Concentration of Metals in Plant Litter Produced in Regions of Caatinga in Southwest Bahia, Brazil

Joaly S. S. O. Lima, Ivaldo S. Gomes, Uillian M. F. M. Cerqueira, Paulo L. S. Carneiro, Hélio R. Sousa Filho, Raildo M. de Jesus and Marcos A. Bezerra

Departamento de Ciências Biológicas, Universidade Estadual do Sudoeste da Bahia, Av. José Moreira Sobrinho, s/n, 45206-191 Jequié-BA, Brazil
Departamento de Ciências e Tecnologias, Universidade Estadual do Sudoeste da Bahia, Av. José Moreira Sobrinho, s/n, 45206-191 Jequié-BA, Brazil
Programa de Pós-Graduação em Desenvolvimento e Meio Ambiente, Universidade Estadual de Santa Cruz, Rodovia Ilhéus-Itabuna, km 16, 45662-000 Ilhéus-BA, Brazil

Plant litter has several functions in a forest ecosystem. Studies about plant litter are of great importance to better understand and conserve ecological environments. The objective of this study was to investigate the concentration of metals (Fe, Co, Ca, Cu, Ni, Mg, Mn and Zn) in Caatinga plant litter in the southwest region of Bahia. Plant litter was collected in summer (January/2017) and winter (August/2017). Concentrations were determined by flame atomic absorption spectrometry (FAAS). The results were submitted to multivariate analysis, principal component analysis (PCA), and hierarchical cluster analysis (HCA). The average amount of plant litter found in January and August was 1190 and 1329 kg ha⁻¹, respectively. The twigs fraction provided the largest amount of biomass to the plant litter since this deposition occurred in greater quantity in August, a period of drought. Metal concentrations (g kg⁻¹) in the analyzed samples ranged from 1.39-257 (Fe), < limit of quantification (LOQ)-0.269 (Co), 0.0036-0.61 (Cu), 1.39-8.76 (Ca), 0.0022-0.56 (Ni), 0.137-1.52 (Mg), 0.033-1.91 (Mn) and 0.0022-0.8 (Zn). The multivariate analysis showed that the composition of plant litter is altered seasonally.

Keywords: plant litter, Caatinga, metals, multivariate analysis

Introduction

Caatinga is an exclusively Brazilian biome, and much of its biodiversity cannot be found anywhere else on the planet. Despite the semi-arid climate, the Caatinga presents a great variety of landscapes and biological wealth. Its biodiversity supports several economic activities aimed at farming-wild-pastoral and industrial purposes, especially in the pharmaceutical, cosmetic, chemical and food sectors, with immense potential for sustainable use and bioprospecting. However, the unsustainable use of natural resources over the years has led this biome to a state of considerable degradation.¹

In this biome, annual average temperatures are in the range of 23 to 27 °C, and relative humidity is generally below 50%. As a consequence, potential evapotranspiration is high, generally above 1500 mm year⁻¹, resulting in negative water balances over seven to eleven months per year.² Its vegetation, adapted to the edaphoclimatic conditions of the semi-arid climate, is characterized by great heterogeneity and endemism, yet it is still one of the unknown biomes in the country.³ Its soils are usually shallow, have low permeability, high surface flow and reduced natural drainage.⁴

Plant litter is the layer of organic waste formed on the soils of forest ecosystems due to the periodic falling of leaves, branches, bark, fruit, and the accumulation of animal debris. It is a fundamental component within a forest ecosystem, mainly for acting in soil protection and nutrient cycling.⁵ Vegetation and litter cover are essential for the protection of the soil. Plant litter protects the soil from the intense sun rays in the dry season, and in the first rains, the concentration of the metal prevents the direct impact of rain droplets.⁶ According to Maia,⁷ plant litter...
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J. Braz. Chem. Soc.

is of great importance not only concerning soil protection and fertilization but also to conserve the local biodiversity. The amount of litter on the ground varies according to the composition of the species, the intensity of the forest cover, the successional stage, the age, the collection season, the type of forest and the site. Several biotic and abiotic factors affect litter production, such as vegetation type, altitude, latitude, precipitation, temperature, luminosity regimes, vegetation deciduousness, successional stage, water availability and soil characteristics.

Knowledge about these natural ecosystems and the cycling of nutrients in native forests in Brazil is still rare. Consequently, the development of new research is of major importance, especially in the regions of the country that are most prone to human impacts, where natural ecosystems are disappearing. The generation of information about the nutrients or potentially toxic substances contained in a litter is an important tool for the understanding and conservation of vegetation, as well as their interrelationships with the environment.

Flame atomic absorption spectrometry (FAAS) is an analytical technique used in the quantification of about 70 elements. It is based on the absorption of radiation by free atoms, in the gaseous phase, and the fundamental state. This technique is widely adopted by many researchers for its large sensibility, being able to determine traces (µg mL⁻¹) and ultra-traces (ng mL⁻¹) with high precision and selectivity. A large variety of samples such as clinical, biological, environmental, food, fuel, geological materials, can be analyzed by FAAS.

Thus, the objective of this study was to determine the concentration of the metals in the litter collected from Caatinga areas of southwest Bahia, located in the municipalities of Jequié, Manoel Vitorino and Boa Nova.

Experimental

Instrumentation

Absorbance measurements were made using a PerkinElmer (Norwalk, CT, USA) flame atomic absorption spectrometer (FAAS), model AAnalyst 200, for the determination of Fe, Co, Cu, Ca, Ni, Mg, Mn and Zn in the samples. The flame was composed of acetylene (2.0 L min⁻¹) and air (13.5 L min⁻¹). The flow used by the nebulizer was 5.0 mL min⁻¹. The wavelength values for the hollow cathode lamps used in the determination of Fe, Co, Cu, Ca, Ni, Mg, Mn and Zn were 248.3, 240.7, 324.8, 422.7, 232.0, 285.2, 279.5 and 213.9 nm, respectively. A laboratory stove (Quimis, Model Q317 M-12, Diadema, São Paulo, Brazil) was used to dry the samples. A Sartorius (model BL D105, Göttingen, Germany) analytical balance was used to establish the sample mass. A Tecnal digester block (model TE 0851, Piracicaba, Brazil) was used for sample decomposition.

Reagents

All reagents used in this work were of analytical purity. Ultrapure water was obtained using a Purelab purification system, model Classic (Elga, High Wycombe, UK). All the glassware was immersed in a 5% (v v⁻¹) nitric acid (HNO₃) solution for decontamination for 12 h and then washed with deionized water and dried in a dust-free environment. Metal solutions were prepared from appropriate dilutions of commercial standards (Merck, Kenilworth, NJ, USA) at concentrations of 1000 mg L⁻¹ in a 1% (v v⁻¹) HNO₃ solution. Concentrated HNO₃ (65% m v⁻¹, Éxodus Científica, São Paulo, Brazil) and 30% (v v⁻¹) hydrogen peroxide (H₂O₂) (Quimica Moderna, São Paulo, Brazil) were used in the sample digestions.

Area characterization

This study was carried out in Caatinga regions located in the southwest of Bahia, in the municipalities of Boa Nova (14°22′05″S and 40°12′24″W), Manoel Vitorino (14°8′42″S and 40°14′34″W) and Jequié (13°51′28″S and 40°05′02″W). In each municipality, three collection points were chosen, with a distance greater than 1000 meters between the chosen points.

In the city of Boa Nova, the climate is hot and temperate. There is significant rainfall throughout the year, the average temperature is 24.2 °C (summer) and 18.1 °C (winter), and the average annual rainfall is 701 mm. In Manoel Vitorino, a tropical climate prevails, summer has much more rainfall than winter, the average temperature is 26.6 °C (summer) and 20.2 °C (winter) and the average annual rainfall is 703 mm. Jequié has a tropical climate, summer has much more rainfall than winter, the average temperature is 29.8 °C (summer) and 23.3 °C (winter) and 703 mm is the average annual rainfall.

Sample collection

The collections were carried out in January/2017 and August/2017 in a unique step for each campaign. It was used a polyvinyl chloride (PVC) square template of 1.0 m² (1.0 × 1.0 m), which was randomly released, with three replicates, inside of a demarcated area of 25 m², at each collection point. In each municipality was collected three samples using this methodology, generating twenty-seven
samples to be analyzed by each season. The collected samples were conditioned in plastic containers and properly identified.

Treatment and sample digestion

The collected material was separated into its constituents: leaves, twigs, reproductive structures, and miscellaneous. After sorting, the fractions were packed in paper bags, identified and dried in a forced circulation oven at 70 °C until reaching constant weight. Afterward, they were weighed on a semi-analytical balance to determine the dry mass. From these data, the percentage of each litter fraction was estimated.

The samples (about 0.2 g) were digested, and the organic matter was efficiently oxidized in a digestion block using 2.0 mL of concentrated HNO₃ and 3.0 mL of H₂O₂. The digestion conditions of the digester block were 5 h and a temperature of 120 °C. After being cooled, the solutions were filtered on filter paper, black band, and their volumes complete to 15 mL.

Calculations of parameters related to plant litter

The dry mass values of accumulated plant litter per collector were converted from g m⁻² to kg ha⁻¹, multiplying the mass of dried plant litter by factor 10. An estimate of litter carbon stock was carried out by multiplying the litter mass in kg ha⁻¹ by a factor of 0.37, considering the reference value for the plant litter, as suggested by the Intergovernmental Panel on Climate Change.²⁰

Data analysis

In the data analysis, multivariate techniques, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), were used to describe the similarity between the samples considering the total set of variables and the correlations between them. In these statistical analyses, the data set was organized in a matrix consisting of 18 samples (lines) and 9 variables (columns) representing the determined metals. Before multivariate analysis, autoscaling of the matrix was carried out, aiming to give the same weight for all variables. The analysis was performed, and graphs were plotted using the Statistica 10 software.²¹

Results and Discussion

Validation of the analytical method

The analytical method adopted for the determination of metal in the litter was validated obtaining some parameters of merit such as the limit of quantification (LOQ), precision expressed as repeatability (percentage of relative standard deviation, %RSD), linearity (expressed as determination coefficient, R²). The results for these parameters are presented in Table 1.

Table 1. Analytical characteristics of the methodology used in the determination of metals in samples of plant litter

| Metal | LOQa (mg kg⁻¹) | RSDb / % | Linearity (R²) |
|-------|---------------|---------|----------------|
| Fe    | 2.6           | 2.2     | 0.9954         |
| Co    | 0.15          | 1.2     | 0.9989         |
| Cu    | 0.74          | 1.8     | 0.9956         |
| Zn    | 2.3           | 3.1     | 0.9985         |
| Ca    | 16            | 1.5     | 0.9866         |
| Mn    | 1.0           | 3.2     | 0.9916         |
| Ni    | 0.15          | 2.4     | 0.9992         |
| Mg    | 3.7           | 3.1     | 0.9964         |

LOQ: limit of quantification for a sample mass of 0.2 g; †RSD: relative standard deviation for a 0.5 mg L⁻¹ metal solution (n = 10).

The LOQ values found were adequate to determine these elements. Relative standard deviation values ranged from 1.2 to 3.2% for the microelements and 3.1 (Mg) and 1.5% (Ca) for macroelements. Typically, methods that quantify compounds in macro quantities require a RSD of 1 to 2%. For trace or impurity analysis methods, RSDs up to 20% are accepted depending on sample complexity. Linearity was evaluated as the correlation coefficient (R). According to the Brazilian National Institute of Metrology, Quality and Technology (INMETRO) R-values above 0.90 are recommended. Thus, these values show that these analytical curves present adequate linearity.²²

Accuracy was accessed by analysis of certified reference material of apple leaves. Table 2 shows the results of the determination of the studied metals in a certified reference material of apple leaves. By the paired t-test application, it is noted that, at a 95% confidence level, there is no significant difference between the two sets of data (t = -0.871 > -2.44). The found recoveries were between 93.3 to 103% for the majority of studied elements. Only Mg has presented a recovery of 114%.

Co accuracy was accessed by addition/recovery tests in three samples. Recoveries in the range of 97-106% were found for this metal.

Evaluation of plant litter contribution to deposited organic matter

The average amount of plant litter deposited was estimated at 1190 kg ha⁻¹, in January and 1329 kg ha⁻¹, in
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August, as shown in Table 3. The amount of litter deposited in the studied areas was very small when compared with other works carried out in Caatinga areas. Results obtained in this study were similar to those reported by Santana and Souto\(^23\) (2,068.6 kg ha\(^{-1}\)) and Lopes et al\(^24\) in a Caatinga area in Ceará (5,366.0 kg ha\(^{-1}\)). However, they are closer to the results found by Alves et al\(^25\) (899.2 kg ha\(^{-1}\)). Litter production in the Caatinga biome, according to Costa et al\(^11\), can vary widely, both in arboreal and shrub forests, and the morphological and physiological characteristics of the plants that make up the biome are the determining factor.

It was observed that the period of greatest deposition, winter, was a period of drought, with one of the lowest rainfalls of the year. Silva et al\(^26\) emphasized the adaptation of the Caatinga to the condition of the high-water deficit, in which plants deposit large amounts of deciduous material to reduce evapotranspiration.

The results obtained about the behavior of litter as a function of rainfall showed a clear relationship between precipitation and deposition of litter. The pattern of litter deposition is directly influenced by the changes in seasons (rainy and dry periods) that occur in the Caatinga biome.

Table 3. Plant litter mass deposited in the areas of litter collection

| Collection local | Sample symbol | Plant litter mass / (kg ha\(^{-1}\)) |
|------------------|---------------|-----------------------------------|
|                  |               | January (summer)      | August (winter)    |
| Boa Nova         | A             | 1283                  | 1313               |
| Manuel Vitorino  | B             | 1421                  | 1536               |
| Jequié           | C             | 865.2                 | 1139               |
| Average          |               | 1190                  | 1329               |

Differences can be observed when comparing the results of this study with those found in other Brazilian biomes. In a study developed by Machado et al\(^27\) in a fragment of Atlantic Forest, 14700 kg ha\(^{-1}\) was obtained for the advanced stage of succession. In a tropical forest located in the Biological Reserve of Poço das Antas (Rio de Janeiro, Brazil), Barbosa and Faria\(^28\) found a total contribution of 6874.3 kg ha\(^{-1}\). Both results are very high when compared to those found in this study, which is justified by the presence of vegetal specimens of large size in this biome.

It is estimated that the average amount of carbon stock (Table 4) in the litter corresponded to 440.2 kg ha\(^{-1}\) in January and 491.7 kg ha\(^{-1}\) in August. Higher results were found by by Souza et al\(^29\) in a preserved abiotic-arboreal Caatinga area in Seridó da Paraíba (1499 kg ha\(^{-1}\). This behavior is expected since the carbon stored in the litter is directly proportional to the total litter supply.

Table 4. The estimated amount of carbon stored in plant litter in the areas of litter collection

| Collection local | Sample symbol | Carbon stored in plant litter / (kg ha\(^{-1}\)) |
|------------------|---------------|-----------------------------------------------|
|                  |               | January (summer) | August (winter) |
| Boa Nova         | A             | 474.8            | 485.6            |
| Manuel Vitorino  | B             | 525.6            | 568.3            |
| Jequié           | C             | 320.1            | 421.3            |
| Average          |               | 440.2            | 491.7            |

The twigs fraction (Figure 1) was predominant in all litter samples studied in the two studied months. This result is unlike the study developed by Silva et al\(^30\) in which the leaf fraction was superior to the others in the litter collected from Caatinga areas in the Desertification Nucleus of Seridó (an interstate area situated in the Brazilian Northeast, between the states of Rio Grande do Norte and Paraíba). This study was similar to another developed by Lima et al\(^31\) in the Caatinga area in the south of Piauí. However, Maciel et al\(^32\) in a Caatinga area of the Pernambuco State semiárid, found superior values for the twigs fraction (about 57.7% more).

Figure 1. Components amount of the plant litter collected from the Caatinga region of southwestern Bahia in January and August.
It can also be verified that the twig fraction (Figure 1), with an average percentage of 84.3%, in January was higher than the 68.8%, found in August. The percentage of the twigs fraction decreased in the winter, while the leaf, reproductive, and miscellaneous parts fractions had higher values in this season. This phenomenon can be attributed to the rainfall index of August which is lower than January. The long dry period in the Caatinga causes losses of plant leaves and even to their more lignified structures. 32

The fraction of reproductive parts was the third largest, accounting for 3.8 and 8.6% of the total plant litter contribution, in January and August, respectively. This is contrary to the findings of Lopes et al. 24 According to these authors, in the dry season, the deposition of reproductive structures is much lower than in the rainy season. Andrade et al. 33 reported that the fraction of the reproductive structures, which included flowers, fruits and seeds, was responsible for only 8.7% of total litter production from Caatinga plants in the municipality of Santa Terezinha (Paraíba State, Brazil). However, Lopes et al. 24 obtained a percentage for reproductive structures of 11.24% in a Caatinga area located in Iguatu (Ceará, Brazil).

The miscellaneous fraction presented the smallest share of the litter produced in the two studied months, accounting for 1.3 and 5.7%, respectively. Despite the small share of total litter, this fraction is of considerable importance. 14 Proctor et al. 34 considers that the miscellaneous components are rich in nutrients and energy, and, as they are associated with a high degree of fragmentation, can be a more accessible source for decomposers.

Metals contribution by plant litter

The metals found in high amounts in the samples were Fe, Ca, Mg and Mn and those found in lower concentrations were Co, Cu, Ni and Zn (Tables 5 and 6). Calcium was the macronutrient with the highest levels. The average values among the samples were 5.55 g kg⁻¹ in January and 4.92 g kg⁻¹ in August. For Dias et al., 35 Ca content tends to show greater variation with positive peaks during the drier months, probably due to a lower metabolic rate in this period, which would further reduce the mobility of this element which is generally considered to be less mobile. This characteristic causes Ca, even in excess, stored in the form of crystals in the leaf, to remain even in its senescence.

Regarding micronutrients, iron presented the greatest amounts in the collected samples. The average value found was 6.7 g kg⁻¹ in January, and the highest concentration was found in sample SA3 (11 g kg⁻¹). The average value found in August was 91 g kg⁻¹, and the highest concentration was found in sample WC3 (257 g kg⁻¹). According to Larcher, 36 the potential site of occurrence of iron is in the leaves, as a result, higher iron content was expected in the August samples because the leaf fraction was higher in that month compared to January. This is due to the participation of leaves in photosynthesis, N₂ fixation, among other functions. 37 However, the concentration of iron in sample WC3 was higher than the other samples collected in the same municipality. It is possible that the iron content, along with other elements in this sample, was a consequence of its location, close to a national highway (BR 116) with heavy traffic of vehicles, which is responsible for dust suspension and the existence of an area for deposited garbage (landfill).

Metal below the LOQ was Co in the samples WB2, WB3, WC1, WC2 and WC3.

There were many variations in the concentrations of elements in the different localities and also in the months collected, showing that there is no well-defined behavior

Table 5. Metal concentrations in plant litter samples from the southeast region collected in the summer

| Sample | Metal concentration (± standard deviation) / (g kg⁻¹) |
|--------|-----------------------------------------------------|
|        | Fe       | Co       | Cu       | Ca       | Ni       | Mg       | Mn       | Zn       |
| SA1    | 7.2 ± 0.2 | 0.019 ±0.009 | 0.006 ± 0.001 | 3.14 ± 0.06 | 0.0022 ± 0.0008 | 0.46 ± 0.02 | 0.061 ± 0.005 | 0.0026 ± 0.0008 |
| SA2    | 1.5 ± 0.7 | 0.003 ± 0.006 | 0.0050 ± 0.0008 | 5.22 ± 0.08 | 0.0044 ± 0.0008 | 0.73 ± 0.020 | 0.033 ± 0.006 | 0.018 ± 0.002 |
| SA3    | 11 ± 2    | 0.019 ±0.005 | 0.021 ± 0.003 | 4.03 ± 0.02 | 0.055 ± 0.009 | 0.74 ± 0.4 | 1.91 ± 0.05 | 0.006 ± 0.002 |
| SB1    | 7.1 ± 0.1 | 0.020 ± 0.005 | 0.011 ± 0.003 | 6.67 ± 0.02 | 0.009 ± 0.001 | 1.4 ± 0.1 | 0.171 ± 0.005 | 0.031 ± 0.005 |
| SB2    | 7.8 ± 0.2 | 0.023 ± 0.007 | 0.024 ± 0.008 | 7.04 ± 2.1 | 0.008 ± 0.002 | 1.06 ± 0.01 | 0.13 ± 0.02 | 0.025 ± 0.005 |
| SB3    | 4.4 ± 0.3 | 0.010 ± 0.001 | 0.0036 ± 0.0008 | 6.71 ± 3.9 | 0.009 ± 0.003 | 1.52 ± 0.06 | 0.11 ± 0.02 | 0.0022 ± 0.0008 |
| SC1    | 4.6 ± 0.1 | 0.011 ± 0.004 | 0.010 ± 0.002 | 6.09 ± 1.3 | 0.011 ± 0.004 | 0.65 ± 0.09 | 0.07 ± 0.01 | 0.19 ± 0.06 |
| SC2    | 8.8 ± 0.1 | 0.029 ± 0.007 | 0.010 ± 0.002 | 6.26 ± 0.73 | 0.011 ± 0.003 | 1.2 ± 0.1 | 0.12 ± 0.02 | 0.010 ± 0.002 |
| SC3    | 7.8 ± 0.2 | 0.025 ± 0.009 | 0.015 ± 0.003 | 4.75 ± 0.64 | 0.016 ± 0.005 | 1.0 ± 0.1 | 0.065 ± 0.004 | 0.0035 ± 0.0005 |

SA: sample of Boa Nova municipality collected in the summer; SB: sample of Manuel Vitorino municipality collected in the summer; SC: sample of Jequié municipality collected in the summer.
for these elements in the analyzed samples. According to Souto et al., these large variations are associated with the presence of different species, with different ages, and also to changes in edaphoclimatic conditions. For Schumacher et al., the nutrient content in plant litter may vary, even for the same species, depending on the site, the characteristics of the plant and the element properties.

PCA verifies that the first three principal components explain 86.89% of the total variation. In addition, it was possible to recognize a tendency of the samples from the same locality and even the month of collection to form groups, with some exceptions. For example, sample WC3, which does not resemble the samples collected in the same month and locality, WC2 and WC1, as well as sample WA3 did not group to the other samples. Besides, it was possible to perceive the formation of two large groups. The first formed by WA1, WA2, WB1, WB2, WB3, WC1 and WC2, all collected in August and the second formed by SA1, SA2, SA3, SB1, SB2, SB3, SC1, SC2 and SC3, collected in January. This behavior evidences the influence of some abiotic factors, which may have influenced the biochemical processes. This seasonality of the plant litter composition may be related to the low water content in the soil, because it is shallow. This is reflected in a reduction in the amount of water infiltrated, since, when compared to the accumulated rain for the two groups, it was verified that in the first group, samples collected in August have less rainfall than the second group. The variables Ca and Mg behaved similarly in the samples, as well as Co, Ni and Mn and also Cu, Fe and Zn.

Evaluating the dendrogram (Figure 2), it can be seen that nutrient contents and distributions discriminate between two main groups. The first group formed only by samples collected in summer and the second group formed by the other samples. This result corroborates with the one found in the principal component analysis (Figure 3), which shows this difference of the samples WC3 and WA3 with the others. Also, the tendency of the samples to be grouped per month of collection, rather than by locality, was verified. This behavior evidences the seasonal behavior in the contents of the metals determined in the plant litter.

### Conclusions

The deposition of plant litter collected in the municipalities from southeast Bahia (Jequié, Manoel Vitorino and Boa Nova) was lower than the values found in other forest ecosystems, as well as previous studies. The twig fraction was the largest contributor to the total plant litter composition analyzed. The micronutrient with the highest content in the samples was Fe, presenting...
higher values in August (dry season). PCA and HCA showed concordant results, evidencing the difference of two samples, WA3 and WC3 with the others. In addition, it was possible to observe that the metal content in the samples was seasonal.

Acknowledgments

Authors acknowledge the financial support of the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant No. 304582/2018-2), and Financiadora de Estudos e Projetos (FINEP).

Author Contributions

Joaly S. S. O. Lima was responsible for development of the experiments and preparation of the manuscript first version. This article is derived from her master’s degree work. Ivaldo S. Gomes was responsible for assistance in the development of the experiments and writing of some sections; Uillian M. F. M. Cerqueira for assistance in the development of the experiments and writing of some sections; Paulo L. S. Carneiro for statistical analysis and writing of some sections; Hélio R. Sousa Filho for writing of some manuscript sections and final formatting; Raildo M. de Jesus for statistical analysis and review of the final manuscript; Marcos A. Bezerra for guidance of developed work and coordination of manuscript writing.

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