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Scientific Article

EFFECT OF INCREASED CARBON DIOXIDE CONCENTRATION ON THE ELEMENTS Ca, Fe, K, Mg, Mn, AND Na IN *Senna reticulata*

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ABSTRACT

The CO_2 is the main gas responsible for the greenhouse effect, and assessing its effects on plant species is crucial for future research. The aim of this study was to test the impact of increased CO_2 on the elements Ca, Fe, K, Mg, Mn, and Na in leaflets, roots, and stems of *Senna reticulata*. The plants were exposed to 360 $mg.kg^{-1}$ and 720 $mg.kg^{-1}$ of CO_2 in a controlled environment. The samples were solubilized with HNO_3 and analyzed by flame atomic absorption spectrophotometry. The increase in CO_2 to 720 $mg.kg^{-1}$ resulted in a 56.05% increase in Ca in the root, with reductions in the leaflet (1.11%) and stem (10.21%); reduction of Fe in all parts (leaflet 1.99%, root 19.16%, stem 41.73%); reduction of K in the leaflet (15.40%) and increases in the root (35.42%) and stem (3.17%); increase in Mg in all parts (leaflet 15.06%, root 111.46%, stem 6.15%); reduction of Mn in the leaflet (19.97%) and stem (16.69%) and increase in the root (1.47%); and reduction of Na in the leaflet (52.59%) and increases in the root (234.73%) and stem (168.38%). Exposure to 720 $mg.kg^{-1}$ of CO_2 induces adaptations in the distribution of elements in *Senna reticulata* compared to exposure to 360 $mg.kg^{-1}$.

Keywords: Nutritional management; Global warming; Climate bioindicators

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EFEITO DO AUMENTO DA CONCENTRAÇÃO DE GÁS CARBÔNICO SOBRE OS ELEMENTOS Ca, Fe, K, Mg, Mn E Na EM *Senna reticulata*

RESUMO - O CO_2 é o principal gás responsável pelo efeito estufa, e avaliar seus efeitos em espécies vegetais é fundamental para futuras pesquisas. O objetivo deste estudo foi testar o impacto do aumento de CO_2 nos elementos Ca, Fe, K, Mg, Mn e Na em folíolos, raiz e caule de *Senna reticulata*. Plantas foram expostas a 360 $mg.kg^{-1}$ e 720 $mg.kg^{-1}$ de CO_2 em ambiente controlado. As amostras foram solubilizadas com HNO_3 e analisadas por espectrofotometria de absorção atômica. O aumento de CO_2 para 720 $mg.kg^{-1}$ resultou em: aumento de 56,05% de Ca na raiz, com reduções no folíolo (1,11%) e caule (10,21%); redução de Fe em todas as partes (folíolo 1,99%, raiz 19,16%, caule 41,73%); redução de K no folíolo (15,40%) e aumento na raiz (35,42%) e caule (3,17%); aumento de Mg em todas as partes (folíolo 15,06%, raiz 111,46%, caule 6,15%); redução de Mn no folíolo (19,97%) e caule (16,69%) e aumento na raiz (1,47%); e redução de Na no folíolo (52,59%) e aumento na raiz (234,73%) e caule (168,38%). A exposição a 720 $mg.kg^{-1}$ de CO_2 induz adaptações na distribuição de elementos em *Senna reticulata* comparada à exposição a 360 $mg.kg^{-1}$.

Palavras-Chave: Manejo nutricional; Aquecimento global; Bioindicadores climáticos

1. INTRODUCTION

Carbon dioxide (CO_2) is naturally found in the atmosphere. The Earth would be a much colder without CO_2, because most of the sunlight absorbed by Earth's surface would be re-radiated back into space without it (Noh & Jeong, 2021). As it stands now, CO_2 forms an insulating blanket over the Earth's surface, allowing short-wavelength ultraviolet radiation and visible sunlight to pass through, but slowing the loss of heat in the form of long-wavelength infrared radiation. (Zhou, 2023).

Plants, algae, and some bacteria capture light energy and convert it into chemical bond energy in carbohydrates. This process is called primary production, and its rate is quantified as primary productivity. Photosynthesis chemically combines two common inorganic compounds, CO_2 and water, to form glucose, releasing oxygen in the process (Machín et al., 2023).

The concentration of CO_2 has been increasing at a rate of 0.4% per year. Of the total emissions, agriculture contributes to approximately 20% of CO_2, along with 50 to 70% of N_2O and CH_4 (El-Hawwary et al., 2022). Its concentration during the pre-industrial era was 280 $mg.kg^{-1}$, and in April 2024, this concentration reached 426.57 $mg.kg^{-1}$, according to data from the National Oceanic & Atmospheric Administration (NOAA) (2024), it is estimated that it will reach 720 $mg.kg^{-1}$ within this century.

Warmer temperatures caused by the greenhouse effect will have various impacts on plant productivity. On the positive side, warmer temperatures extend the growing season and accelerate metabolism, thus tending to enhance production in humid environments (Choudhary et al., 2022).

Environmental stress factors, such as increased CO_2, affect plant growth and pose a growing threat to sustainable agriculture (Ahuja et al., 2010). Some authors have studied the effect of increased CO_2 on plants. Ai et al. (2018) demonstrated that elevated CO_2 levels affect plant physiology and soil microbes, and these changes may threaten crop quality, with unknown implications for food security and human health in future climate scenarios.

Cruz et al. (2014) evaluated how increased CO_2 concentration, using different soil treatments with nitrogen species, affects the growth and photosynthetic responses of *Manihot esculenta* Crantz (cassava). The results indicated that cassava responded with increased biomass accumulation in response to elevated atmospheric CO_2 levels.

Singh et al. (2020) conducted a study on the effect of CO_2 in a greenhouse using a nutrient film technique (NFT) system to quantify the impact of two different CO_2 levels (800 mg.kg^{-1} and 410 mg.kg^{-1}) on the growth and nutritional quality of *Ocimum basilicum* L. (basil), *Lactuca sativa* L. (lettuce), and *Beta vulgaris* L. (chard). The findings suggest that CO_2 supplementation could increase the yield of green leaves grown hydroponically and have a variable impact on different mineral concentrations across species.

AbdElgawad et al. (2015) evaluated how increased atmospheric CO_2 might affect plant growth, including mitigating stress impact across various species. These effects varied considerably between groups of species, such as grasses and legumes. Elevated CO_2 reduced stress impact; in grasses, photosynthesis and chlorophyll levels were better protected by CO_2 compared to legumes.

The species *Senna reticulata*, introduced in India and naturalized in Brazil, is now subspontaneous from the Amazon to Rio de Janeiro, Minas Gerais, and Goiás. In the Amazon region, it is popularly known as mata pasto, due to its high competitive capacity, rapid growth, establishment in pasture areas, adaptation to flooded plains, and has become a pioneer plant in open areas. For this reason, it is considered an invasive plant in pastures, periodically eliminated to preserve the cultivation of grasses (Parolin, 2005).

The leaves are compound, with glands between the lower leaflets, 24 pairs of leaflets, 2-6 cm long, 1-3 cm wide, rounded obovate or obtuse mucronate, glabrous on both sides or pubescent on the lower ones; solitary axillary flowers or two in number, the upper ones very close together, yellow, glabrous or pubescent; pod 8-20 cm long and 5 mm wide, linear curved, numerous seeds (Neves et al., 2017). This species can reach up to 12 meters, but individuals exceeding 8 meters are rarely found, due to pruning control carried out for pasture maintenance. Its flowers are large, yellow, and are pollinated by insects. The fruits are long legumes that produce an average of 14 seeds each, dispersed by wind or water (Parolin, 2005).

Senna reticulata is a legume that fixes nitrogen in the soil, which increases its fertility. *Senna reticulata* can be used as feed for cattle and poultry. Although *Senna reticulata* is not appreciated by ruminants when green, it is widely consumed when naturally dried. Therefore, it can be used as hay, to reduce food shortages in regions with scarcity. The green plant can be used as hay and can also be ensiled, which reduces the amount of tannins and polyphenols that cause animals to reject the plant. *Senna reticulata* has high rates of photosynthesis at the leaf level. Despite their unpleasant odor, the leaves and flowers are used in food in Ceylon and India. Grandis et al. (2021) mention that the species can serve as a promising source of biofuel and has medicinal properties (antifungal, antimalarial, and antioxidant properties).

The initiative to assess the effects of increased CO_2 on plant species is crucial for understanding how this rise influences the nutritional aspects of plants. Evaluating the impact of elevated CO_2 on Ca, Fe, K, Mg, Mn, and Na in native Amazonian species is particularly important due to the vast biodiversity in this region, which could be affected by global warming.

The objective of this work was to verify the effect of increased CO_2 on the elements Ca, Fe, K, Mg, Mn, and Na in leaflets, roots, and stems of *Senna reticulata*. The elements evaluated were chosen due to their nutritional properties that guarantee the good development of living beings.

The main contribution of this work was to generate data on the behavior of *Senna reticulata* regarding its adaptation to high CO_2 concentration and to analyze the extent of the damage to nutritional elements caused by the increase in global warming.

2. MATERIAL AND METHODS

2.1 Experimental design of the development of *Senna reticulata*

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Fifty adult *Senna reticulata* seeds were collected from Embrapa, located at Avenida Perimetral, number 211, in the Terra Firme neighborhood in Belém-PA. The samples were sent for germination to the São Paulo Botanical Garden, where experiments were carried out in greenhouses with different CO_2 atmospheres.

The *Senna reticulata* seeds were selected and before germination, scarification was performed by wearing down the outside with sandpaper and placed in a petri dish with filter paper and vermiculite. The plates were then placed in a vegetation chamber, where they were conditioned at 29°C for 12 hours without light and another 12 hours with light at the same temperature.

The experiment was carried out from January to March (3 months), when, according to Parolin (2005), there is a high absorption of CO_2 at this time of year.

To avoid nutrient deficiency in the soil, Hogland solutions (mixture of $MnCl_2$, $ZnSO_4$, $CuSO_4$, H_3BO_3, KH_2PO_4, $MgSO_4$ and $CaCl_2$), NaOH and $FeSO_4.7H_2O$ solutions and EDTA were added.

When removed from the vegetation chamber, the seeds were transferred to pots and fed with Hogland solution and Fe-EDTA solution. The pots were placed in the chambers at 360 and 720 mg.kg^{-1} CO_2. The pots were filled with washed sand and vermeculite in a 3:2 ratio.

The Hogland solution was added to the soil and occasionally to the aerial part, weekly. Irrigation was done daily to the aerial part. On days when it was necessary to add extracting solutions, this was done after irrigation to avoid dragging in the most superficial part. The pots were permuted weekly to ensure that solar availability was distributed homogeneously.

All growth measurements were performed at the Botanical Institute of São Paulo. At the end of the desired growth period, the individuals were selected and leaf area, leaf mass, stem mass, root mass, and total biomass were measured.

All measurements were taken with a ruler. Leaf area was obtained by measuring the length and width of the leaflets. To correct these values, an adjustment curve (real area x length*width) was made on plants grown in parallel with the experiment in the greenhouse. Fifty leaflets of each type were used for the adjustment. Leaf mass was obtained by adding the masses of all leaflets of each individual leaf measured on an analytical balance with a precision of 4 decimal places.

All mass measurements were taken with a dry matrix on an analytical balance. The drying treatment was carried out in an oven at 60°C for five days.

2.2 Sample collection of leaflet, root, and stem

Four individuals were chosen and underwent experiments in 2 atmospheres (360 and 720 mg.kg^{-1} of CO_2) for the removal of parts of *Senna reticulata* (root, stem, and leaflet), and the material was sent to the Laboratory in Belém for the purpose of analyzing the elements Ca, Fe, K, Mg, Mn, and Na by atomic absorption spectrophotometry.

2.3 Sample treatment

Upon receiving the samples of leaflets, roots, and stems of *Senna reticulata* germinated at 360 and 720 mg.kg^{-1} of CO_2, they were ground, and a mass of 0.1 g from each sample was weighed on an analytical balance in triplicate. After weighing, the samples were solubilized in a microwave oven using ultrapure concentrated HNO_3 and H_2O_2. The microwave oven used was a Provecto model DGT-100, with time and power settings as recommended by the manufacturer for the evaluated matrices.

2.4 Element analysis

After the samples were opened, they were transferred to 25 mL volumetric flasks and filled to the mark with ultrapure water. Subsequently, atomic absorption analyses were performed on all samples in triplicate, including a blank prepared using the same reagents and procedure as the sample opening mentioned earlier.

The analyses of Ca, Fe, K, Mg, Mn, and Na were conducted using a Varian model SpectrAA 55 flame atomic absorption spectrophotometer. The analyses of the elements were performed in triplicate. Standard solutions were prepared for the calibration of the atomic absorption spectrophotometer using the multi-element standards Standard 19 QC-019.5 and Dionex No. 040187.

2.5 Analytical quality

The analytical curves were generated during the calibration of the equipment, and calculations for the limit of detection and quantification were performed by analyzing 15 blanks over the course of the analytical development.

The method validation was performed using the recovery of the standard reference material 1515 apple leaves from the National Institute of Standards & Technology (NIST). A recovery test was conducted for the elements under study.

2.6 Statistical treatment

The results obtained in the evaluation of the essential elements were treated using descriptive statistics with the aid of the Statistica software, where preliminary calculations were performed based on Pearson's linear correlation, analysis of variance (ANOVA), and variability graphs (box-plot). The multivariate analysis was performed with the aid of the Statistica software, where the techniques of principal component analysis (PCA) were applied to the data.

3. RESULTS

The descriptive statistical results found for the elements in the root (R), leaf (F) and stem (C) of *Senna reticulata* after three months of growth in atmospheres of 360 and 720 mg.kg^{-1} CO_2 are shown in Table 1. For Ca, only the root (R) of *Senna reticulata* had an increase in concentration of 56.05 % when the plant was subjected to an atmosphere of 720 mg.kg^{-1}. For the leaflet (F) there was a 1.11 % reduction in Ca concentration and in the stem (C) a 10.21 % reduction.

Using the boxplot analysis (Figure 1) which showed the variability of the concentration of Ca, Fe, K, Mg, Mn and Na, it was possible to observe that for Ca the concentration was highest in the leaflet in the atmosphere of 360 mg.kg^{-1} CO_2 (largest size of box) with an anomalous result in the stem at 360 mg.kg^{-1} CO_2.

The Pearson correlation matrix for germinated species at 360 and 720 mg.kg^{-1}

Table 1. Descriptive statistics of elements in *Senna reticulata* germinated at 360 and 720 mg.kg^{-1} of CO2 (N=9)
Tabela 1. Estatística descritiva dos elementos em *Senna reticulata* germinada a 360 e 720 mg.kg^{-1} de CO_2 (N=9)

	Mean	Median	SD	Minimum	Maximum		Mean	Median	SD	Minimum	Maximum
			Ca						K		
F1	16905	17030	270.2	16532	17193	F1	15386	15391	199.7	15055	15643
F2	16718	16715	197.0	16443	17050	F2	13016	12984	162.2	12816	13277
R1	2560	2569	58.41	2461	2669	R1	10940	10959	205.5	10623	11252
R2	3995	3999	37.70	3945	4061	R2	14815	14819	270.6	14487	15358
C1	3802	3797	59.64	3673	3877	C1	13664	13660	261.8	13206	14072
C2	3414	3431	52.01	3320	3474	C2	14097	14212	160.4	13855	14252
			Mg						Fe		
F1	4514	4554	179.7	4248	4834	F1	135.7	137.2	2.57	132.0	138.0
F2	5194	5211	192.3	4995	5529	F2	133.0	129.3	7.45	126.0	143.9
R1	2382	2384	42.37	2320	2447	R1	423.3	417.1	10.18	412.7	437.7
R2	5037	5048	64.25	4910	5124	R2	342.2	336.1	9.64	334.2	357.4
C1	1626	1631	53.44	1531	1694	C1	41.60	41.86	1.37	39.37	43.27
C2	1726	1726	26.89	1677	1762	C2	24.24	23.77	1.26	22.72	26.40

Cont...

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	Mean	Median	SD	Minimum	Maximum		Mean	Median	SD	Minimum	Maximum
			Mn						**Na**		
F1	32.49	31.80	3.29	27.64	38.03	F1	249.5	247.8	9.83	236.0	266.3
F2	26.00	25.54	2.90	21.38	29.69	F2	118.3	118.3	6.55	110.0	130.1
R1	44.18	44.18	4.86	37.96	52.47	R1	267.8	269.1	11.70	250.7	285.9
R2	44.83	43.70	5.04	39.59	53.95	R2	896.4	902.9	26.56	843.2	929.4
C1	69.27	69.95	3.53	63.83	76.07	C1	94.27	96.46	7.82	81.63	104.7
C2	57.71	57.48	2.14	54.89	61.41	C2	253.0	256.1	8.97	240.3	270.4

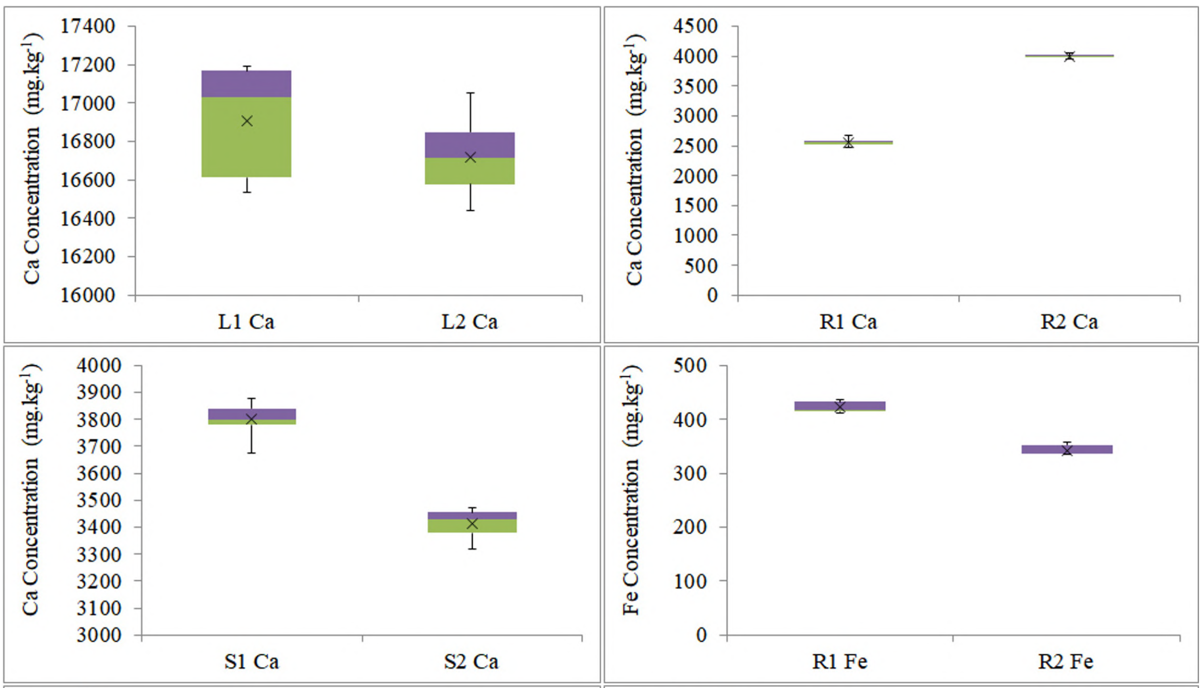
Legend: SD: Standard deviation; F1: Follicle germinated at 360 mg.kg^{-1}; F2: Follicle germinated at 720 mg.kg^{-1}; R1: Root germinated at 360 mg.kg^{-1}; R2: Root germinated at 720 mg.kg^{-1}; C1: Stem germinated at 360 mg.kg^{-1}; C2: Stem germinated at 720 mg.kg^{-1}.
Legenda: SD: Desvio padrão; F1: Folículo germinado a 360 mg.kg^{-1}; F2: Folículo germinado a 720 mg.kg^{-1}; R1: Raiz germinada a 360 mg.kg^{-1}; R2: Raiz germinada a 720 mg.kg^{-1}; C1: Caule germinado a 360 mg.kg^{-1}; C2: Caule germinado a 720 mg.kg^{-1}.

for all elements is presented in the supplementary material 1 and 2.

The significant correlations ($p<0.5$) of Ca, in the atmosphere of 360 mg.kg^{-1} CO_2, with the other elements were for Ca (root) with a negative correlation with Mn (stem) and Ca (stem) with a positive correlation with Fe (leaf).

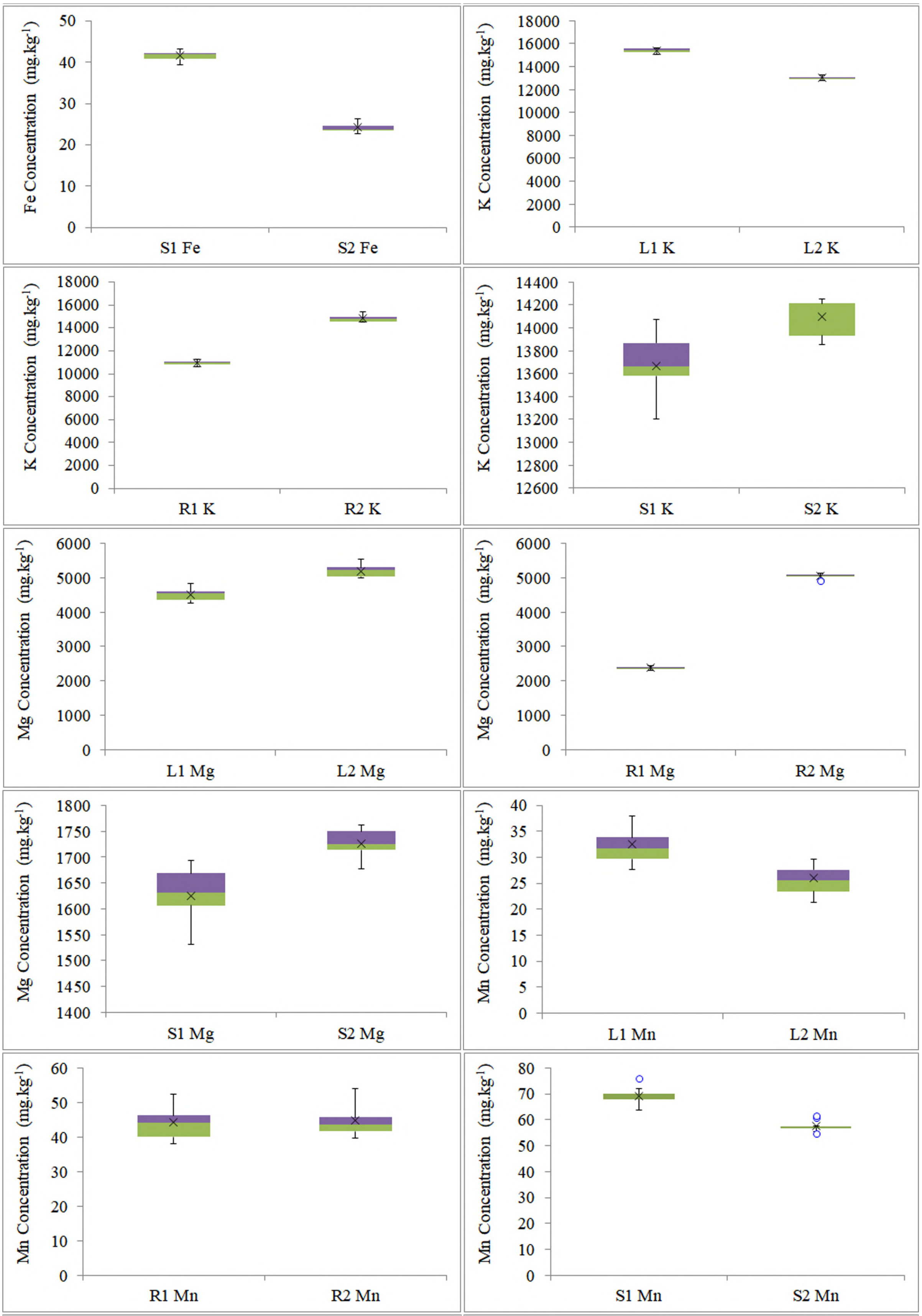
The significant correlations of Ca, in the atmosphere of 720 mg.kg^{-1} CO_2 with the other elements were for Ca (leaf) with a negative correlation with Fe (leaf), Ca (leaf) with a positive correlation with Mg (leaf) and Ca (root) with a positive correlation with Fe (stem).

Figure 1. Variability of the Ca, Fe, K, Mg, Mn and Na in *Senna reticulata* germinated at 360 and 720 mg.kg^{-1} of CO_2
Figura 1. Variabilidade de Ca, Fe, K, Mg, Mn e Na em *Senna reticulata* germinada com 360 e 720 mg.kg^{-1} de CO_2



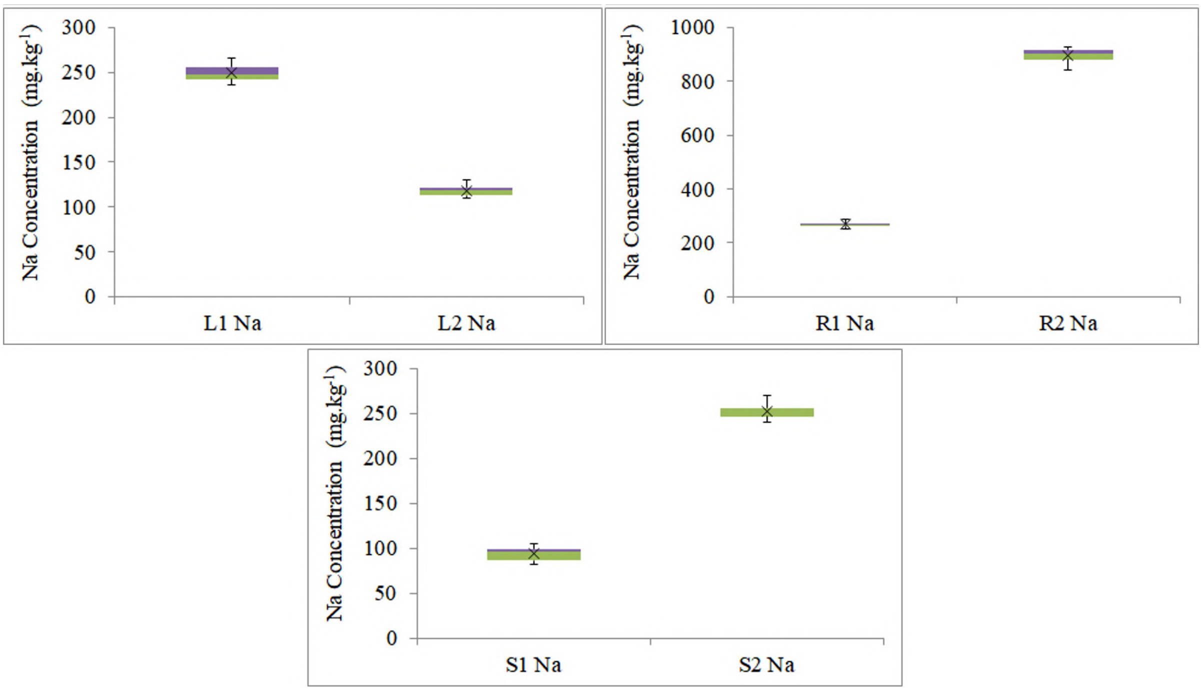
Cont...

...Cont.


Fe Concentration (mg.kg^{-1})
S1 Fe
S2 Fe
K Concentration (mg.kg^{-1})
L1 K
L2 K
K Concentration (mg.kg^{-1})
R1 K
R2 K
K Concentration (mg.kg^{-1})
S1 K
S2 K
Mg Concentration (mg.kg^{-1})
L1 Mg
L2 Mg
Mg Concentration (mg.kg^{-1})
R1 Mg
R2 Mg
Mg Concentration (mg.kg^{-1})
S1 Mg
S2 Mg
Mn Concentration (mg.kg^{-1})
L1 Mn
L2 Mn
Mn Concentration (mg.kg^{-1})
R1 Mn
R2 Mn
Mn Concentration (mg.kg^{-1})
S1 Mn
S2 Mn

Cont...

...Cont.



Legend: L: Leaf; R:Root; S: Stem; 1: CO_2 atmosphere at 360 $mg.kg^{-1}$; 2: CO_2 atmosphere at 720 $mg.kg^{-1}$
Legenda: L: Folha; R: Raiz; S: Caule; 1: Atmosfera de CO_2 a 360 $mg.kg^{-1}$; 2: Atmosfera de CO_2 a 720 $mg.kg^{-1}$

The change in atmosphere for Ca in the leaflets did not present averages that differed significantly by the simple factor ANOVA calculation (supplementary material 3). In all the other averages there were significant differences showing that the atmosphere altered the concentration of the element when the plant was subjected to a condition of 720 $mg.kg^{-1}$ CO_2.

For Fe, the variability was greater in the leaflets subjected to an atmosphere of 720 $mg.kg^{-1}$, with an anomalous result in the stem at 720 $mg.kg^{-1}$. The significant correlations of Fe ($p<0.05$), in the atmosphere of 360 $mg.kg^{-1}$ CO_2, were for Fe (leaf) with a positive correlation with Fe (stem), Fe (leaf) with Na (root), Fe (leaf) with Na (stem), Fe (stem) with Na (root), Fe (stem) with Na (stem) and Fe (stem) negative correlation with Mg (leaf). The significant correlations of Fe in the atmosphere of 720 $mg.kg^{-1}$ CO_2 were for Fe (leaf) with a positive correlation with Na (stem), Fe (root) with Mn (stem) and with a negative correlation of Fe (stem) with Na (root).

The behavior of Fe in *Senna reticulata* germinated at 360 and 720 $mg.kg^{-1}$ CO_2 was of reduction in its concentration in all parts, when the plant was subjected to an atmosphere of 720 $mg.kg^{-1}$ CO_2. In the leaflet the reduction in Fe concentration was 1.99%, in the root the reduction was 19.16% and in the stem 41.73%.

In the single-factor ANOVA analysis (supplementary material 3), Fe presented means does not differ significantly only for the leaflets in both atmospheres. The influence of the atmosphere on the root and stem was confirmed by the ANOVA calculation which showed that all the means differed significantly.

The behavior of K in *Senna reticulata* germinated at 360 and 720 $mg.kg^{-1}$ CO_2 was reduced by 15.40% in the leaf when the plant was subjected to an atmosphere of 720 $mg.kg^{-1}$ CO_2. There was a 35.42% increase in concentration in the root and a 3.17% increase in the stem. In the single factor ANOVA analysis, K showed means that did not differ significantly when comparing the means of the concentrations of the plant parts in both atmospheres. The variability of the K concentration was greater in the stem subjected to an atmosphere of 360 mg.kg^{-1} with no extreme or anomalous results.

K did not correlate significantly with the other elements ($p<0.05$), with the exception of K (leaf) with a negative correlation with Mn (leaf), in the atmosphere of 360 mg.kg^{-1} CO_2. In the atmosphere of 720 mg.kg^{-1} CO_2 the K (leaf) showed positive correlations of with Mn (root) and K (root) with a positive correlation with Mn (leaf).

The influence of the atmosphere on the leaf, root and stem was confirmed by the ANOVA calculation (supplementary material 3), for the elements K, Mg, and Na, which showed that all means differed significantly for all parts evaluated.

For Mg, all parts of *Senna reticulata* increased their concentration. In the leaflet the increase was 15.06%, in the root 111.46% and in the stem 6.15%, when the plant was subjected to an atmosphere of 720 mg.kg^{-1} CO_2 proving that the increase in CO_2 in the atmosphere favored the absorption of the element by the plant.

The greatest increase in Mg occurred in the roots, with a gain of over 100%, a greater increase than that shown by Ca and K. The change of atmosphere altered the Mg results in all the parts evaluated, as proven by the ANOVA calculation which showed that all the averages were different. The greatest variability in Mg in *Senna reticulata* occurred in the leaflet subjected to an atmosphere of 720 mg.kg^{-1} CO_2, with anomalous results for the root in the 720 mg.kg^{-1} atmosphere.

The significant correlations of Mg, in the atmosphere of 360 mg.kg^{-1} CO_2, were for Mg (root) with a negative correlation with Mn (stem), Mg (leaf) with a negative correlation with Na (root), Mg (leaf) with a negative correlation with Na (stem), Mg (stem) with a positive correlation with Na (stem). In the 720 mg.kg^{-1} atmosphere, Mg (stem) correlated positively with Mn (leaf) and there was a negative correlation between Mg (leaf) and Na (stem).

For Mn there was a 1.47% increase in its concentration only in the root when the plant was subjected to an atmosphere of 720 mg.kg^{-1} CO_2, in the other parts there was a reduction in the concentration of the element when the concentration of CO_2 was increased. There was a 19.97% reduction in its concentration in the leaf and a 16.69% reduction in its concentration in the stem.

The greatest variability of results for Mn was for the root in the atmosphere of 360 mg.kg^{-1} CO_2, with anomalous results in the stem in both atmospheres and in the root at 720 mg.kg^{-1} CO_2.

Mn did not show significant correlations with the other elements in the atmospheres of 360 mg.kg^{-1} and 720 mg.kg^{-1} CO_2. It was observed that for Na, there was a 52.59% reduction in the content of the leaflets alone as the concentration of the element increased by 234.73% for the root and 168.38% for the stem.

The increase in Mn was not significant according to ANOVA calculation, according supplementary material 3, only in the root, since the averages did not differ significantly. For all the other parts there was a significant reduction, which indicated the influence of the increase in CO_2 for the leaflet and stem in relation to the element.

The Na averages differed significantly for all parts of *Senna reticulata* germinated at 360 and 720 mg.kg^{-1} CO_2, indicating that the change in the CO_2 atmosphere altered the plant's Na absorption. The greatest variability in Na was for the root subjected to the atmosphere of 720 mg.kg^{-1} CO_2, with an anomalous result in the stem at 720 mg.kg^{-1} CO_2.

The significant correlations of Na in the atmosphere of 360 mg.kg^{-1} CO_2 were for Na (root) with a positive correlation with Na (stem) and in the atmosphere of 720 mg.kg^{-1} CO_2 Na (leaf) correlated positively with Na (root).

Table 2 shows the most significant factors in the concentration results of the elements analyzed at 360 and 720 mg.kg^{-1} CO_2 when applying multivariate analysis to the results found.

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Based on this data, the most significant factors are 1 and 2, which account for 99.07% of all the factors added together. For factors 1 and 2, the elements that contribute most to their importance are shown in Table 3.

Table 2. Contribution of statistical factors of *Senna reticulata* germinated at 360 and 720 mg.kg^{-1} of CO_2
Tabela 2. Contribuição dos fatores estatísticos da *Senna reticulata* germinada a 360 e 720 mg.kg^{-1} de CO_2

Factor	Eigenvalue	% Total	Eigenvalue accumulation	% Total accumulation
		360 mg.kg^{-1}		
1	3.42	57.03	3.42	57.03
2	2.52	42.04	5.94	99.07
3	0.035	0.589	5.98	99.66
4	0.010	0.174	5.99	99.83
5	0.007	0.119	6.00	99.95
6	0.003	0.049	6.00	100.0
		720 mg.kg^{-1}		
1	3.36	56.05	3.36	56.05
2	2.54	42.40	5.91	98.44
3	0.046	0.771	5.95	99.21
4	0.043	0.719	6.00	99.93
5	0.003	0.050	6.00	99.98
6	0.001	0.017	6.00	100.0

Table 3. Contribution of elements to the importance of factors for *Senna reticulata* germinated at 360 and 720 mg.kg^{-1} of CO_2
Tabela 3. Contribuição dos elementos na importância dos fatores para *Senna reticulata* germinada a 360 e 720 mg.kg^{-1} de CO_2

Element	360 mg.kg^{-1} CO_2		720 mg.kg^{-1} CO_2	
	Factor 1	Factor 2	Factor 1	Factor 2
Ca	-0.918	-0.390	-0.963	0.247
Fe	-0.037	0.997	0.382	0.922
K	-0.558	-0.827	0.966	0.187
Mg	-0.993	-0.070	-0.341	0.936
Mn	0.905	-0.396	0.797	-0.579
Na	-0.678	0.729	0.778	0.623

4. DISCUSSION

Senna reticulata is popularly known as mata pasto in the Amazon region. Due to its high capacity for competition, growth and establishment in pastures and open areas, it is considered an invasive species that is periodically eliminated in order to preserve the cultivation of grasses. This species can reach up to 12 meters, but individuals that exceed 8 meters are rarely found, due to the pruning control carried out to maintain pastures (Arenque et al., 2014).

Senna reticulata has the highest photosynthetic assimilation measured in the trees of the Amazon plain. Mature leaves are composed of 8 to 14 pairs of leaflets which are oval-oblong, obtuse, glabrous on both sides, and thin (Parolin, 2005). According to the results of the elements evaluated, there was irregular behavior between the elements for the two CO_2 atmospheres studied.

For Ca there was a reduction in its concentration in an atmosphere of 720 $mg.kg^{-1}$ and this could affect its function as a secondary messenger. Ca can bind to calmodulin, a protein found in the cytosol of plant cells, this calmodulin-Ca complex regulates many metabolic processes, with a reduction in Ca these processes are compromised (Yamniuk et al., 2007).

Characteristic symptoms of Ca deficiency include necrosis of young meristematic regions, such as root apices or young leaves, in which cell division and wall formation are faster (Machado et al., 2014).

Necrosis in slow-growing plants can be preceded by generalized chlorosis and a downward curving of the leaves. Young leaves may also appear deformed. The root system of a Ca deficient plant may appear brownish, short and highly branched. There can be a severe reduction in growth if the meristematic regions of the plant die prematurely (Taiz et al., 2016; Wang et al., 2020).

Ca ions are cofactors for various enzymes, but rarely if ever with much specificity. Ca can competitively inhibit the activating effect of Mg, evidently by displacing it from its functional sites (Jeong et al., 2020).

Ca is indispensable for pollen grain germination and pollen tube growth, which may be due to its role in cell wall synthesis or the functioning of the plasmalemma (Kim et al., 2019).

Most of the total Ca in leaves is present in chloroplasts; the accumulation of the element in these organelles is dependent on energy supply, as is the case with mitochondria (Carraretto et al., 2016).

The regulation of gene expression by cellular Ca is also crucial for plant defense against various stresses. When the level of Ca increases, it is recognized by some sensors or Ca-binding proteins, which can activate element-dependent kinases. These kinases regulate the function of many genes, such as those regulating stress tolerance. Ca is also involved in regulating cell cycle progression in response to abiotic stress (Tuteja & Mahajan, 2007).

The behavior of K in *Senna reticulata* showed leaf reduction when subjected to an atmosphere of 720 $mg.kg^{-1}$. The first visible symptom of K deficiency is chlorosis in spots or margins, which then develops into necrosis, mainly at the leaf apexes, margins and between the veins. In many monocots, these necrotic lesions can form initially at the leaf apexes and margins and then extend towards the base (Asif et al., 2017).

As K can be remobilized to the younger leaves, these symptoms initially appear on the more mature leaves at the base of the plant. The leaves may also curl and dry out. The stems of K-deficient plants can be thin and weak, with abnormally short internodal regions. In K-deficient maize, the roots may have an increased susceptibility to root rot fungi present in the soil, and this susceptibility, together with the stem defects, results in a greater tendency for the plant to topple to the ground (lodging) (Taiz et al., 2016).

K plays an important role in regulating the osmotic potential of plant cells. It also activates many enzymes involved in respiration and photosynthesis (Shah et al., 2024). K is the only monovalent cation essential for all higher plants and, in fact, for all living beings, except for some microorganisms where Ru can replace it (Sardans & Peñuelas, 2021). Unlike Ca, K is highly mobile in the phloem. Its utilization is therefore efficient in the sense that it is readily redistributed from the older leaves to the new growing organs. As a result, deficiency symptoms appear first in the older leaves (Lucas et al., 2013).

For Mg, all the parts of *Senna reticulata* increased their concentration in the two atmospheres evaluated. Mg was the only element in which there was unanimity of behavior in the parts analyzed, showing an increase in concentration in the two atmospheres studied.

Singh et al. (2020) found that Mg

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concentrations were generally lower in plants grown in an environment supplemented with CO_2, but the results were not consistent for each species evaluated. The Mg concentration was reduced only for B*eta vulgaris* L. (chard) when CO_2 was supplemented, in the other species studied *Ocimum basilicum* L. (basil) and *Lactuca sativa* L. (lettuce), the Mg concentrations increased.

Mg activates more enzymes than any other element. It is a cofactor for almost all enzymes that act on phosphorylated substrates, forming a bridge between ATP pyrophosphate or ADP (adenosine tri- and diphosphate, respectively) and the enzyme molecule (Kleczkowski & Decker, 2022).

The transfer of these two compounds is fundamental in the processes of photosynthesis, respiration, organic compound synthesis reactions (carbohydrates, lipids, proteins), ion absorption and mechanical work carried out by the plant. In some phosphate group transfer reactions, Mg^{+2} can be replaced by other ions, such as Mn^{+2}, mainly and to a lesser extent by other divalent cations. However, it is often more efficient than its substitute (Alejandro et al., 2020).

Chlorophyll is the compound responsible for capturing sunlight and ensuring that photosynthesizing organisms are able to produce their food through the process of photosynthesis. It was possible to observe a positive correlation between Mg and the increase in CO_2. Since Mg is the central element of chlorophyll, it was to be expected that there would be a positive correlation between the increase in CO_2 and the increase in the element, since plants use CO_2 in photosynthesis (Yilmaz et al., 2017).

The behavior of Fe in *Senna reticulata* was a reduction in its concentration in all parts when the plant was subjected to an atmosphere of 720 $mg.kg^{-1}$ CO_2. Singh et al. (2020) subjected *Ocimum basilicum* L. (basil), *Lactuca sativa* L. (lettuce) and *Beta vulgaris* L. (chard) to 2 atmospheres of CO_2 (800 $mg.kg^{-1}$ and 410 $mg.kg^{-1}$) and found that the concentration of Fe was increased under CO_2 supplementation only for *Ocimum basilicum* L. (basil). In the other species there was a reduction in Fe proving what was found in this study for *Senna reticulata*.

Fe is an essential micronutrient for plants, since many cellular processes, including photosynthesis, respiration and the elimination of reactive oxygen species depend on adequate levels of Fe, however, uncomplexed Fe ions can be dangerous for cells, as they can act as pro-oxidants (Murgia et al., 2022).

A characteristic symptom of Fe deficiency is inner-rib chlorosis, such symptoms initially appearing in young leaves because Fe cannot be readily mobilized from older leaves. Under conditions of extreme or prolonged deficiency, the veins can also become chlorotic, causing the entire leaf to turn white (Taiz et al., 2016). When there is a deficiency of this micronutrient, the chlorophyll content drops, the number of chloroplasts decreases and there is less green in the chloroplasts, as much of the Fe in the leaf is in the chloroplasts (Li et al., 2021).

Rodriguez et al. (2011) evaluated the effects of high concentrations of CO_2 (400 and 600 $mg.kg^{-1}$) on some essential and non-essential elements in the roots, stems and seeds of *Glycine max* (L.) Merr (soybean). The results showed that at high levels of CO_2 the plant showed an increase in biomass. For most of the elements analyzed, the accumulation in the plants was greater at high levels of CO_2 than at ambient concentrations. The highest concentrations of Fe were found in the roots, a different conclusion to this study which found a reduction in Fe in this part of *Senna reticulata*.

For Mn, there was a reduction in the concentration of the element when the concentration of CO_2 was increased, with the exception of the root subjected to an atmosphere of 720 $mg.kg^{-1}$ CO_2, where there was an increase. Rodriguez et al. (2011) found that the highest concentrations of Mn were found in the roots, when they studied the effect of CO_2 at 400 and 600 $mg.kg^{-1}$ on the roots, stems and seeds of *Glycine max* (L.) Merr (soybean), in line with the results found in this study.

Mn ions activate various enzymes in plant cells. Decarboxylases and dehydrogenases involved in the tricarboxylic acid cycle (Krebs cycle) in particular are specifically activated by Mn (Schmidt & Husted, 2019). The best defined function of Mn is that of photosynthetic reduction by which oxygen is produced from water. The main symptom of Mn deficiency is inner-vein chlorosis, associated with the development of small necrotic spots. This chlorosis can occur in young or older leaves, depending on the plant species and growth rate (Taiz et al., 2016).

The metabolic disturbance caused by Mn deficiency is severe; several metabolites are affected. This is to be expected; Mn plays an important role in many reactions of the Krebs cycle and, due to its central position in respiration, a deficiency of this element has repercussions on other metabolic consequences (Schmidt & Husted, 2019).

Mn and other heavy metal ions, when present in the environment in high concentrations, can induce iron deficiency in plants. This phenomenon depends on competitive effects on iron absorption and translocation as well as competition for functional sites that bind to iron (Angulo-Bejarano et al., 2021). There was a reduction in Na only in the leaves as the concentration increased in the other parts of the plant.

Most species that use the carbon fixation pathways require Na ions. In these plants, Na appears to be vital for the regeneration of phosphoenolpyruvate, the substrate for the first carboxylation in the C4 and CAM pathways (Kronzucker et al., 2013).

Under Na deficiency, these plants show chlorosis and necrosis or stop flowering. Many C3 species also benefit from exposure to low levels of Na ions. Na stimulates growth through greater cell expansion and can partially replace potassium as an osmotically active solute (Taiz et al., 2016).

The multivariate analysis showed that at a concentration of 360 $mg.kg^{-1}$ CO_2 for factor 1, the most significant metals were Ca, Mg and Mn, while for factor 2, Fe and K were the most important. In this case, Na did not show a value above 0.75, but was very close, and was therefore of lower value than the others.

In the 720 $mg.kg^{-1}$ atmosphere, the behavior was also similar to that of the plants germinated at 360 $mg.kg^{-1}$ CO_2, with the factors that contributed most being numbers 1 and 2, which represent 98.44% of all the factors; the others were therefore disregarded for the analysis of the main components. In this case, the metals Ca, K, Mn and Na are more influential in factor 1, while in factor 2, the metals Fe and Mg are given priority. In the PCA representation, 3 groups were separated, represented by the leaf, root and stem groups in both atmospheres.

5. CONCLUSION

The increase in CO_2 concentration was not significant for Ca and Fe in the leaflet and for Mn in the root. However, for the other elements and other parts of *Senna reticulata*, the increase in CO_2 concentration significantly altered the evaluated means.

The results showed that the species did not remain indifferent when subjected to different levels of CO_2 concerning the studied nutrients. This indicates that in the future, this species will be adapted to absorb larger amounts of Na, K, Ca, and Mg through the roots, which will likely cause nutrient deficiencies in the soil due to the increased demand by the plant. Consequently, this will lead to greater competition among individuals.

The root was the only part analyzed that showed a growth trend for most of the evaluated elements, with the exception of Mn, which did not undergo significant changes, and Fe, which showed a slight reduction. This may indicate that the number of stomata decreased due to the lack of necessity to absorb CO_2 in the same proportion, given that the availability of this gas is twice as high. This would also suggest that the plant transpires less, as there are fewer stomata and even fewer open stomata. In this case, there is likely an accumulation of nutrients in the root due to reduced transpiration and consequently less upward movement within the plant.

Regarding the variation of the other elements, it is not possible to precisely assert a trend of increase or decrease for each metal; there may simply be a redistribution of elements throughout the plant.

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AUTHOR CONTRIBUTIONS

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