Micronutrient content and accumulation in leaves and bunches of black pepper during two crop cycles[1]

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ABSTRACT

A comprehensive knowledge about micronutrient dynamics in leaves and bunches of black pepper is needed in order to proper diagnose nutrient needs, and thus facilitate fertilization management. This study aimed to evaluate micronutrient concentration and accumulation in leaves and bunches of black pepper bunches to understand the crop nutritional demand. The experiment was carried out on in a commercial field of Piper nigrum 'Bragantina' located in São Mateus, Espírito Santo, Brazil. Composite samples of leaves and bunches were taken periodically during two crop cycles and the concentration of Fe, Zn, Mn, Cu, and B were assessed. The micronutrient accumulation curves behaved linearly and quadratically, reaching the maximum accumulation point during the time of harvesting. Fe, Mn and B were the micronutrients with highest concentration in both bunches and leaves of black pepper. The nutrient concentration in leaf tissues varied seasonally. These results may contribute to a more sustainable agriculture, in which fertilization rates should consider different needs through the crop stage.

Keywords: *Piper Nigrum* L.; nutrient dynamics; mineral composition; nutritional management.

INTRODUCTION

Black pepper (*Piper nigrum*) is one of the most popular spices in the world, also considered as "the king of spices" (Thangaselvabal *et al.*, 2008). It is extensively grown in tropical regions, and Ethiopia was the main producer of black pepper in 2019, followed by Vietnam, Brazil, Indonesia and India (FAOSTAT, 2021).

The black pepper production increased 356% in the last twenty years, from 309,940 tons in 1999 to 1,103,024 tons in 2019 (FAOSTAT, 2021). Such increased may be related to a higher market demand, in which black pepper is used for culinary and medicinal purposes, also because of its health-promoting elements, such as Fe, Cu, Mn, and Zn, which are nutraceutically valued (Bhat *et al.*, 2010). These elements, among a few others, are considered as micronutrients for crops, as they are essential elements required in small quantities.

Despite the low concentration of micronutrients in a plant dry weight, micronutrients are vital for crop growth and development, and therefore it influences crop yield, as well as crop quality (Laviola *et al.*, 2007), in which a micronutrient mixture specific to black pepper is commonly recommended for higher yields (Lijo & Rajeev, 2011). Micronutrients participate in several biochemical and physiological reactions, e.g., in the formation of cell walls (B), cell membranes (B, Zn), enzymes (Fe, Mn, Cu, Ni), enzyme activators (Mn, Zn) and in photosynthesis (Fe, Cu, Mn, Cl) (Kirkby & Römheld, 2007).

Regarding some micronutrient requirements, Sadanandan *et al.* (2000) suggested a DRIS (Diagnosis

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and Recommendation Integrated System) norms to classify ‘deficient’, ‘low’, ‘optimum’, ‘high’ and ‘excess’ nutrient concentrations in black pepper, which was specifically assessed for South India. Moreover, further studies about micronutrient concentrations in black pepper is needed, especially for other pepper-producing countries in order to address site-specific conditions, such as different soil groups and fertility status (Srinivasan *et al.*, 2007). In relation to sampling, studies assessing leaves and bunches of black pepper are needed, as most (e” 60%) nutrients are distributed within leaves and bunches in relation to roots, stems and branches (Paduit *et al.*, 2018).

Considering that assessments of micronutrient concentration in leaves and bunches of black pepper is required to recommend and adjust black pepper fertilization, as well as information regarding nutrient accumulation through the crop cycle, this study aimed to evaluate micronutrient concentration and accumulation in black pepper leaves and bunches in order to understand the crop nutritional needs.

MATERIALS AND METHODS

Study site

The experiment was carried out during two crop cycles, in 2017 and 2018, in a commercial field of black pepper (*Piper nigrum* ‘Bragantina’) in the municipality of São Mateus, Espírito Santo State, Brazil (18°46’48.4" S, 39°52’31.5" W; alt 23 m asl), with smooth to undulating relief. The crop management included weed control with herbicides, liming, top dressing fertilization, pruning and drip irrigation. Annual fertilization included 400 kg ha^{-1} of N, 80 kg ha^{-1} of P_2O_5 and 320 kg ha^{-1} of K_2O.

The regional climate, according to Köppen’s classification, is Aw, tropical with dry winters and rainy summers (Alvares *et al.*, 2013). The maximum, average and minimum air temperature, global solar radiation, rainfall and relative humidity during the experiment period (Figure 1) were determined at the meteorological station from Federal University of Espírito Santo (CEUNES-UFES), located 15 km away from the experimental area.

During the establishment of the experiment, on April 2017, the black pepper plants were approximately three years old. The field was cultivated under a full sun farming, at a spacing of 3.5 x 1.8 m, on a soil classified as a Latossolo Amarelo Distrófico according to the Brazilian classification (Santos *et al.*, 2018), which corresponds to a Ferralsol in the World Reference Base (IUSS Working Group WRB, 2015). Chemical properties (Teixeira *et al.* 2017) and particle size distribution (Flint & Flint 2002) for the 0 – 20 cm soil layer are as follows: pH in water (1:2.5 v v^{-1}) = 5.05; P (Mehlich-1) = 52 mg dm^{-3}; K = 95 mg dm^{-3}; S = 8 mg dm^{-3}; Ca^{2+} = 4 cmol$_c$ dm^{-3}; Mg^{2+} = 0.35 cmol$_c$ dm^{-3}; Al^{3+} = 0.5 cmol$_c$ dm^{-3}; titratable acidity (H + Al) = 5.9 cmol$_c$ dm^{-3}; organic matter = 2.2 dag dm^{-3}; base saturation =4.62 cmol$_c$ dm^{-3}; cation exchange capacity = 5.12 cmol$_c$ dm^{-3}; sand = 679.5 g kg^{-1}; silt = 60.5 g kg^{-1}; and clay = 260 g kg^{-1}.

Sampling strategy and micronutrients assessments

Ten plants were marked and studied during each one of the two crop cycles, which totaled twenty plants marked overall. Sampling was conducted in a completely randomized design, in a time plot arrangement. For the analyses of pepper bunches, 11 samplings were performed in the first crop cycle and nine in the second. The difference between the number of samplings in the two cycles was because the second cycle was 42 days shorter than the first. In each sampling date, nine bunches were randomly selected to constitute three composite samples, i.e., three replicates per sampled period.

During the first crop cycle, the first bunches were marked on 04/17/2017, when 200 inflorescences of 10 plants were labeled, approximately 28 days after inflorescence emergence. This date was also the day of the first sampling. Thereafter, samplings occurred at intervals of about 21 days, until the last one on 11/13/2017, 238 days after inflorescence emergence. In the second crop cycle, 10 other plants were labeled by using the same pattern for bunch marking. Bunch sampling then began on 11/13/2017 and ended on 04/30/2018 (196 days after inflorescence emergence). The last sampling of bunches, in both crop cycles, occurred at full fruit maturation, the stage when the fruits were completely filled, however, with the husk color still green.

For leaf assessments, a total of 20 samplings were performed throughout the year. Each sample consisted of 40 freshly matured leaves with three replicates per sampled period. The leaves were collected from the upper part of the middle third of the plants. The evaluations covered one year to assess the pattern of the foliar nutrient concentration in an annual cycle.

The collected samples (with either leaves or bunches) were washed in running water and then rinsed with distilled water. Thereafter, the samples were dried in a forced ventilation oven at 70 ºC to constant weight and weighed on a precision scale (0.001 g). The concentration s of iron, zinc, manganese, copper and boron in bunches and leaves were determined according to Malavolta *et al.* (1997). In order to calculate nutrient accumulation, the nutrient concentration and dry weight throughout the year were taken into account, from 28 to 238 days after inflorescence emergence.

Data analyses

Differences between each micronutrient concentration within leaves and within bunches, as well as the nutrient accumulation in bunches were subjected to analysis of variance (p d” 0.05), considering micronutrient means as source of variation. The analyses were performed using the Sisvar 5.6 software (Ferreira, 2019). Regression analysis for nutrient accumulation in bunches was also performed. The diagrams were based on the means and mean standard error, using software SigmaPlot, version 11.0.

RESULTS AND DISCUSSION

Significant differences in micronutrient concentration of leaves were found during the one-year period, as well as for micronutrient concentration in the bunches over both crop cycles, with the exception of copper (Cu) in the first crop cycle (Table 1).

Micronutrients concentration and accumulation in the leaves

Micronutrient concentration in the leaves varied throughout the year (Figure 2A and B). Manganese concentration in the leaves were higher than those of the other elements, even in April, May and December 2017, when Mn concentration were lowest (Figure 2A). Iron was the element with the second highest concentration, although the Fe concentration varied greatly over time; the periods of highest and lowest concentration, respectively, were in April and October 2017. Zinc, copper and boron were the nutrients with lowest concentration in the leaves (Figure 2B). The fluctuations of B and Cu concentration over time were higher than those of Zn.

The variation for micronutrient concentration in leaves found in this study corroborates with the results of Bataglia *et al.* (1976) that studied 96-months-old pepper plants. However, it diverges from that of Veloso *et al.* (1998),

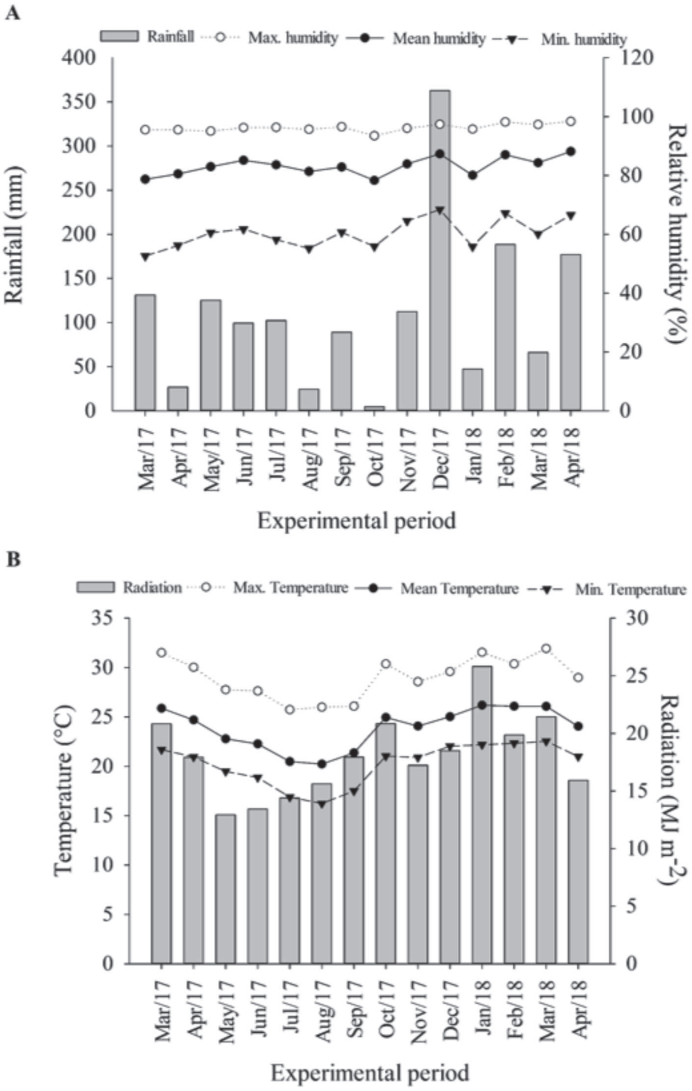

Figure 1: Total precipitation and mean values of maximum, mean and minimum (A), maximum, mean and minimum temperature, radiation (B) recorded at the meteorological station of São Mateus, ES, Brazil, from March 2017 to April 2018.

which also studied black pepper (var. Bragantina), and that of Viégas *et al.* (2013), for seedlings of long pepper plants (*Piper hispidinervum*) grown in a protected environment. Both reported variations in the order of nutrients, with higher Fe than Mn concentration, followed by Zn, B and Cu, and differences between the nutrient concentration, with higher Fe, Zn and Cu values than observed in this study. Such differences might be related to different phenological development stage of the sampled plants. According to Yap (2012), there are three main phenological development stages during a 30-months period of black growth in Malaysia: *i)* immature stage, the first 18 months of planting; *ii)* flowering to harvest stage, from 18 to 26 months of planting; and *iii)* a recovery stage, from 28 to 30 months. The same authors found that the greater nutrient demand was around 12 to 20 months after planting, as more than half of nutrients are taken up during fruit development.

According to the regional references for micronutrient concentration in pepper leaves, appropriate Fe, Zn, Cu, Mn and B concentration during the flowering period are 200; 30; 8; 60; and 25 mg kg 1, respectively (Prezotti *et al.*, 2007). These values show that only the Zn and Cu concentration are within the normal range in both cycles. The Mn and B concentration were higher (or excessive), while Fe, during both cycles, contained a much lower concentration than the recommended (Figure 2). The lower Fe concentration might be a response to the higher B concentration, as B toxicity may lead to Fe deficiency in pepper plants due to the oxidation reduction equilibrium in cells (Sarafi *et al.*, 2018). Furthermore, it is important to note that both B and Mn are nutrients with low phloem mobility, differently from Fe (Etienne *et al.*, 2018). However, in spite of the great difference between the recommended and observed concentration, no deficiency or even toxicity symptoms due to micronutrient imbalance were observed.

Micronutrients concentration and accumulation in the bunches

Regarding micronutrient concentration in the bunches, Fe concentration varied greatly during the first cycle. In the first 84 days between the first sampling (at 28 days after inflorescence emergence) and early fruit development, a decreasing trend of Fe concentration was observed, followed by a sharp increase after 105 days, thereafter slight oscillations until the end of the samplings, when the peppercorns were totally filled, indicating the ideal time for harvesting (Figure 3A). However, the same behavior was not observed in the second crop cycle, when the Fe concentration in the bunches was highest in the first sampling (at 21 days), followed by a steady decline until reaching the lowest level at the end of the experiment, at the time of harvesting (Figure 3A). Although Fe concentration greatly varied between cycles, such difference has been previous found for other spices (Ozkutlu, 2008; Gupta *et al.*, 2003), and it may not follow any detectable pattern (Ancuceanu *et al.*, 2015).

The curves for Zn, Mn, B concentration in both cycles and for Cu in the second cycle only had a similar pattern to that of Fe in the second crop cycle, with a more intense decline from 21 to 63 days after inflorescence emergence, followed by a less intense decrease until the end of the samplings, with a tendency to stabilization of some nutrients (Figure 3B, D and E). This behavior was already expected and also observed in other crops, e.g., *Coffea canephora* (Covre *et al.*, 2018; Dubberstein *et al.*, 2019). This performance was due to the low dry matter accumulation and a given quantity of nutrients during early fruit formation. As soon as the cellular expansion begins, the size increases, causing a so-called "dilution effect" of the amount of nutrients contained in the fruit, reaching the lowest proportions in the stage when the peppercorns were totally filled and at maturity (Laviola *et al.*, 2006).

Table 1: Summary of analysis of variance (ANOVA) for micronutrient concentration in leaves and pepper bunches throughout the experimental period

Source of variation	Iron	Zinc	Copper	Manganese	Boron
			Leaf concentration		
			Mean Square Value		
Overall experimental period	108.24**	24.44**	10.93**	1,103.26**	50.80**
CV (%)	6.16	10.44	9.72	12.63	11.40
				Bunch concentration	
Cycle 1	1,064.42**	410.50**	2.95^{ns}	895.32**	1,884.29**
Cycle 2	588.57**	264.90**	20**	247.59**	1,487.93**
CV_1 (%)	4.63	13.32	9.89	13.45	13.39
CV_2 (%)	8.23	9.72	9.56	13.58	8.54

^{ns}, ** non-significant and significant at 1% error probability, respectively.

Although studies relating micronutrient concentration of the peppercorn bunches throughout reproductive period are scarce, other nutritional aspects have been investigated. In a study on the nutritional composition of black pepper fruits, for example, Abukawsar *et al.* (2018) found 67.86 and 124.2 mg kg^{-1} Fe in fruits of two *P. nigrum* varieties Indigenous and Kerala, respectively. These values differ from those found in the last samplings (45 and 93 mg kg^{-1}), at the ideal harvest time.

In this study, Mn concentration in the bunches (60 mg kg^{-1} in the second cycle) is similar to Pradeep *et al.* (1993), who reported 63.3 mg kg^{-1} Mn. However, Bhat *et al.* (2010) found a concentration of 16.3 mg kg^{-1}, lower than in the other studies. The Zn concentration (14 and 18 mg kg^{-1}) corroborates with those found by Bhat *et al.* (2010) (14.6 mg kg^{-1}) and by Nwofia *et al.* (2013), in a study with nine *P. nigrum* in Nigeria, where Zn levels ranged from 14.5 to 16.3 mg kg^{-1}. The Cu concentration (11 mg kg^{-1}) was much lower than that of 46.9 mg kg^{-1} found by Bhat *et al.* (2010).

The variation in elemental composition between the results of this study and those described elsewhere is very common. This variable is influenced by biotic and abiotic factors, e.g., cultivated variety, plant matrices, soil composition, climate, agricultural practices and harvesting, which affect the biochemical synthesis of the plant (Saleh-e-In *et al.*, 2017).

Based on the accumulation curves plotted in Figure 4 (A; B; C; D and E), the dataset fitted linear and quadratic regression models by regression analysis of

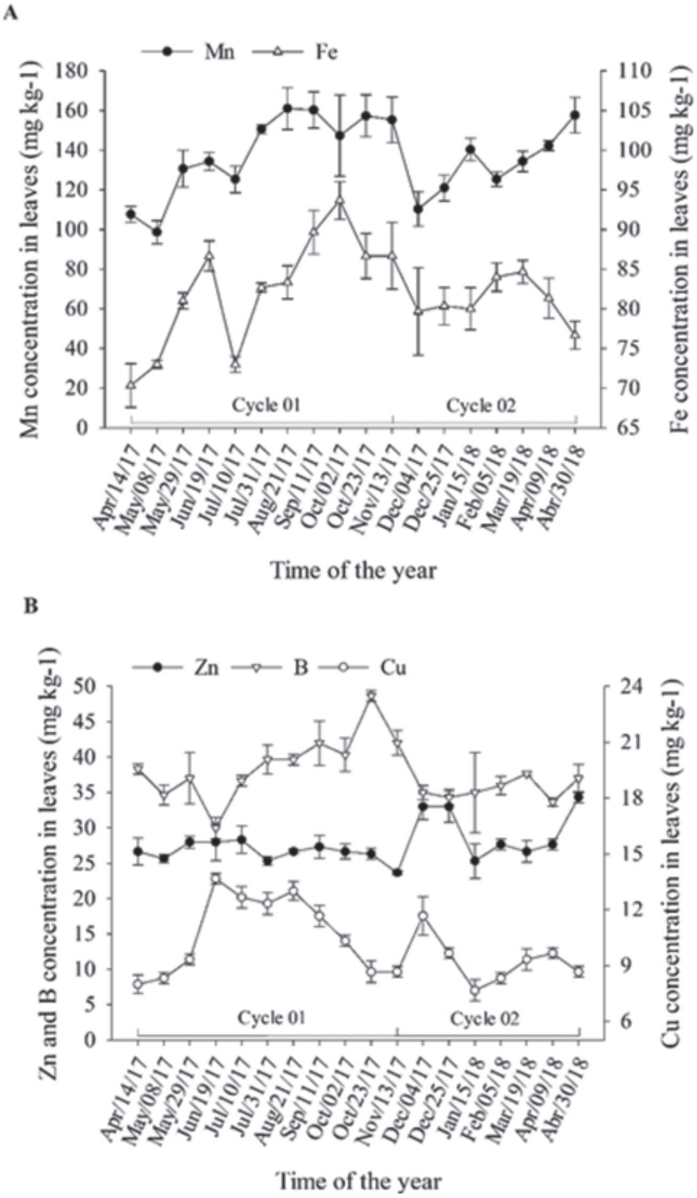

Figure 2: Fe, Mn (A), Zn, Cu and B (B) concentration in black pepper leaves over one year.

variance at a significance of 1% and with a high coefficient of determination. Of the total micronutrients accumulated by the peppercorn bunches, at 238 and 196 days after inflorescence emergence, in the first and second cycle, respectively, 44.40% and 33.92% corresponded to Fe, in other words, the most accumulated nutrient was iron. The accumulation had an increasing pattern over time, reaching highest means in the last sampling of both cycles (463.4 and 174.7 mg kg^{-1}, respectively). These values correspond to 100% of the total accumulated Fe (Figure 4A).

Among micronutrients, Fe accumulated the highest, probably due to its role on photosynthesis and in the biosynthesis of protein and chlorophyll (Bragança *et al.*, 2007). Manganese was the second most accumulated micronutrient by the bunches, in both cycles, representing 28.36 and 32.15% of the accumulated total, respectively, at the end of the

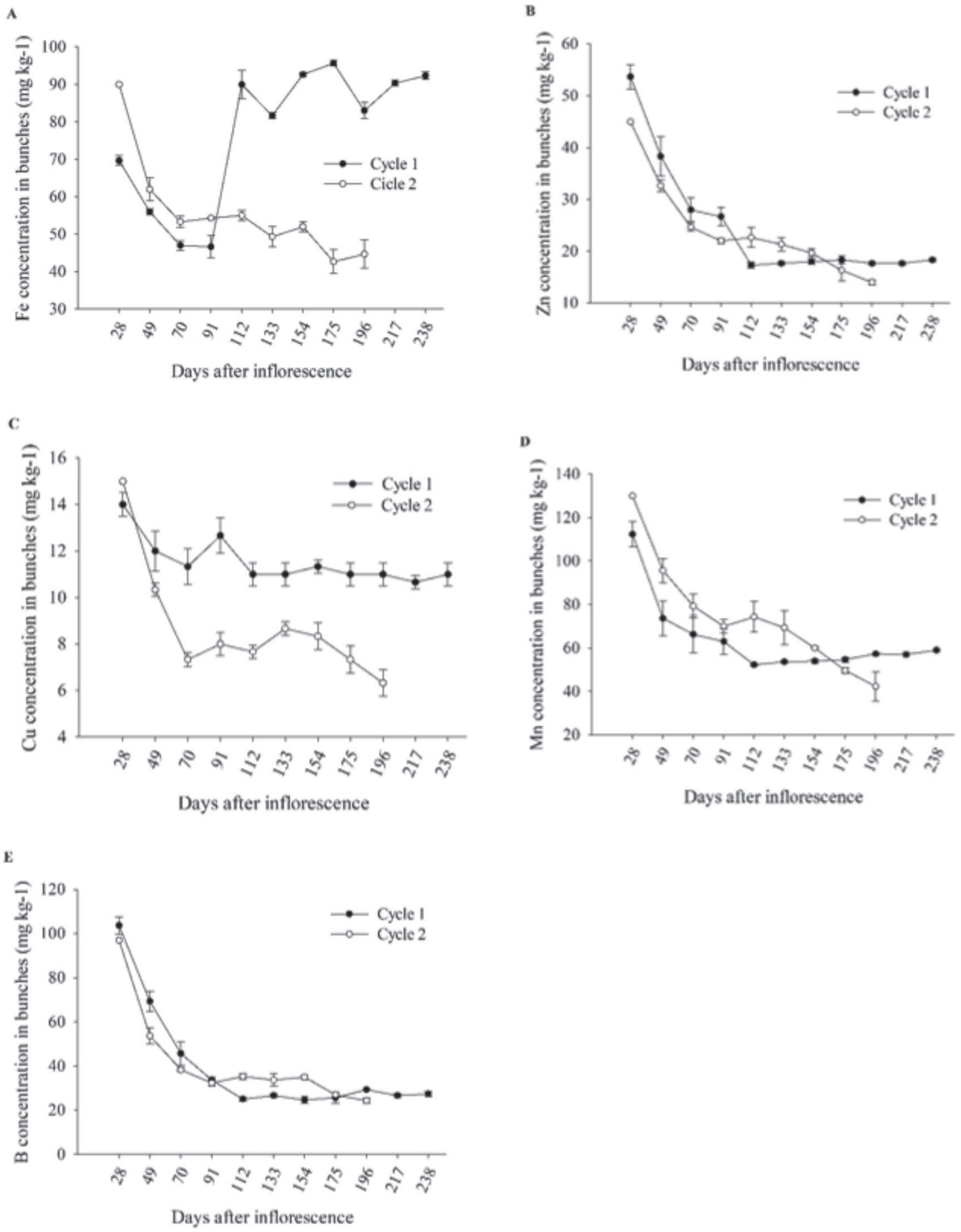

Figure 3: Fe (A), Zn (B), Cu (C), Mn (D) and B (E) concentration in black pepper bunches over two crop cycles; bars indicate the standard error.

experiment (Figure 4 B), which is important for enzyme activation and for photosynthesis (Kirkby & Römheld 2007; Taiz *et al.*, 2014).

The accumulation of B in the pepper bunches was highest 228 days after inflorescence emergence in the first cycle (137.17 mg kg^{-1}) and 189 days after inflorescence emergence in the second (95.17 mg kg^{-1}), corresponding to 13.14% and 10.63% of the total nutrients accumulated in the bunches at harvest (Figure 4C). Boron accumulation in the bunches increased linearly in both cycles, with a higher increase in the first, as observed for Fe. Boron fulfills an important function both in the floral development and fruit and seed set, since it is involved in the maintenance of the structural integrity of the cell wall and membranes (Bragança *et al.*, 2007; Zhang *et al.*, 2014; Borghi & Fernie, 2017).

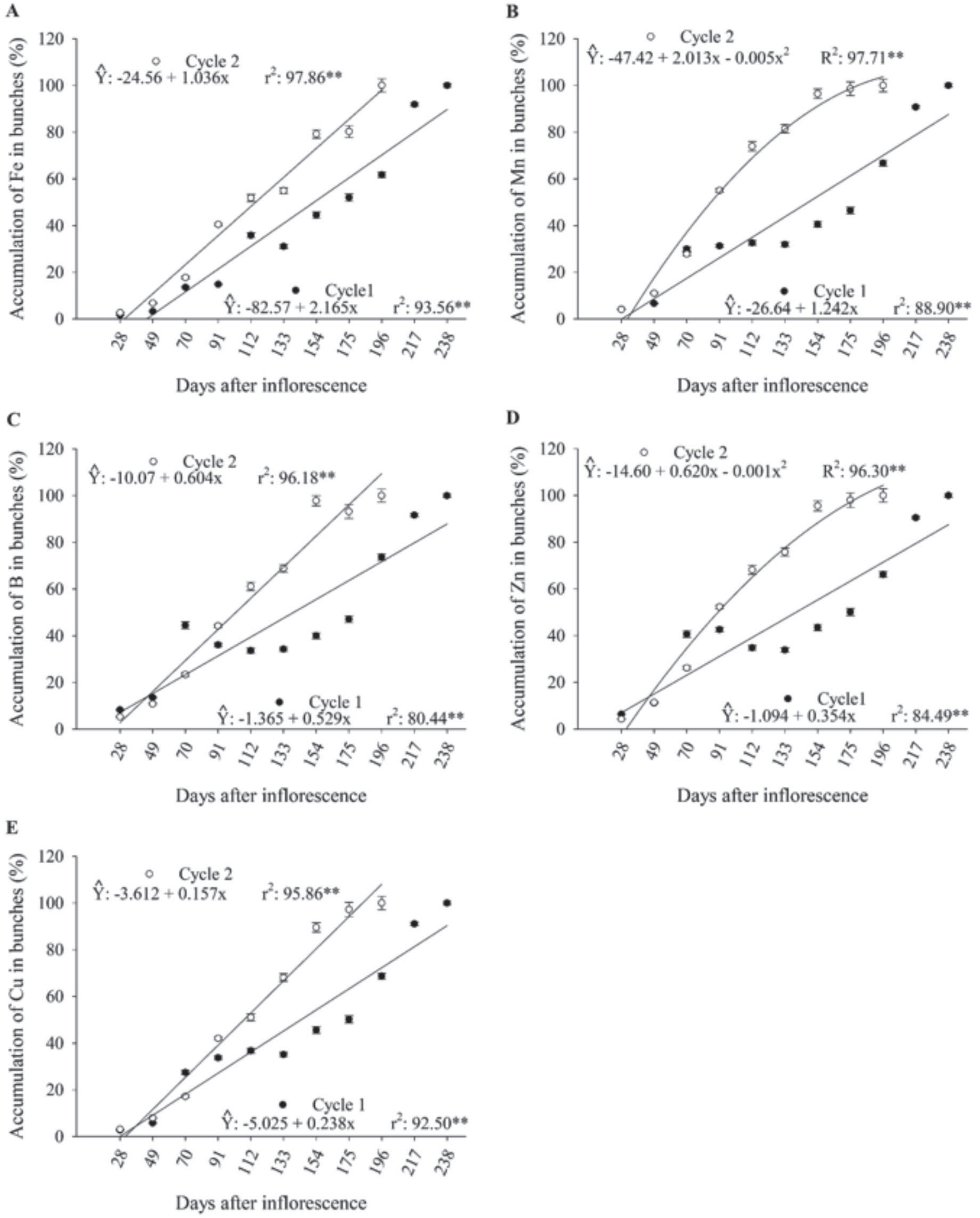

Figure 4: Fe (A), Mn (B), B (C), Zn (D) and Cu (E) accumulation in black pepper bunches over two crop cycles; bars indicate the standard error.

Zinc and copper were the nutrients accumulated in lowest quantity in the bunches. The lowest accumulation rates were observed in the first samplings, followed by an increasing behavior until the end of the evaluation period. At that time, 100% of the accumulated total was observed, i.e., 92.01 and 57.76 mg kg^{-1} Zn; and 24.76 and 55.20 mg kg^{-1} Cu, in the first and second cycles, respectively. The performance of Zn, which plays an important role in increasing cell division (Hamza & Sadanandan, 2005), was similar to that of Mn. Copper, which affects photosynthesis, respiration, N metabolism and plant reproduction (Mattos Jr. *et al.*, 2010), was the least accumulated element in the peppercorn bunches (Figure 4D and E).

In spite of the great importance of this information for an improved management of black pepper, no studies were found in the literature related to the accumulation of micronutrients over time in black pepper bunches. However, studies with micronutrient accumulation in *Coffea canephora* fruits in the southwestern Amazon (Dubberstein *et al.*, 2019), northwestern Espírito Santo (Bragança *et al.*, 2007) and southern Bahia (Covre *et al.*, 2018), stated that Fe, Mn and B are the elements most needed by the fruits, whereas Zn and Co were required at lower amounts, differing only in the accumulation pattern, with a sigmoidal performance.

The before-mentioned response was assumedly due to the time of the last drupe sampling, when the grains were completely filled and green, which is the ideal harvest point of the fruits. It is expected that thereafter, the accumulation will tend to stabilize and then decline, due to the transformations associated with fruit ripening, e.g., the degradation of chemical compounds. According to Whitehead & Bowers (2014), a natural strategy of plants is the alteration of the chemical composition of the mature fruits to avoid metabolic expenses; these changes may include a reduction in the chemical concentration of the fruits.

CONCLUSIONS

The highest concentrations of micronutrients, in the bunches, occurred in the inflorescence phase, with less expressive rates of nutrients at the end of the fruit formation cycle.

The most abundant micronutrients in the peppercorn bunches were Fe, Mn and B, followed by Zn and Cu.

There is seasonal variation in micronutrient concentrations in black pepper leaves throughout the year.

The decreasing order of the concentration of micronutrients in the leaf tissues of black pepper was Mn > Fe > Zn > Cu > B.

These results may contribute to a more sustainable agriculture, in which fertilization rates should consider different needs through the crop stage.

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The authors declare that there is no conflict of interest to disclosure.

REFERENCES

Abukawsar MM, Saleh-e-In MM, Ahsan MA, Rahim MM, Bhuiyan MNH, Roy SK, Ghosh A & Naher S (2018) Chemical, pharmacological and nutritional quality assessment of black pepper (*Piper nigrum* L.) seed cultivars. Journal of Food Biochemistry, 42:e12590.

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM & Sparovek G (2013) Köppen's climate classification map for Brazil. Meteorologische Zeitschrift, 22:711-728.

Ancuceanu R, Dinu M, Hovaneþ MV, Anghel AI, Popescu CV & Negreº S (2015) A survey of plant iron content - A semi-systematic review. Nutrients, 7:10320.

Bataglia OC, Gallo JR & Cardoso M (1976) The influence of applications of fertilizer on foliar concentrations of nutrients of black pepper. Bragantia, 35:405-411.

Bhat R, Kiran K, Arun AB & Karim AA (2010) Determination of mineral composition and heavy metal content of some nutraceutically valued plant products. Food Analytical Methods, 3:181-187.

Borghi M & Fernie AR (2017) Floral metabolism of sugars and amino acids: Implications for pollinators' preferences and seed and fruit set. Plant Physiology, 175:1510-1524.

Bragança SM, Martinez HEP, Leite HG, Santos LP, Sediyama CS, Alvarez V VH & Lani JA (2007) B, Cu, Fe, Mn and Zn accumulation by Conilon coffee plant. Revista Ceres, 54:398-404.

Covre AM, Partelli FL, Bonomo R & Gontijo I (2018) Micronutrients in the fruits and leaves of irrigated and non-irrigated coffee plants. Journal of Plant Nutrition, 41:1119-1129.

Dubberstein D, Partelli FL, Espindula MC & Dias JRM (2019) Concentration and accumulation of micronutrients in robust coffee. Acta Scientiarum. Agronomy, 41:e42685.

Etienne P, Diquelou S, Prudent M, Salon C, Maillard A & Ourry A (2018) Macro and micronutrient storage in plants and their remobilization when facing scarcity: the case of drought. Agriculture, 8:10.3390/agriculture8010014f.

FAOSTAT (2021) Crop production: Pepper. Available at: http://www.fao.org/faostat/en/#data/QC/visualize. Accessed on: April 14th, 2021.

Ferreira DF (2019) Sisvar: A computer analysis system to fixed effets split plot type designs. Revista Brasileira de Biometria, 37:529-535.

Gupta KK, Bhattacharjee S, Kar S, Chakrabarty S, Thakur P, Bhattacharyya G & Srivastava SC (2003) Mineral compositions of eight common spices. Communications in Soil Science and Plant Analysis, 34:681-693.

Flint AL & Flint LE (2002) Particle Density. In: Dane JH & Topp GC (Eds.) Methods of Soil Analysis: Part 4 - Physical Methods. Madison, Soil Science Society of America. p.229-240.

Hamza S & Sadanandan AK (2005) Effect of source and method of application of zinc on yield and quality of black pepper (Piper nigrum L.). Journal of Spices and Aromatic Crops, 14:117-121.

IUSS Working Group WRB (2015) World Reference Base for Soil Resources: International soil classification system for naming soils and creating legends for soil maps. Rome, FAO. 192p.

Kirkby EA & Römheld V (2007) Micronutrientes na fisiologia de plantas: funções, absorção e mobilidade. Piracicaba, International Plant Nutrition Institute. 249p.

Laviola BG, Martinez HEP, Salomão LCC, Cruz CD, Mendonça SM & Rosado LDS (2007) Nutrient accumulation in coffee fruits at two at two plantation altitudes: micronutrients. Revista Brasileira de Ciencia do Solo, 31:1439-1449.

Laviola BG, Martinez HEP, Souza RB & Venegas VHA (2006) Dynamics of N and K in leaves, flowers and fruits of arabic coffee (*Coffea arabica* L.) using three manuring levels. Bioscience Journal, 22:33-47.

Lijo T & Rajeev P (2011) Black Pepper. Kozhikode, Indian Institute of Spices Research. 24p.

Malavolta E, Vitti GC & Oliveira SA (1997) Avaliação do estado nutricional das plantas: princípios e aplicações. Piracicaba, Associação Brasileira para Pesquisa da Potassa e do Fosfato. 319p.

Mattos Jr D, Ramos UM, Quaggio JA & Furlani PR (2010) Nitrogen and copper for citrus nursery production on two different rootstocks. Bragantia, 69:135-147.

Nwofia GE, Kelechukwu C & Nwofia BK (2013) Nutritional composition of some *Piper nigrum* (L.) accessions from Nigeria. International Journal of Medicinal and Aromatic Plants, 3:2249-4340.

Ozkutlu F (2008) Determination of Cadmium and Trace Elements in Some Spices Cultivated in Turkey. Asian Journal of Chemistry, 20:1081-1088.

Paduit N, Pampolino M, Aye TM & Oberthür T (2018) Nutrient uptake and distribution in black pepper. Better Crops with Plant Food, 102:24-27.

Pradeep KU, Geervani P & Eggum BO (1993) Common Indian spices: nutrient composition, consumption and contribution to dietary value. Plant Foods for Human Nutrition, 44:137-148.

Prezotti LC, Gomes JA, Dadalto GG & Oliveira JA (2007) Liming and fertilization recommendation for the State of Espírito Santo, Brazil. Vitória, CEDAGRO. 305p.

Sadanandan A, Hamza S, Bhargava B & Raghupathi H (2000) Diagnosis and Recommendation Integrated System (DRIS) norms for black pepper (*Piper nigrum* L.), growing soils of South India. In: Muraleedharan N & Kumar PR (Eds.) Recent Advances in Plantation Crops Research. New Deli, Allied Publishers Limited. p.203-205.

Saleh-e-In MM, Sultana N, Rahim MM, Ahsan MA, Bhuiyan MNH, Hossain MN, Rahman MM, Kumar Roy S & Islam MR (2017) Chemical composition and pharmacological significance of *Anethum Sowa* L. Root. BMC Complementary and Alternative Medicine, 17:10.1186/s12906-017-1601-y.

Santos HG, Jacomine PKT, Anjos LHC, De Oliveira VÁ, Lumbreras JF, Coelho MR, Almeida JA, Araújo Filho JC, Oliveira JB & Cunha TJF (2018) Brazilian Soil Classification System. 5ª ed. Brasília, Embrapa. 356p.

Sarafi E, Siomos A, Tsouvaltzis P, Therios I & Chatzissavvidis C (2018) The influence of boron on pepper plants nutritional status and nutrient efficiency. Journal of Soil Science and Plant Nutrition, 18:653-667.

Srinivasan V, Dinesh R, Hamza S & Parthasarathy VA (2007) Nutrient management in black pepper (Piper nigrum L.). CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 2:01-14.

Taiz L, Zeiger E, Møller IM & Angus M (2014) Plant Physiology and Development. 6º ed. Sunderland, Sinauer Associates. 761p.

Teixeira PC, Donagemma GK, Fontana A & Teixeira WG (2017) Methods for Soil Analysis. 3ª ed. Brasília, Embrapa Solos. 574p.

Thangaselvabal T, Justin CGL & Leelamathi M (2008) Black pepper (*Piper nigrum* L.) the king of spices. Agricultural Reviews, 29:89-98.

Veloso CAC, Muraoka T, Malavolta E & De Carvalho JG (1998) Micronutrient deficiency in black pepper. Pesquisa Agropecuaria Brasileira, 33:1883-1888.

Viégas I de JM, De Sousa GO, Da Silva AF, De Carvalho JG & Lima MM (2013) Mineral composition and visual symptoms of nutrients deficiencies in long pepper plants (*Piper hispidinervum* C. DC.). Acta Amazonica, 43:43-50.

Whitehead SR & Bowers MD (2014) Chemical ecology of fruit defence: Synergistic and antagonistic interactions among amides from Piper. Functional Ecology, 28:1094-1106.

Yap CA (2012) Impact of different fertilization methods on the soil, yield and growth performance of black pepper (*Piper Nigrum* L.). Malaysian Journal of Soil Science, 16:71-87.

Zhang D, Zhao H, Shi L & Xu F (2014) Physiological and genetic responses to boron deficiency in Brassica napus: a review. Journal of Soil Science and Plant Nutrition, 60:304-31.