal component analysis for the micronutrients (B, Cu, Fe, Mn, Zn, and Na) explained 43.21 % data variance in the first two principal components (PC). Zinc (45.00 %), Fe (23.04 %), and Na (22.00 %) were the micronutrients with the largest contributions to PC1 (Fig. 10B). With regard to PC2, B, and Fe had the largest contributions, with 51.99 % and 27.30 %, respectively (Fig. 10C). In the distribution of the variables in the multivariable plan, Fe had a significant contribution to the first two components, with similar distribution between the planting densities (Figs. 10D and 10E).

The vectors related to Cu and Mn are smaller and closer to the PCA

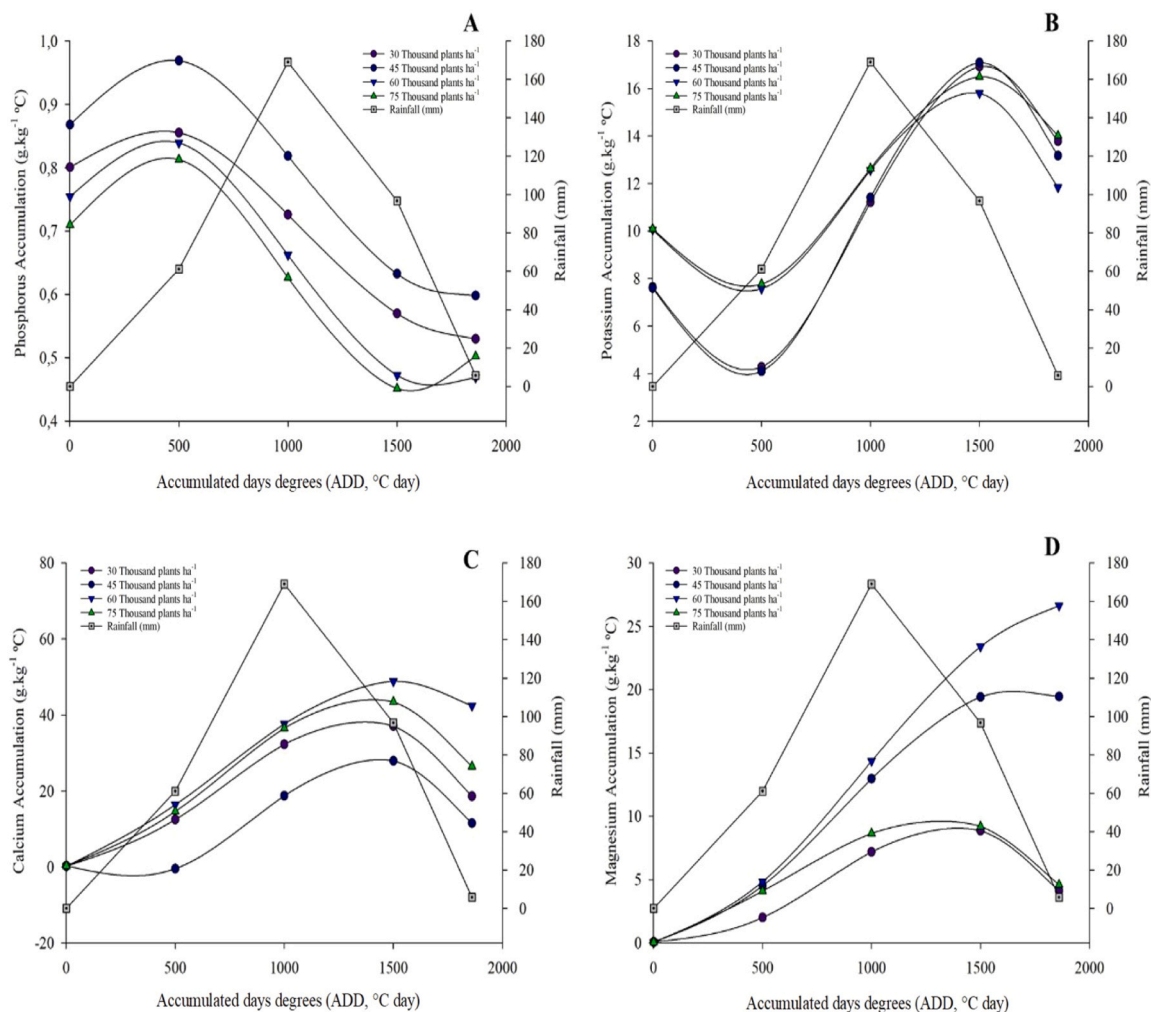


Fig. 6. Accumulation of phosphorus (A), potassium (B), calcium (C) and magnesium (D) in cactus pear under different planting densities.

axis, indicating a lower influence of the treatments on these minerals within the multivariate space (Fig. 10E). The angle between Fe and B suggests similarity in their behavior across the samples (Fig. 10E). Meanwhile, the observed angle between Fe and Na implies that these minerals exhibit similar behavior in the distribution of treatments (Fig. 10E).

The PCA for the macrominerals explained 75.02 % data variance in the first two components (Fig. 11A), obtaining greater contributions to the PC1 with the minerals K (27.40 %), Ca (28.54 %), and Mg (29.56 %) (Figs. 11B and 11D). Concerning PC2, S was the most representative mineral, with 80.0 % contribution (Figs. 11C and 11D). Demonstrating equal contribution of densities on principal components (Fig. 11E).

The distribution of the vectors in the multivariate plane demonstrates that K, Ca, and Mg are close to each other, indicating a high correlation between these minerals. Additionally, the proximity of the angle shows that these variables exhibit similar variation patterns among the treatments (Fig. 11E). However, the P vector shows equal contribution to PC1 and PC2 due to its intermediate position. Its angle demonstrates that the variation in its content across treatments is not independent but has a partial correlation with the other minerals (Fig. 11E). The opposing vectors between S and K, Ca, and Mg indicate that these variables are inversely proportional (Fig. 11E).

#### 4. Discussion

The nutritional assessment strategy according to the days after

planting and in relation to the different densities was carried out in order to obtain information about the best period for cutting cactus pear, with a view to disseminating this information to producers in the Brazilian semi-arid region. This information can guide producers about the nutritional composition of cactus pear, thus ensuring that animals are offered food with greater nutritional intake, reducing losses related to the non-use of nutrients by the animals.

The increase in planting density reduces the DM content. This effect is associated with greater competition among plants for light, water, and nutrients, which does not result in further increments (Fonseca et al., 2020). Cavalcante et al. (2014) investigated densities of 10,000, 20,000, 40,000, and 80,000 plants. $\text{ha}^{-1}$  and observed lower DM accumulation as planting density increased. The lowest DM content at 240 DAP is attributed to the climatic conditions during this period (Fig. 1), characterized by concentrated rainfall, which led to greater water accumulation in the plant, thereby reducing the DM concentration. The DM contents observed in this study are similar to those reported by Melo et al. (2022) and Nascimento Junior et al. (2022), with values of 74.80  $\text{g.kg}^{-1}$  NM and 192.70  $\text{g.kg}^{-1}$  NM, respectively.

The reduction in MM as a function of DAP is associated with lower rainfall during this period, resulting in decreased mineral absorption and, consequently, reduced mineral accumulation due to lower soil moisture and mineral availability (Pastorelli et al., 2022). As MM content increases, the concentration of carbon-based constituents (OM and C) decreases, as they are inversely proportional. The MM and OM contents in this study are similar to those reported by Silva et al. (2022),

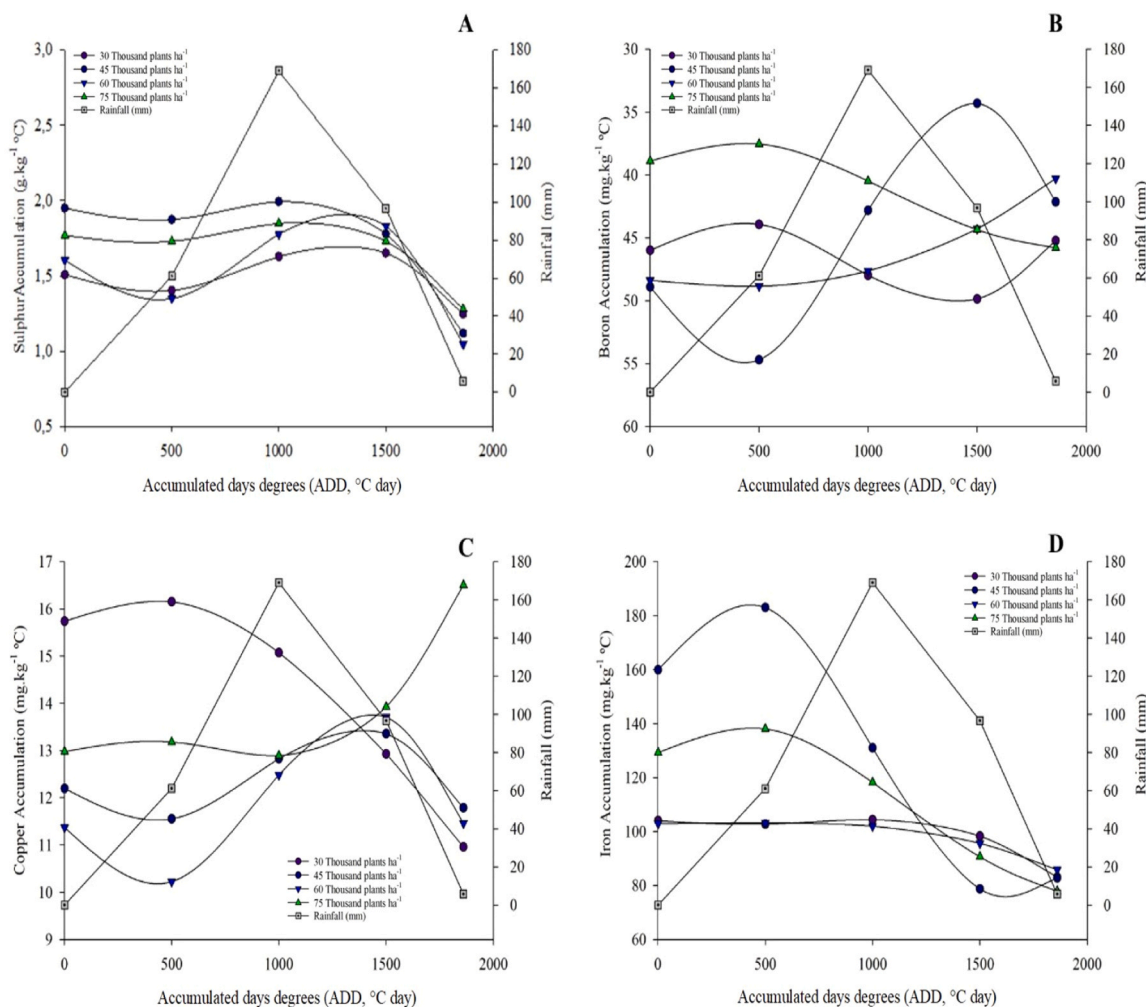


Fig. 7. Accumulation of sulphur (A), boron (B), copper (C) and iron (D) in cactus pear under different planting densities.

with MM contents ranging from 141 to 189 g.kg<sup>-1</sup> DM and OM contents between 810 and 858 g.kg<sup>-1</sup> DM.

Competition for light affects plant development in denser crops. Light availability and photon wavelengths influence photosynthetic reactions and pigment composition (Araújo et al., 2021). In this context, the response observed in this study regarding EE content in relation to planting density may be associated with the emergence of cladodes. Increased planting density limits the number of cladodes per plant (Cavalcante et al., 2014), thereby reducing EE content. Silva et al. (2022) evaluated the effect of cactus pear phenology by analyzing the nutritional characteristics of young, intermediate, and mature cladodes and found that young cladodes contain higher EE (20.13 g.kg<sup>-1</sup> DM) compared to intermediate (15.59 g.kg<sup>-1</sup> DM) and mature (16.46 g.kg<sup>-1</sup> DM) cladodes. The values found for intermediate and mature cladodes in this study are similar to those reported in the literature.

The increase in planting density reduced the CP content. A study by Dubeux Junior et al. (2006) found that the highest nitrogen concentrations in cactus pear were obtained with a population of 5000 plants ha<sup>-1</sup>, reaching 11.2 g.kg<sup>-1</sup>, whereas populations of 40,000 plants ha<sup>-1</sup> presented contents of 9.0 g.kg<sup>-1</sup>. Araújo Júnior et al. (2021) analyzed irrigated cactus pear systems and observed CP levels of 35.80 g.kg<sup>-1</sup> in Mexican elephant ear, lower than those found in the present study, which was conducted under rainfed conditions.

The harvest at 360 DAP was carried out during the driest period, which alters plant composition. During drought and higher temperatures, cactus pear mucilage exhibits improved quality, with a higher composition of soluble fiber and lower neutral and acid detergent fiber

contents due to the plant's high water retention capacity (Du Toit et al., 2018). The NDF, ADF, TC, and NFC contents are consistent with values reported in the literature: 248–263 g.kg<sup>-1</sup> DM of NDF; 179–207 g.kg<sup>-1</sup> DM of ADF; 625–690 g.kg<sup>-1</sup> DM of TC, and 398–462 g.kg<sup>-1</sup> DM of NFC (Dubeux Junior et al., 2021).

The increase in ADL as a function of DAP is attributed to plant aging, which leads to higher concentrations of cell wall components (NDF, ADF, and ADL). Lignin plays a crucial role in plant development, providing structural strength to support cladodes and increasing cell wall resistance to withstand negative pressures during transpiration (Meents et al., 2018). The reduction in ADL with increasing planting density may be related to anatomical changes in cladode cells, as these modifications reduce the lignification rate in cactus pear (Reyes-Rivera et al., 2017). The ADL content observed in this study was lower than the 11.3 g.kg<sup>-1</sup> DM reported by Magalhães et al. (2021) for *Opuntia stricta* Haw at 620 DAP.

The increase in glucose, sucrose, and starch as a function of planting density is associated with the genetic characteristics of the cactus pear clone used. *Opuntia* species exhibit high concentrations of chlorophyll "a" and total chlorophyll, which enhance the performance of phosphoenolpyruvate carboxylase (PEPC), enabling the production of soluble sugars (Falcão et al., 2013). Another contributing factor is that CAM plants use PEPC to catalyze oxaloacetate and produce energy (Walker et al., 2011).

Thus, according to our observations, higher planting densities of cactus pear (genus *Opuntia*) under semi-arid conditions result in a greater proportion of carbohydrates. This factor, combined with

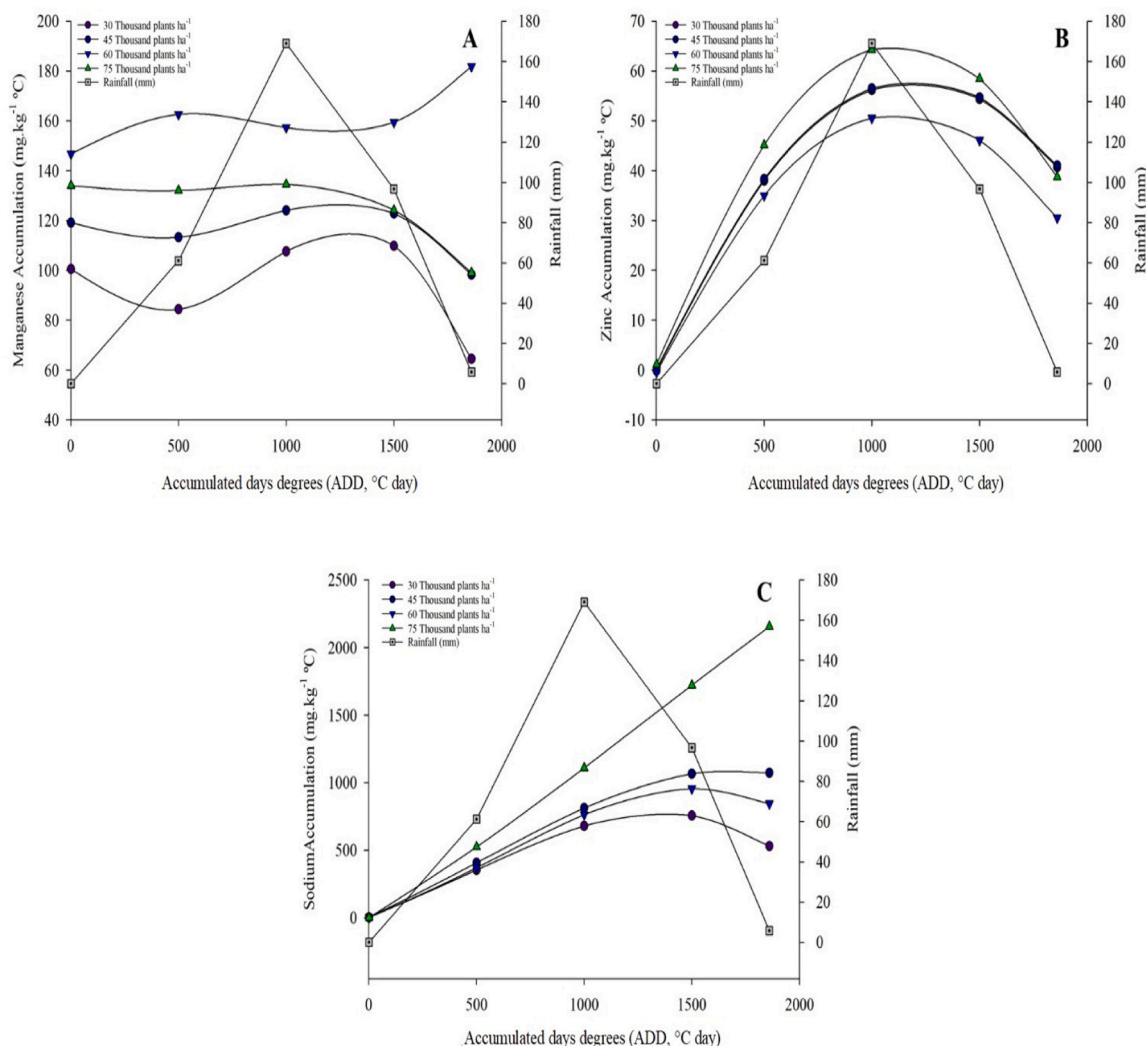


Fig. 8. Accumulation of manganese (A), zinc (B) and sodium (C) in cactus pear under different planting densities.

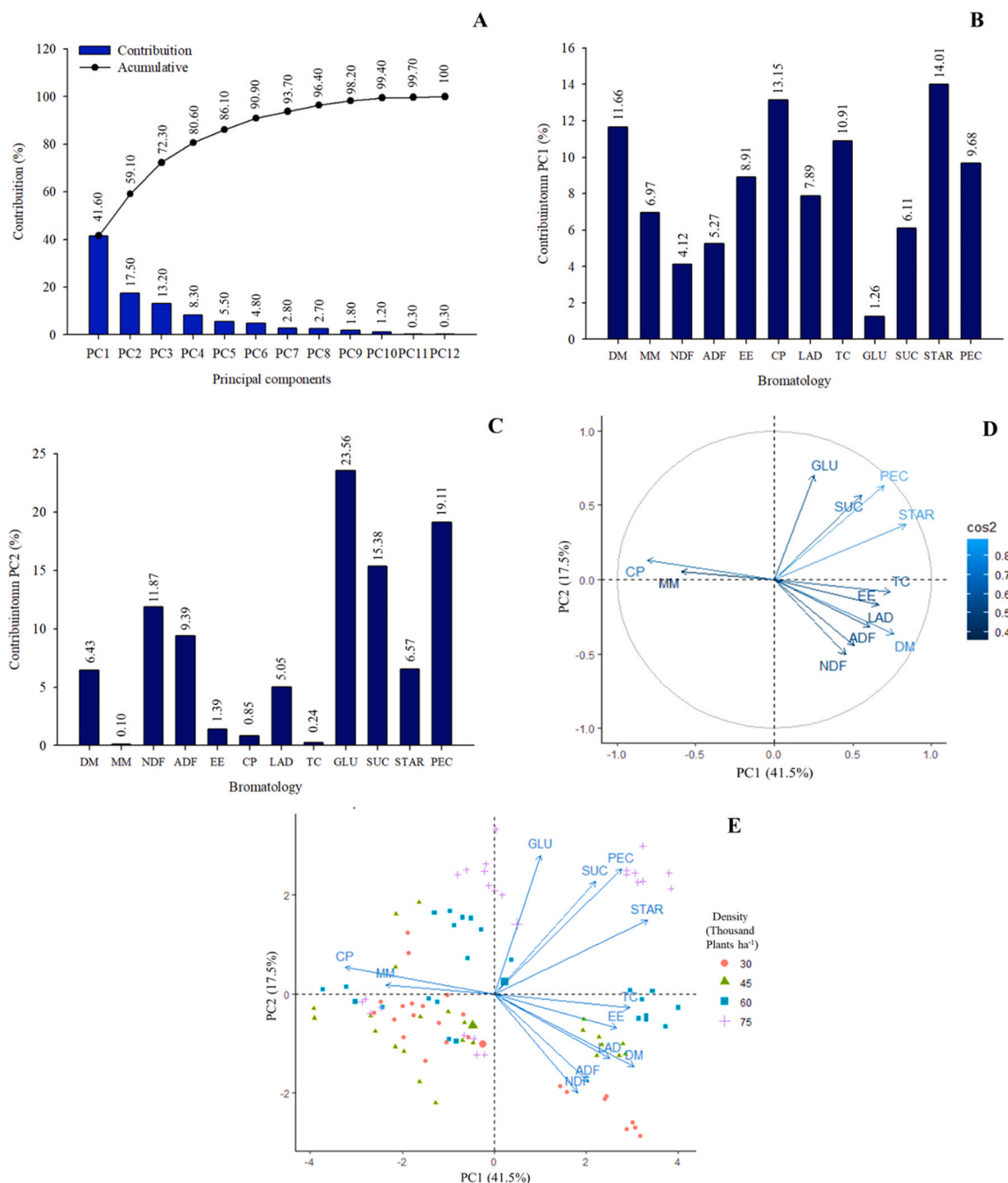
increased competition for light, water, nutrients, and space, drives the plant to use physiological mechanisms to store energy for periods of drought. Glucose and fructose are the main sugars in cactus pear (Santiago et al., 2018). When the plant is cultivated at low temperatures, fructose, glucose, and sucrose contents increase (Nobel et al., 1995). The higher activation of Rubisco contributes to an increased electron transport rate through photosystem II, leading to greater sugar production and the mobilization and accumulation of carbohydrate reserves (Gonçalves et al., 2020). The starch content observed in this study was higher than that reported by Rocha Filho et al. (2021), who recorded values between 135 and 252 g.kg<sup>-1</sup> DM.

Pectins are polysaccharides with a defensive role against plant pathogens due to their anionic nature (Morales-Martínez et al., 2018). They act directly in ion transport regulation, influence cell wall porosity, and control wall permeability through enzymatic activity (Voragen et al., 2009). Studies by Magalhães et al. (2021) demonstrated that PEC represents 25.8 % of non-fibrous carbohydrates in *O. stricta* Haw cactus pear crops at 730 DAP. In our study, this proportion was higher, ranging from 30.63 % to 36.12 % at the lowest and highest planting densities, respectively. Additionally, the effect of DAP resulted in higher PEC proportions, reaching 35.15 % of non-fibrous carbohydrates. The highest ion retention was observed at 240 DAP, which may be associated with increased ion content, particularly calcium (Table 3). Notably, an increase in Ca<sup>2+</sup> leads to the formation of softer pectin gels (Morales-Martínez et al., 2018).

The P content in plant tissue has a direct effect on photosynthetic performance, influencing various photochemical parameters, including potential and effective quantum efficiencies of the photosystem and the overall photosynthetic rate (Singh et al., 2013). Additionally, P availability affects the electron transport rate, as this process is positively correlated with P content (Lopes et al., 2021). Phosphorus plays a crucial role in plant development, particularly in root system growth, enhancing water absorption efficiency (Cavalcante et al., 2014).

In plant metabolic reactions, K has a regulatory function, influencing stomatal activity (opening and closing of stomata) and water absorption capacity. Additionally, it contributes to defense mechanisms against environmental stress (Chakraborty et al., 2016), provides tissue firmness by participating in protein metabolism, and enhances photosynthesis (Galizzi et al., 2004). Phosphorus and K are highly mobile minerals within plants; thus, at advanced developmental stages, their concentrations decrease in the aerial parts (Teles et al., 2004). This effect was observed in the accumulation graphs, which demonstrated a reduction at 1800 ADD. Silva et al. (2022) evaluated the vegetation stages of cactus pear (young, intermediate, and mature) and reported K contents ranging from 24.22 to 39.18 g.kg<sup>-1</sup> DM and P contents between 4 and 9 g.kg<sup>-1</sup> DM, values higher than those found in this study.

Several factors influence Ca levels in cactus pear, including cultivation conditions (soil physicochemical properties and salinity), the presence of thorns, and the plant's maturity stage (Contreras-Padilla et al., 2012). The highest Ca content in cactus pear is related to its



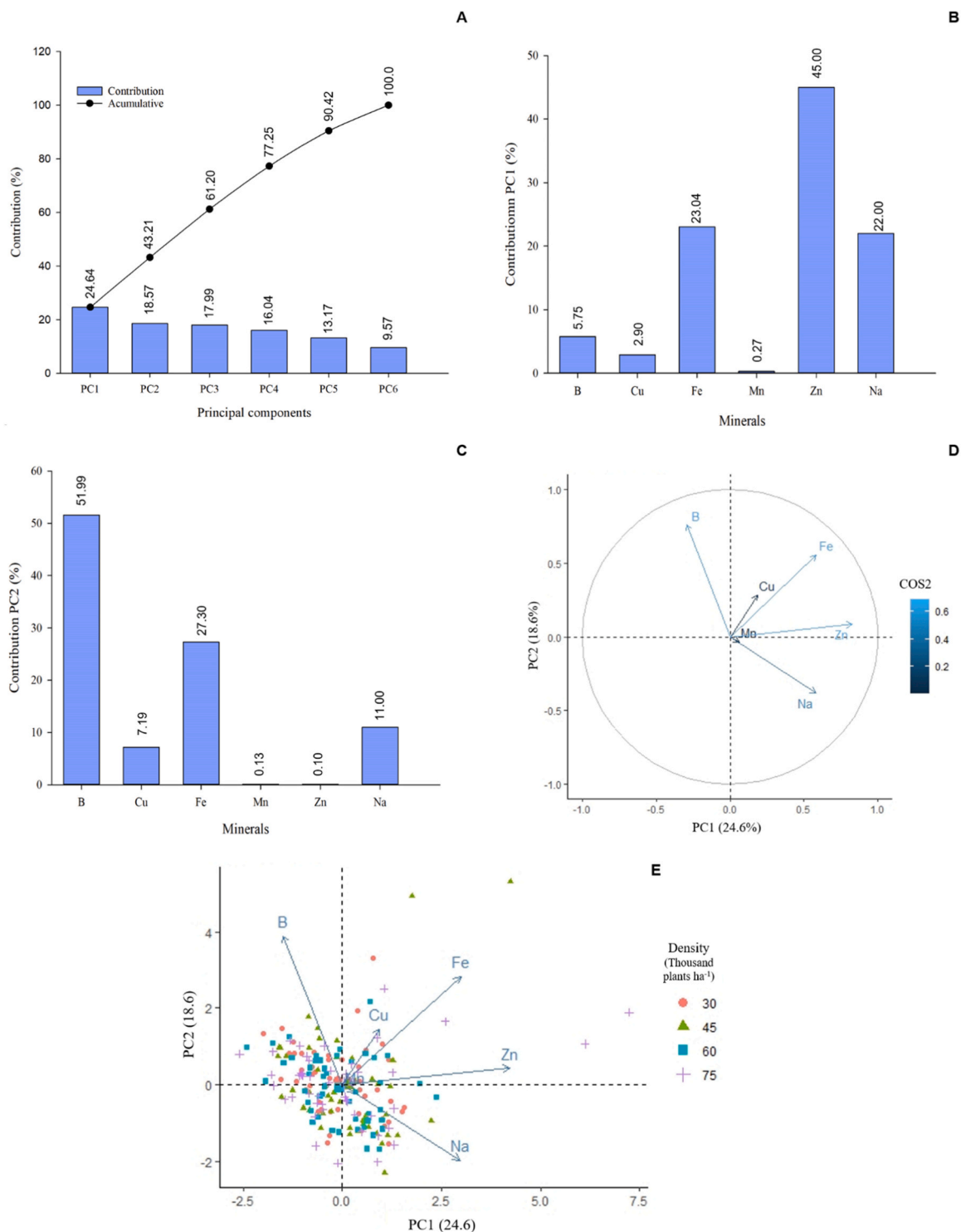
**Fig. 9.** Analysis of principal components of bromatological analysis of cactus pear under different planting densities: A) individual and cumulative contribution of principal components - PC; B) Individual contribution of variables to PC1; C) Individual contribution of variables to PC2; D and E) distribution of variables in the multivariate plan.

capacity for tissue expansion, and its accumulation follows the rainfall pattern. In this study, the greatest Ca accumulation was observed at 240 DAP, coinciding with the highest water accumulation period. Notably, cactus pear is considered a good source of Ca for both human and animal nutrition. However, it also accumulates calcium oxalate, which is regarded as an anti-nutritional factor due to its potential to form calcium oxalate stones (Missouai et al., 2020).

Calcium is involved in several chemical reactions, acting as a messenger under abiotic stress conditions through intracellular stimulation (Parvin et al., 2019). In addition, a considerable amount of Ca is found in the form of inorganic crystals associated with cell structures. These crystals, synthesized within cells, consist of biomacromolecules such as proteins, carbohydrates, and nucleic acids (Elejalde-cadena

et al., 2020). Hybrid nanocomposites, in which proteins and polysaccharides (chitin, collagen, and other sugars) are chemically bonded to various calcium salts—namely calcium oxalate, calcium phosphate, and calcium carbonate—have been reported (Cuéllar-Cruz et al., 2020). The formation of these insoluble crystals is considered a defense mechanism against herbivorous animals (Cuéllar-Cruz et al., 2020), as it can lead to urolithiasis by increasing the concentration of crystals and calcium oxalate in the urine (T.S. Silva et al., 2021; T.G.P. Silva et al., 2021; K.B. Silva et al., 2021).

According to Valadez-Moctezuma et al. (2021), in CAM metabolism plants, Ca, Mg, and Mn levels tend to be higher than in other cultivated plants. Blanco-Macías et al. (2010) reported that these plants accumulate Ca, Mg, P, Mn, Fe, and Zn in decreasing order, a pattern similar to

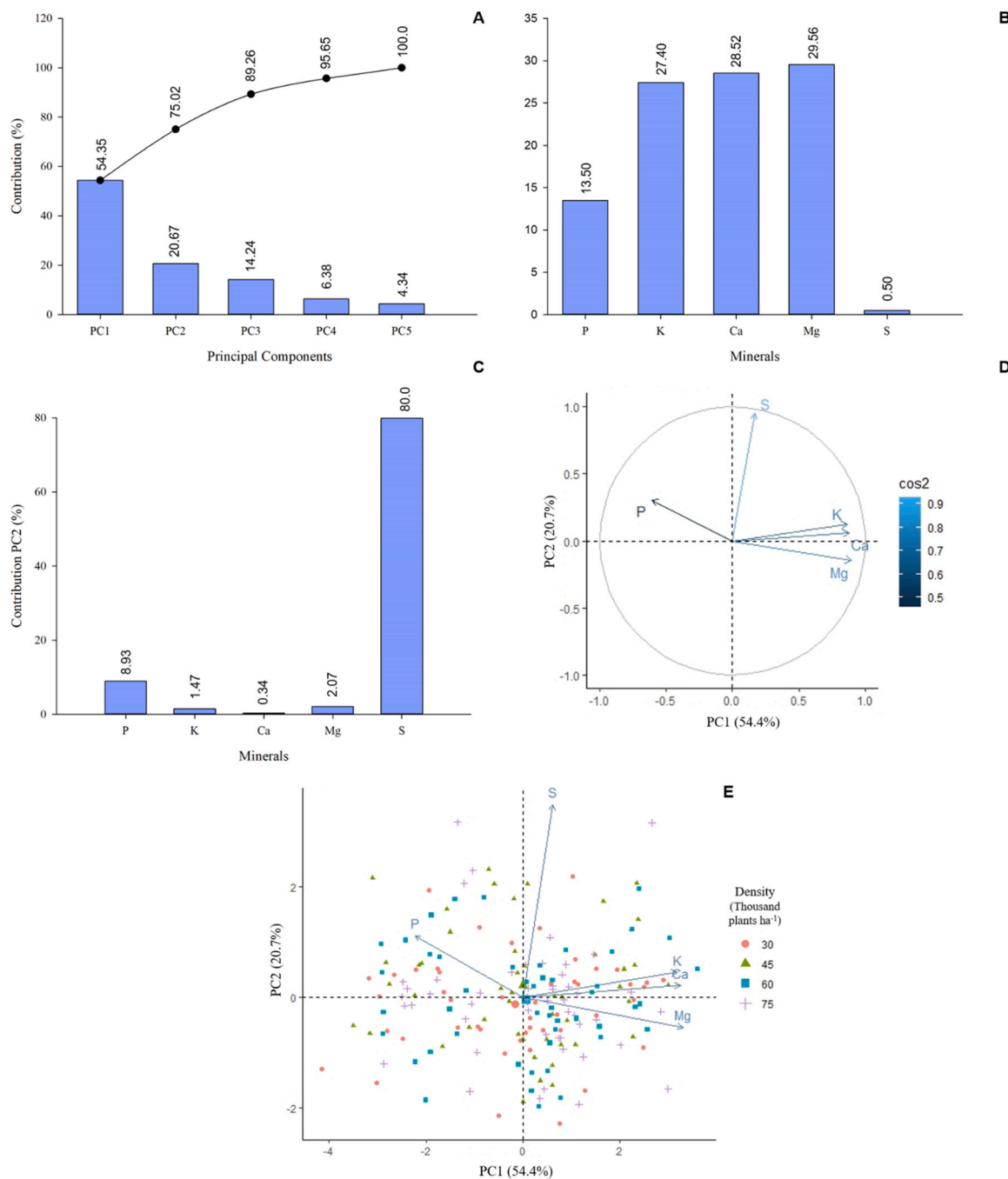


**Fig. 10.** Analysis of principal components of forage cactus microminerals under different planting densities: A) individual and cumulative contribution of principal components - PC; B) Individual contribution of microminerals to PC1; C) Individual contribution of microminerals to PC2; D and E) distribution of variables in the multivariate plan.

the findings of this study. In this context, Mg, together with K and Na, serves as a reserve for the anabolism of carboxylates, which have an osmotic stabilization function to maintain adequate water levels (Broadley et al., 2012). This effect was observed at 240 DAP, the period with the highest water accumulation in the plant, requiring osmotic adjustment.

Magnesium ions are essential for photosynthesis. Estimates suggest that approximately 15–35% of a plant's Mg is associated with

chloroplasts, where it plays a key role in energy transfer, as it is one of the main constituents of chlorophyll (Cakmak & Yazici, 2010; Chen et al., 2018). A study by Silva et al. (2022) on cactus pear grown in a semi-arid climate at an altitude of 683.9 m, with an average annual temperature of  $24.9 \pm 1.97$  °C, reported average Mg values ranging from 6.89 to 7.39 g·kg<sup>-1</sup> DM, which were higher than those found in our study. According to Dubeux Junior et al. (2021), variations in plant mineral content depend on soil and climatic conditions.



**Fig. 11.** Analysis of principal components of forage cactus macrominerals under different cultivation densities: A) individual and cumulative contribution of principal components - PC; B) Individual contribution of macrominerals to PC1; C) Individual contribution of microminerals to PC2; D and E) distribution of variables in the multivariate plan.

In cactus pear, harvesting frequency influences Fe, Cu, and Mn content. Our results demonstrate a reduction in Cu and Mn levels, corroborating the findings of [Gomes et al. \(2021\)](#), who observed that annual harvesting reduces Cu and Mn content while increasing Fe content. However, in our study, Fe content was also reduced, a result that may vary depending on soil and climatic conditions. In the previous study by [Araújo et al. \(2023\)](#), when using principal component analysis on the soil mineral profile, it was reported that the main minerals limiting cactus pear production in a semi-arid region under different density levels are calcium, phosphorus, potassium, manganese, iron, and zinc. This demonstrates that the removal of each chemical element from the soil by the plant enhances its biochemical functions. The authors

recommend that in crops where fertilization practices and stability are not planned, lower densities should be used, such as 30 thousand plants. ha<sup>-1</sup>.

Iron and other micronutrients can associate with proteins, such as superoxide dismutase isoenzymes that contain a metal component—Fe, Cu, Zn, or Mn—which helps detoxify superoxide anion free radicals ([Broadley et al., 2012](#)). In agreement with our results, [Alves et al. \(2017\)](#) reported Zn, S, Fe, and Mn values for cactus pear ranging from 19.19 to 91.14; 36.67 to 1315.59; 59.38 to 208.21, and 102.50 to 704.57 mg. kg<sup>-1</sup>, respectively.

Plants with older cladodes have larger CO<sub>2</sub> assimilation surfaces, which are responsible for the distribution of nutrients to other organs. In

this context, Na concentrations tend to be higher in older cladodes due to the importance of this mineral in the photosynthesis of CAM plants (Alves et al., 2017). However, our results showed higher Na values in cladodes harvested at 240 DAP. Thus, Na is considered an essential micronutrient for cactus pear (Epstein & Bloom, 2006), as it plays a fundamental role in the carbon fixation pathway, particularly in the regeneration of phosphoenolpyruvate, the substrate of the first photosynthetic reaction (Lemos et al., 2018).

The PCA of micronutrients in cactus pear under different cultivation densities revealed a clear pattern of variability and association among the elements. Observing the individual contribution of variables to PC1, it is possible to note that Zn, Fe, and Na exert a dominant influence on this component, probably due to their role in enzymatic functions and plant metabolism. On the other hand, in PC2, the minerals B and Fe stood out, indicating that these elements may vary independently of those contributing to PC1. The presence of Fe in both principal components (PC1 and PC2) can be explained by different factors related to its dynamics in the soil and the plant. In PC1, iron may have a strong influence due to its essential role in plant metabolism, including photosynthesis, respiration, and the formation of enzymes and proteins, such as ferritin and cytochromes (Ning et al., 2023; Rai et al., 2021).

In PC2, however, the presence of iron may reflect specific interactions with other minerals, such as Cu, Mn, and Zn, which compete for the same absorption transporters in the roots. According to Rai et al. (2021), the homeostasis of Fe, Cu, Zn, and Mn is vital for plant metabolism since the deficiency of these nutrients impairs cellular metabolism, significantly reducing plant growth and development. However, to the best of our knowledge, there are gaps regarding how these minerals interact and influence each other in cactus pear, making it possible to increase crop yield, and future research is needed to elucidate such mechanisms. What we can infer, according to the experimental conditions, is that the variation may be associated with differences in nutrient absorption and availability in the soil, impacted by cactus pear planting density.

Similarly, the PCA of macrominerals in cactus pear for PC1 indicates that K, Ca, and Mg are the main contributors to the variation along this axis, which aligns with their roles in cellular functions, water regulation, and structural integrity of the plant (Hasanuzzaman et al., 2018; Hu et al., 2023). On the other hand, contributions to PC2 suggest that S plays a more distinct role, possibly influenced by differences in root absorption efficiency and soil availability. Sulfur is essential for plant growth and development, as well as metabolic activities such as the formation of amino acids (cysteine and methionine), biosynthesis of sulfolipids, antioxidants, and cofactors (Abou Seeda et al., 2020).

The differential clustering of variables in the multivariate plane suggests that specific cultivation strategies can optimize the availability and utilization of essential minerals in cactus pear production systems.

## 5. Conclusion

Higher planting densities, such as 75 thousand plants.ha<sup>-1</sup>, result in a higher dry matter content, greater carbohydrate storage capacity, and do not alter the mineral composition of cactus pear, confirming the feasibility of high-density planting as a strategy to optimize production in semi-arid cultivation systems. However, the harvest period affects the mineral content, and mineral accumulation follows water dynamics, allowing for mineral dilution.

As future perspectives, studies evaluating the interaction between planting density and management practices, such as irrigation and fertilization, are suggested to determine more efficient strategies for maximizing cactus pear productivity. Additionally, investigations on solar radiation interception and water use efficiency at different densities may provide valuable insights for improving the cultivation of this species in environments with low water availability.

The findings of this research contribute to the knowledge of cactus pear agronomic management and reinforce the importance of strategies

that promote sustainable production, aiming to ensure greater efficiency in the use of cultivable areas and forage production in semi-arid regions.

## Authorship statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

## CRediT authorship contribution statement

**Kacya Araújo:** Methodology, Formal analysis. **Gherman Araújo:** Visualization, Validation, Supervision, Funding acquisition. **Cleyton Araújo:** Writing – original draft, Methodology, Formal analysis, Data curation. **André Magalhães:** Supervision, Formal analysis. **Thieres Freire:** Supervision, Resources, Funding acquisition. **Tadeu Voltolini:** Supervision, Project administration. **Gláyciane Gois:** Writing – review & editing, Writing – original draft. **Deneson Lima:** Methodology, Formal analysis. **Fleming Campos:** Supervision, Methodology, Formal analysis. **Janiele Araújo:** Methodology, Formal analysis.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Declaration of Competing Interest

The authors declare that there is no conflict of interest, and that the article has not been submitted to another journal. Thus, I, Cleyton de Almeida Araújo as the main author and correspondent declare that we have no conflict of interest.

## Data Availability

Data will be made available on request.

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