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# ACCUMULATION AND PARTITION OF Fe, Zn, Cu, MN AND Na IN MACRO AND MICRONUTRIENT-DEFICIENT COWPEA PLANTS

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## ABSTRACT

Cowpea (*Vigna unguiculata*) is an important crop for people living in the semi-arid tropics where it is used as food, animal feed and forage. Researches related to nutritional status of plants are important, principally in crops commonly grown in nutrient-poor soils. This study aimed to analyze the growth and accumulation/distribution of some micronutrients in cowpea plants submitted to macro and micronutrient deficiency. Cowpea seeds were germinated in grit and after five days, ten uniform seedlings were transferred to complete nutrient solution for an acclimatization period. After three days, the plants were grown in nutrient solution containing all macro and micronutrients or in nutrient solution without N, Ca, K, Mg, P, S, Fe, B or aeration. In all case, omission of mineral nutrients and the aeration caused reductions in the dry mass of cowpea plants, except for B; however, the absence of Ca was the most limiting for plant growth. Interestingly, the root Fe content significantly increased in Mg-, P- and S-deficient plants. Yet, Fe-deficient plants displayed a significant increase in Cu and Mn content, regardless of plant organ. Our data clearly demonstrate that the Ca is the most important nutrient for *V. unguiculata* growth, followed by N and Fe. The accumulation and partition of Fe, Zn, Cu and Mn in cowpea vary differentially in root, stem and leaf as affected by nutritional deficiency.

**Key words:** nutrient accumulation; nutritional deficiency; *Vigna unguiculata.*

## Academic Discipline And Sub-Disciplines

Plant Physiology

## SUBJECT CLASSIFICATION

Plant Sciences

## TYPE (METHOD/APPROACH)

Scientific research

## INTRODUCTION

Mineral nutrients are essential components for normal plant growth and metabolism. Several elements are essential for maintaining the electrochemical equilibrium among cell compartments [24] and cell osmotic potential [4]; [8]; whereas others play a vital role as cofactors in biochemical reactions [16]; [1] and as structural components in complex biological molecules [10]. Nonetheless, previous reports have shown that the overaccumulation of some nutrients may cause deleterious effects on cell through disrupt the biochemical and physiological processes [10].

In soils and plants, changes in mineral nutrient content are common, which can mainly occur because of (i) alterations in soil chemical environment caused by plants (acidification or releasing of organic compounds) or environmental factors (rain, animals, deposition of organic matter, among others), (ii) alterations in plant morphology, including the root structure and the plant developmental stage, (iii) changes in the plant's capacity to uptake ions and promote allocation of them to specific cell compartments, and (iv) variations in the content of chelators, such as organic acids, peptides and proteins [6] [8]; [10]; [15].

In response to nutritional imbalance, plants exhibit an elaborate and complex network of genetic and physiological alterations, which control the absorption, transport and accumulation of mineral nutrients. When a given element is absent or insufficient, plants change the content of other nutrients in an attempt to partially overcome its limitation [2].

Cowpea (*Vigna unguiculata*) is an important crop for people living in the semi-arid tropics where it is used as food, animal feed and forage. It is the principal legume grown in the Northeast of Brazil [18] and cowpea grains are rich in proteins and iron. Besides, cowpea plants are characterized by their excellent ability to adapt to drought and to fix nitrogen, and are relatively undemanding with regard to soil fertility [13].

There are many studies aimed at understanding plant responses to nutritional imbalance [8]; [9]; [11]; [14]; [23]. However, there is no report regarding the physiological processes that lead to nutritional balance in cowpea plants subjected to nutrient deficiency conditions. Therefore, our investigative study aimed to analyze the nutritional status of


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cowpea plants under deficiency of essential mineral elements, and more specifically, to evaluate the accumulation and partition of some micronutrients in roots, stems and leaves.

## MATERIALS AND METHODS

Cowpea [*Vigna unguiculata* (L.) Walp.] seeds of Pitiúba cultivar were sown in grit washed with distilled water. After five days, the seedlings were transferred to a 10 L tray containing half-strength Hoagland's nutrient solution, and acclimated for 3 days. At eight days after sowing, uniform seedlings were transferred to 3.5 L pots (one plant per pot), and grown in complete nutrient solution (N, Ca, K, Mg, P, S and micronutrients) or in nutrient solution without nitrogen, calcium, potassium, magnesium, phosphorus, sulfur, boron or iron. To characterize the aeration lack effects, plants growing in nutrient solution (containing all macro and micronutrients) were carried without aeration. All nutrient solutions were renewed weekly. Solution pH values were checked daily and adjusted to 5.5-6.0 with 1.0 M NaOH or 1.0 M HCl, as needed. The experiment was carried under greenhouse conditions during 21 days after start the treatments.

Three plants from each treatment were individually harvested and divided into roots, stems and leaves. The plant material was put in paper bags and placed in an oven at 65°C, until constant weight and; thereafter, the dry mass (g) of roots, stems and leaves was determined. For the determination of mineral nutrients, 0.5 g of plant material was submitted to nitro-perchloric digestion at 3:1 (v/v). The Fe, Zn, Cu and Mn contents were determined by atomic absorption spectrometry [19], while the Na contents were determined by flame photometry [12]. The total content of each micronutrient was calculated taking into account the dry mass and the micronutrient content of the roots, stems and leaves.

The experimental design was completely randomized with three replicates, being each experimental unit composed by one plant. The data were subjected to analysis of variance (test *F*) and the means were compared using Tukey's test at 5% probability. Statistical analyzes were performed using the SISVAR program [3].

## RESULTS AND DISCUSSION

The nutritional deficiency promoted significant changes in the dry mass of cowpea plants (Table 1). The absence of N, Ca and Fe in the growth medium severely decreased the dry mass of roots, stems and leaves, and, consequently, the total dry mass; however, the most deleterious effects of nutrient deficiency was registered in Ca-deficient plants. The K, Mg, P and S omissions in the nutrient solution, in turn, caused conspicuous less reduction in dry mass of the different organs. Surprisingly, the absence of B did not alter the plant biomass in comparison to control, except for root dry mass (Table 1). In concordance with, the N and Ca omissions in the nutritive solution were the treatments most damaging to productivity of *Spondias tuberosa*, with the latter being the most limiting [5]. Otherwise, our findings disagree with those found in *Myrciaria dubia* [23], in which Ca deficiency was not harmful for plant growth.

**Table 1. Dry mass of cowpea plants grown in complete nutrient solution (Control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg), phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) or aeration.**

| Treatment | Dry mass (g/plant) | | | |
|---|---|---|---|---|
| | Roots | Stem | Leaves | Total |
| Control | 1.37a | 0.43ab | 3.86[a] | 5.65a |
| - N | 0.34d | 0.10d | 0.31d | 0.75de |
| - Ca | 0.08e | 0.04d | 0.15d | 0.27e |
| - K | 0.72bc | 0.17cd | 1.62bc | 2.51bc |
| - Mg | 0.64c | 0.20cd | 1.93b | 2.76b |
| - P | 0.66c | 0.12d | 0.77cd | 1.55cd |
| - S | 0.73bc | 0.32bc | 1.74b | 2.79b |
| - B | 0.94b | 0.51[a] | 3.27[a] | 4.71a |
| - Fe | 0.22de | 0.14d | 0.54d | 0.90de |
| - Aeration | 0.76bc | 0.42ab | 2.02b | 3.20b |
| F | 67.250** | 21.970** | 52.068** | 54.906** |
| CV (%) | 12.11 | 24.50 | 18.32 | 16.14 |

Mean values followed by same letter in the same column do not differ by Tukey's test ($p \leq 0.05$). ** ($p < 0.01$)

Accumulation of Fe, Zn, Cu, Mn and Na in cowpea plants was differentially modulated by nutritional deficiency as compared to control plants. The most striking effects in the Fe content occurred in roots of the S-deficient plants, whose mean accumulation values were 10-fold higher than that observed in the control plants (Table 2). Except for plants grown without aeration, which showed a stem Fe content smaller than that in the control plants, the deficiency of all macro and

micronutrients did not promoted significantly differences in the Fe content of stems and leaves as related to controls (Table 2). In plants grown without N, P, S and Fe, the root was the organ which accumulated more Fe, whereas in control plants and in plants grown without Ca, K, Mg, B and aeration, the leaves accumulated more than 50% of total Fe (Fig 1).

**Table 2. Iron (Fe) and zinc (Zn) contents of cowpea plants grown in complete nutrient solution (Control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg), phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) or aeration.**

| Treatment | Fe content (mg kg$^{-1}$) | | | | Zn content (mg kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Roots | Stem | Leaves | Total | Roots | Stem | Leaves | Total |
| Control | 82c | 158abc | 179ab | 153bc | 64[a] | 58ab | 65b | 64ab |
| - N | 223bc | 206a | 132ab | 184bc | 45[a] | 77a | 157a | 96a |
| - Ca | 95c | 37cd | 176ab | 131c | 24[a] | 82a | 42b | 43bc |
| - K | 81c | 47bcd | 238a | 182bc | 52[a] | 37ab | 43b | 46bc |
| - Mg | 417b | 177ab | 204ab | 251b | 38[a] | 46ab | 28b | 32bc |
| - P | 386b | 158abc | 178ab | 265ab | 28[a] | 36ab | 44b | 37bc |
| - S | 916a | 50bcd | 205ab | 374[a] | 45[a] | 60ab | 32b | 38bc |
| - B | 236bc | 53bcd | 107ab | 128c | 32[a] | 36ab | 26b | 28c |
| - Fe | 232bc | 202a | 54b | 119c | 58[a] | 67ab | 56b | 58bc |
| - Aeration | 265bc | 24d | 145ab | 157bc | 34[a] | 14b | 22b | 24c |
| F | 38.492** | 8.172** | 3.071* | 12.528** | 1.489ns | 3.579** | 5.528** | 9.286** |
| CV (%) | 23.59 | 40.62 | 32.86 | 20.17 | 44.42 | 37.59 | 56.33 | 26.00 |

Mean values followed by same letter in the same column do not differ by Tukey's test ($p \leq 0.05$). ** ($p < 0.01$) * ($p < 0.05$)

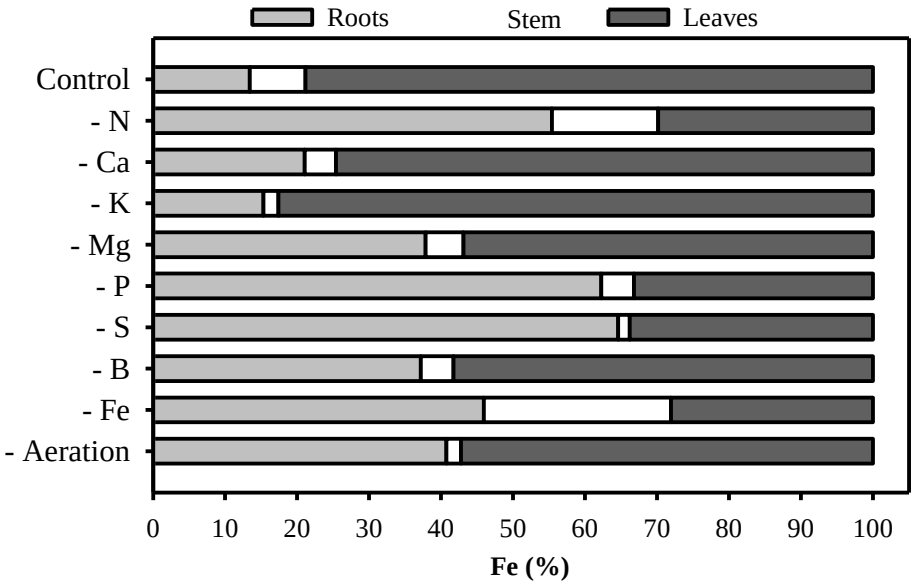


**Figure 1.**

Percentage accumulation of iron (Fe) in roots, stems and leaves of cowpea plants grown in complete nutrient solution (control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg), phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) or aeration. Measurements were performed at 21th day after starting deficiency treatments.

Iron and sulfur constitute Fe-S proteins, acting in electron transfer reactions for energy conservation in the electron transport chain [10]. Probably, the S deficiency reduced the iron demand, particularly in leaves, which resulted in the higher accumulation of Fe in roots (Fig 1 and Table 2). Our results differ from those observed in *Ricinus communis*, in which the Fe content was significantly reduced in plants grown without this nutrient [9].

The total Zn content was more affected in plants grown without B and aeration, as shown in Table 2. Nutrient deficiency did not affect the Zn content in roots, stems and leaves, except for leaf Zn content of plants grown in N absence, which showed a value 142% higher than that in control. In addition, the highest accumulation of Zn was registered in the leaves of cowpea plants, regardless of treatment (Fig 2). Zinc has an important function as a prosthetic group of $N_2$-fixing enzymes [10]. Thus, the highest Zn content in leaves of N-deficient plants can be correlated with the high demand for nitrogen assimilation.

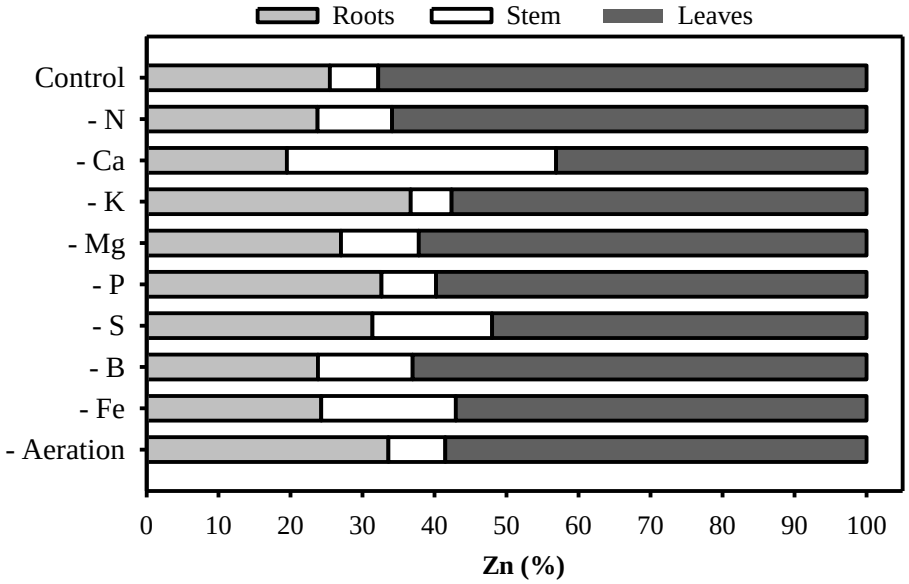


**Figure 2.** Percentage accumulation of zinc (Zn) in roots, stems and leaves of cowpea plants grown in complete nutrient solution (control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg), phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) and aeration. Measurements were performed at 21th day after starting deficiency treatments.

Plants grown in the nutrient solutions without K, Mg, P, S, B and aeration showed no significant changes in the total Cu content in comparison to control (Table 3). On the other hand, plants grown without N, Ca and Fe showed increases in total Cu content, and the highest content of Cu was observed in roots, stems and leaves of Fe-deficient plants. Under control conditions, it was only possible to detect the Cu content in roots, which contributed 100% for the total Cu content (Fig 3 and Table 3). In contrast, in the plants deficient of K, Mg, P, S, B and without aeration, the stems were the main organ that contributed to total Cu content, while in the Ca-deficient plants the leaves were the main contributor. Similarly, the roots were the main accumulator organ of Cu in *Psidium guajava* under normal growth conditions [17].

**Table 3. Copper (Cu) and manganese (Mn) contents of cowpea plants grown in complete nutrient solution (Control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg), phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) or aeration.**

| Treatment | Cu content (mg kg$^{-1}$) | | | | Mn content (mg kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Roots | Stem | Leaves | Total | Roots | Stem | Leaves | Total |
| Control | 4.1b | 0.0c | 0.0c | 1.0c | 1148bc | 57bcde | 339c | 513bc |
| - N | 11b | 14bc | 13b | 12b | 1192bc | 88b | 643b | 822b |
| - Ca | 0.0b | 9.4bc | 19ab | 12b | 256c | 22e | 78e | 121d |
| - K | 3.8b | 25b | 1.7c | 3.9c | 1605b | 88b | 547b | 831b |
| - Mg | 0.0b | 8.1bc | 0.3c | 0.8c | 1533b | 82bc | 306cd | 572bc |
| - P | 0.0b | 13bc | 0.0c | 1.0c | 658bc | 49cde | 231cde | 399cd |
| - S | 1.1b | 8.4bc | 0.0c | 1.2c | 782bc | 71bcd | 346c | 430cd |
| - B | 0.0b | 12bc | 0.0c | 1.3c | 970bc | 37de | 257cde | 376cd |
| - Fe | 59a | 51a | 22a | 35a | 2236[a] | 196a | 1189a | 1275a |
| - Aeration | 0.0b | 20bc | 0.0c | 2.5c | 522bc | 21e | 136de | 215cd |
| F | 28.854** | 8.358** | 38.389** | 96.612* | 5.006** | 48.280** | 65.785** | 22.534** |

| | | | | * | | | | |
|---|---|---|---|---|---|---|---|---|
| CV (%) | 74.14 | 52.39 | 44.13 | 26.82 | 41.69 | 17.78 | 16.95 | 22.38 |

Mean values followed by same letter in the same column do not differ by Tukey's test ($p \leq 0.05$). ** ($p < 0.01$)

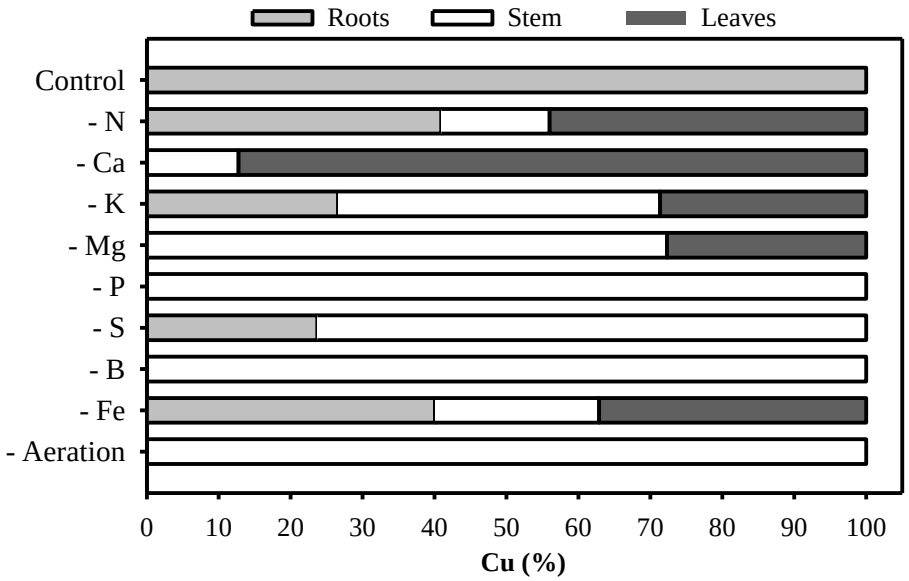


**Figure 3.** Percentage accumulation of copper (Cu) in roots, stems and leaves of cowpea plants grown in complete nutrient solution (control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg), phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) and aeration. Measurements were performed at 21th day after starting deficiency treatments.

The total, root, stem and leaf Mn content significantly increased in Fe-deficient plants when compared to control plants (Table 3), suggesting that the Mn is an important nutrient in attempt to partially overcome the Fe limitation. In addition, although the root in Fe-deficient plants have showed the highest accumulation of Mn in cowpea plants, the leaves were the main organ which contributed to total Mn content (Fig 4). On the other hand, the leaf Mn content increased in the N- and K-deficient plants and while it decreased in the ones grown without Ca and aeration (Table 3).

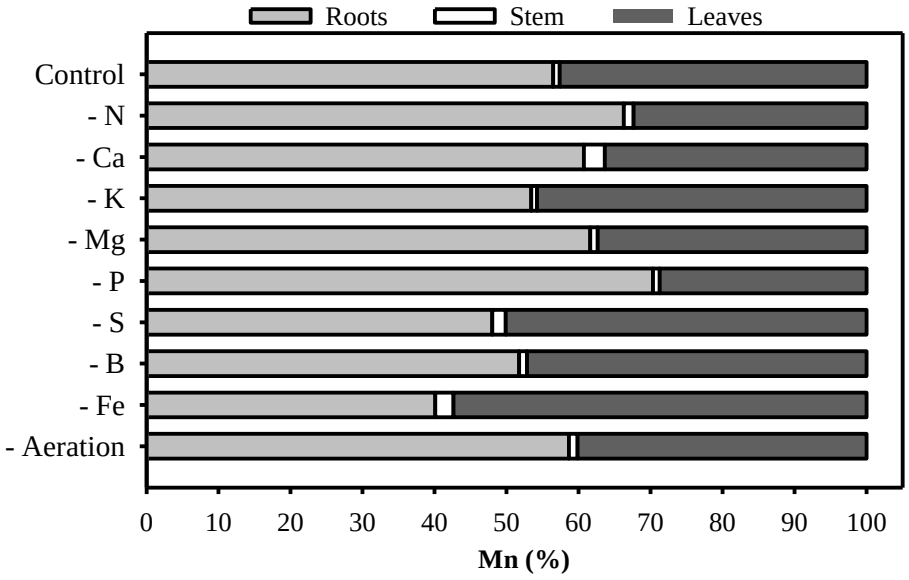


**Figure 4.** Percentage accumulation of manganese (Mn) in roots, stems and leaves of cowpea plants grown in complete nutrient solution (control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg), phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) or aeration. Measurements were performed at 21th day after starting deficiency treatments.

The iron ion is part of the heme groups of cytochromes and it makes up the active site (as prosthetic group) of nitrogenases together with molybdenum. Besides, the iron is extremely important in nitrogen assimilation (in the conversion of nitrate to ammonium), photosynthesis and respiration [21]. The Fe transport in the xylem is driven by transpiration and the internal redistribution of Fe hardly occurs in developing and adult plants [20]. Several biochemical reactions and physiological processes are affected in Fe-deficient plants. In this work, we observed a significant increase in the micronutrient content in Fe-deficient plants, mainly Cu and Mn, in an attempt to compensate the Fe deficiency (Figs 3, 4 and Table 3). It may be explained by the function of Cu and Mn, which are required for activity of several enzymes and component of proteins involved in redox reactions, such as cytochrome oxidase and plastocyanin [21]. Similarly, *Ricinus communis* plants also displayed a massive increase in Mn accumulation under deficiency of Fe [9].

The total Na content of cowpea plants was not altered by both nutritional deficiency and lack of aeration (Table 4). The contents of Na in the root and stem were increased by absence of Ca, K and aeration, except for root of Ca-deficient plants. In contrast, in leaves, only the Fe-deficient plants showed significant increases in the Na content. In the N-, K- and P-deficient plants, the roots were the organs most accumulated; whereas in the plants from other treatments, the highest Na accumulation occurred in the leaves (Fig 5).

**Table 4. Sodium (Na) content of cowpea plants grown in complete nutrient solution (Control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg), phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) or aeration.**

| Treatment | Na content (mg kg$^{-1}$) | | | |
|---|---|---|---|---|
| | Roots | Stem | Leaves | Total |
| Control | 1127c | 833c | 633b | 768a |
| - N | 1133c | 960c | 720ab | 942a |
| - Ca | 1207bc | 1887[a] | 780ab | 1072a |
| - K | 2287a | 1580ab | 547b | 1113a |
| - Mg | 1220bc | 1233bc | 593b | 785a |
| - P | 1347bc | 1173bc | 593b | 960a |
| - S | 1333bc | 1227bc | 607b | 869a |
| - B | 1273bc | 1353abc | 593b | 811a |
| - Fe | 1487bc | 887c | 953[a] | 1068a |
| - Aeration | 1733ab | 1613ab | 707ab | 1081a |
| F | 9.147** | 7.965** | 3.711** | 3.353ns |
| CV (%) | 14.42 | 16.42 | 16.33 | 12.94 |

Mean values followed by same letter in the same column do not differ by Tukey's test ($p \leq 0.05$). ** ($p < 0.01$) ns ($p > 0.05$)

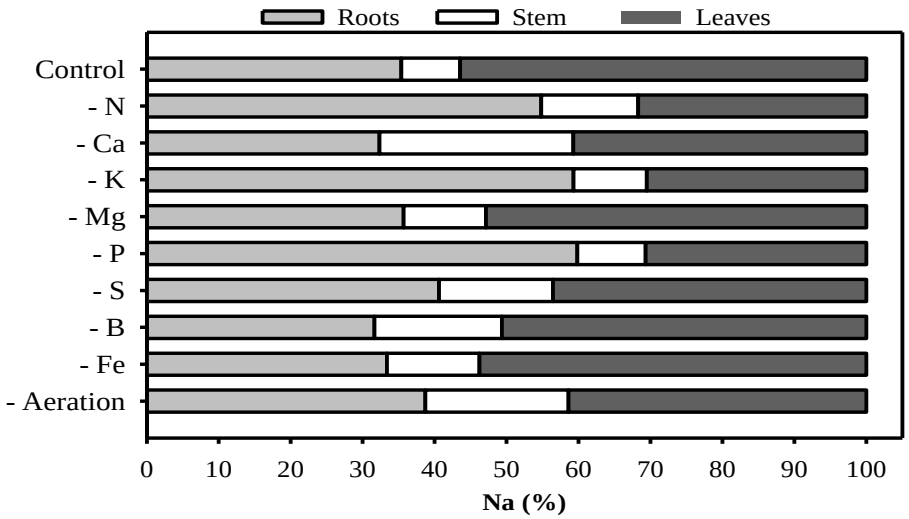


**Figure 5.** Percentage accumulation of sodium (Na) in roots, stems and leaves of cowpea plants grown in complete nutrient solution (control) or in nutrient solution without nitrogen (-N), calcium (-Ca), potassium (-K), magnesium (-Mg),

phosphorus (-P), sulfur (-S), boron (-B), iron (-Fe) or aeration. Measurements were performed after at 21th day after starting deficiency treatments.

Potassium plays a dominant role in turgor pressure and water homeostasis of cells and it is fundamental for pressure potential generation in xylem and phloem vessels and the stomatal opening [10]. When the soil is deficiency of K, there is a rapid decrease of its concentration in plant tissues. Thus, to maintain the load balancing, plants uptake larger amounts of other cations such as Na, Ca and Mg [17]; [25]. This phenomenon may explain the increased root Na content in the K-deficient plants (Fig 5 and Table 4).

In cell, the calcium may acts as enzymatic cofactor, structural component and second messenger [8]. The Ca also turns in activation of SOS signaling pathway for $Na^+$ exclusion by roots when it is present at toxic levels [22]. Thus, the Ca deficiency could have caused a reduction in activity of the antiporter $Na^+$/ $H^+$, resulting in a higher accumulation and transport of $Na^+$, especially for the stems and leaves (Fig 5 and Table 4).

In conclusion, the deficiencies of Ca, N and Fe were the most damaging to biomass production of *V. unguiculata*, but the Ca is the most important for plant growth. The root is the main accumulator of Cu and Mn, while the leaves accumulate the highest accumulators of Fe, Zn and Na under normal conditions. Under nutritional deficiency conditions, the Fe, Zn, Cu and Mn contents vary differentially between root, stem and leaf of *V. unguiculata*.

## AUTHOR CONTRIBUTIONS STATEMENT

This study was developed under supervision of F. B. Sudério, who also helped in the data analyses. R. S. Miranda planned and carried out the experiment and drafted the manuscript. E. C. Marques helped with biochemical analysis and drafting the manuscript. E Gomes-Filho contributed with biochemical analyses and corrected some parts of the manuscript. All authors have read and approved the final manuscript.

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## CONFLICT OF INTEREST

The authors declare that they have no conflitcs of interest.

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