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Efficient detection of nutrient deficiencies and development of corrections in avocado through the Compositional Nutrient Diagnosis (CND)

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Abstract

Mexico is the main producer of avocado (*Persea americana* Mill.), contributing 31% of the world supply, which provides the country with an annual income of more than 2 billion dollars. The increase in national production is the result of a larger cultivated area, and not an increase in yields. In the State of Mexico, Mexico around 10 thousand hectares are cultivated with avocado trees, although 77% of the producers do not have specialized technical advice that offers reliable information on crop nutrition. This lack of advice and technical support detracts from volume and quality of production. The objective of this research was to carry out the nutritional diagnosis of an avocado orchard in Tejupilco, State of Mexico, Mexico, through the Compositional Nutrient Diagnosis (CND), and to generate specific fertilization recommendations for the study area, for which leaf and soil analyses were carried out. Each nutrient determined in the leaf analysis (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B) was compared with its optimal concentration according to literature. The CND yielded relative deficiency or excess indices that determined the order of nutritional limitation. Zn, B, S, and K deficiencies were detected in all sampled areas, while P, Mn, and Cu were deficient only in some areas. N, Ca, Mg, and Fe did not show nutritional limitation. Recommendations were formulated to address each of the nutritional deficiencies and the problem of sodicity revealed by the soil analysis. This is the first work in which the CND is used to assess the nutritional situation of avocado orchards in the world.

Keywords: chemical soil analysis; correction of nutritional deficiencies; nutritional sufficiency; *Persea americana* Mill.; yield

Introduction

Avocado (*Persea americana* Mill.), belonging to the Lauraceae family, is native to Mexico and Guatemala. There are approximately 400 varieties of avocado, which differ in the shape, color, and weight of the fruit and are classified into three horticultural races: Mexican, Guatemalan, and Antillean (Rebolledo-Roa and Dorado-Guerra, 2007). It is an evergreen tree that exceeds 12 m in height and 14 m in crown diameter,

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due to the jungle conditions of its center of origin, where it is in permanent competition for light and space. The 'Hass' cultivar is one of the most commercialized and distributed genotypes worldwide, which produces a pyriform fruit (berry), with thick skin, somewhat rough and green in color, which turns black as it ripens (Lemus *et al.*, 2010).

The essential elements have a determining influence on the growth and development of avocado trees, and under conditions of nutritional deficiencies, physiological damage and visual symptoms are observed (Salazar-García *et al.*, 2014; Novoa, *et al.*, 2018; Hurtado *et al.*, 2019; Selladurai and Awachare, 2020).

The analysis of plant tissue, is a diagnostic technique that allows to use the mineral concentration of the plants as an indicator of their nutritional status, associated with the achievement of high yields and betterquality characteristics of the harvested product, in relation with the degree of nutrient supply and availability of the substrate, generally the soil (Alcántar *et al.*, 2016). This analysis is the result of the integration of several factors, such as soil, climate, age, type of crop, and management (Espinosa, 1994). Leaf analysis assumes that the part of the plant sampled (generally the leaf) is the organ that reflects the nutritional status of the plant. Furthermore, it is assumed that there are close relationships among: a) soil nutrient supply and yield; b) soil nutrient supply and leaf concentration; and c) leaf concentration and yield (Torri, 2005).

The results of leaf analyses can be interpreted through various approaches. For this, critical or standard values have been generated, either by means of mathematical models or by qualitative examination of the responses to fertilizers (Salazar-García and Lazcano-Ferrat, 1999).

Leaf analysis does not exclude soil analysis but rather, both complement each other for a successful solution of the nutritional problem. A correct interpretation of the leaf analysis is required, as well as the knowledge of the physical and chemical characteristics of the soil to arrive at good recommendations on fertilization (Alcántar *et al.*, 2016).

The determination of the nutritional needs of the fruit trees must be carried out before establishing the yield potential. To do this, leaf analysis helps in the precise identification of these needs, based on the assumption of the positive relationship among the applied dose of some nutrient, the leaf concentration, and the yield (Savita *et al.*, 2016).

Mexico is the main avocado producer in the world, contributing 31% of the world supply, being the state of Michoacán the one that produces three out of every four avocados produced on Mexican soil. In second place is Jalisco with 10%, and in third place the State of Mexico with 5%. In the State of Mexico, about 10 thousand hectares of avocado are cultivated, and Coatepec Harinas is the most important municipality in this aspect. Despite the potential importance that the State of Mexico represents for this crop, 77% of producers do not have specialized technical advice to provide them with information on crop nutrition, which negatively impacts the volume of avocado produced and the quality of the production of this fruit (SIAP, 2019).

The objective of this research was to perform the nutritional diagnosis of avocado trees in the producing district of Tejupilco, located in the State of Mexico, Mexico, as well as to generate a fertilization recommendation considering the nutrient availability of the soil. As compared to other avocado producer areas in the State of Mexico, the introduction and establishment of avocado orchards is relatively recent, with avocado trees being younger than 15 years on average.

Materials and Methods

Site description

The avocado growing region of the municipality of Tejupilco is located in the community of Pueblo Nuevo Tenería (18.973 N, -100.093 W, 1765 masl), Mexico, with a subtropical climate with dry winters and warm summers (Cwa). The average annual temperature is 18.3 °C, while the maximum and minimum are 25.5 °C and 11.0 °C, respectively. Annual rainfall is 1340 mm, with a marked rainy season from June to October (Figure 1). The relief is mountainous with dominant pine and oak vegetation.

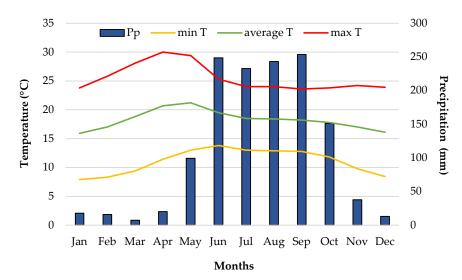


Figure 1. Climograph of the community of Tenería, Tejup ilco, the State of Mexico, Mexico Pp = Rainfall in mm; min T = Minimum temperature in °C; average T = Average temperature in °C; max T = Maximum temperature in °C

The study was carried out in a 3-ha commercial orchard, with 11-year-old trees of the 'Hass' variety, grafted on native Mexican avocado (*Persea americana* Mill.) var. 'Drymifolia', established at 6 × 6 m, cultivated with irrigation on acrisol soils. The region has an annual rainfall of 1340 mm, distributed from June to October. The orchard is located in the lower area of the community of Pueblo Nuevo Tenería, municipality of Tejupilco, State of Mexico (18.985 N, -100.102 W; 1769 masl).

The orchard is young (11 years), with yields that do not exceed 10 t ha⁻¹ on average. For years, a fertility gradient has been observed that goes in the direction of the slope (9%), being the eastern part (area 3, lower) the one that shows the highest production (Figure 2).

Soil and leaf sampling

Soil sampling. The division of sampling units within the orchard was done considering similar productivity characteristics and in a transverse direction to the slope (west-east), in order to know the variation in the amount of nutrients available for the crop (Figure 2a). In each unit, five trees equidistant from each other were chosen as sampling points, from which four subsamples were obtained per point, according to each cardinal point, following the methodology described in the Official Mexican Standard NOM-021-RECNAT-2000 (DOF, 2002).

Leaf sampling. For plant sampling, the three soil sampling units (Figure 2a) were subdivided in a northsouth direction, resulting in nine sampling units (Figure 2b), following the protocol described by Campos and Calderón (2015). The leaf samples were taken in the month of August 2020. The samples corresponded to mature leaves (including leaf blade and petiole), but not senescent, from terminal shoots without fruiting originating from the spring flow, healthy (without physical or chemical damage, nor affected by pests or diseases), seven months old and oriented in the four cardinal points (Maldonado, 2002).



Figure 2. Sampling sites within the avocado (*Persea americana* Mill.) orchard in Tejupilco, State of Mexico, Mexico

(a) Delimitation of soil sampling units and location of sampling sites. (b) Leaf tissue sampling units

Soil and plant chemical analyses

Soil chemical analysis. The determination of the pH and electrical conductivity of the soil were carried out using a portable pH, conductivity, and temperature meter (Corning, model PC18, Puebla, Mexico) in saturated paste extract, following the methodology referred to in the Official Mexican Standard NOM-021-RECNAT-2000 (DOF, 2002). Likewise, the $SO_4^{2^2}$ concentration was measured in the saturated paste extract by turbidimetry, in accordance with what is indicated in the aforementioned Official Mexican Standard.

The quantifications of total inorganic N, P, exchangeable cations $(Ca^{2+}, Mg^{2+}, K^+, and Na^+)$ and extractable metals (Fe, Cu, Zn, and Mn) were done with the micro Kjeldahl, Bray, ammonium acetate, and extraction with diethylenetriaminepentaacetic acid (DTPA) methodologies, respectively, according to the Official Mexican Standard NOM-021-RECNAT-2000 (DOF, 2002).

The extractions of NO_3^- and NH_4^+ were made using a 2 N KCl solution and quantified by steam distillation and titration (Brenmer, 1965).

The determination of soluble B in hot water (water:soil ratio 10:1, w:v) was carried out using an Inductively Coupled Plasma - Optical Emission Spectroscopy equipment (ICP-OES Liberty 725; Mulgrave, Australia) (ALS Environmental, 2019).

The apparent density of the soil was estimated following the paraffin method and the soil texture was determined with the Bouyoucos method (DOF, 2002).

Plant chemical analysis. In the laboratory, the leaf samples were rinsed with distilled water; later they were dried for 48 h in a forced air oven (Riossa HCF-125; Guadalajara, Mexico) at a temperature of 70 °C. Subsequently, they were ground to a particle size of 2 mm using a stainless-steel mill (Wiley 4; Swedesboro, NJ, USA) fitted with a 40-mesh sieve (425 µm).

The leaf N concentration was determined using the semi-micro Kjeldahl method, in the extract resulting from the wet digestion of dry and ground tissue, using a 3.3% solution of $C_7H_6O_3$ in concentrated H_2SO_4 (Alcántar and Sandoval, 1999).

The concentrations of P, K, Ca, Mg, S, Fe, Cu, Zn, Mn, and B were measured by ICP-OES in the aforementioned equipment, in the extract from digestion of dry leaf material with HNO_3 : $HClO_4$ (2:1, v:v).

Nutrient diagnosis

First of all, we carried out a literature search and analysis on leaf nutrient concentrations, associated with an optimal nutritional status of avocado trees and high yields. Our results are displayed in Table 1. With the nutritional sufficiency data obtained, we estimated the corresponding arithmetic mean and the standard deviation for each nutrient. These data were considered as a sufficiency reference.

With the nutrient sufficiency reference and the leaf concentration data obtained in this study, the nutritional diagnosis was made using the Compositional Nutritional Diagnosis (CND). The CND considers that the composition of the plant tissue forms an arrangement (S^D), D dimensional of nutrients; that is, the tissue is composed of d nutrients plus a filling value X_D that represents the undetermined nutrients (Parent and Dafir, 1992; Khiari *et al.*, 2001; Blanco-Macías *et al.*, 2006; Arroyo-Vargas *et al.*, 2013):

$$S^{D} = [(N, P, K, ..., X_{D}) : N > 0, P > 0, K > 0, ..., X_{D} > 0; N + P + K + ... + X_{D} = 100]$$

Where 100 is the total concentration of elements on a dry matter basis (%); N, P, K, ..., are the proportions of nutrients and X_D is the filling nutrient, estimated by considering 100% and the sum of the d proportions of nutrients as follows:

$$X_{D} = 100 - (N + P + K + \cdots)$$

These proportions become invariant in scale after they are divided by the geometric mean g:

$$g = (N * P * K * ... * X_D)^{1/I}$$

Then, the Z_i values (variable without units) are determined, calculating the logarithms of the proportions of each nutrient:

$$\begin{split} & Z_{N} = \log\left(\frac{N}{g}\right); Z_{P} = \log\left(\frac{P}{g}\right); Z_{K} = \log\left(\frac{K}{g}\right); \ Z_{X_{D}} = \log\left(\frac{X_{D}}{g}\right) \\ & \text{So that:} \\ & Z_{N} + Z_{P} + \ Z_{K} + \dots + Z_{X_{D}} = 0 \end{split}$$

Lastly, the z_i expressions of the norm are subtracted from the Z_i of the leaf sample and divided by the standard deviation (S) to obtain the I_{zi} indices. Negative values indicate relative deficiency; positive values indicate relative excess. Zero indicates nutritional balance.

$$I_{zi} = \frac{Z_i - Z_i}{S}$$

Table 1. Nutrient sufficiency levels in avocado (Persea americana Mill.) leaf tissue

		References								
Nutrient	Lemus <i>et</i> <i>al.</i> , 2010	Salazar- García and Lazcano- Ferrat, 1999	Embleton and Jones, 1964; 1972; Chapman, 1973; Lahav and Kadman, 1980	Goodall <i>et</i> <i>al.</i> , 1981	Rowley, 1992	Salazar- García, 2002	Maldonado- Torres <i>et al.</i> , 2007	Ruiz and Ferreyra, 2011	Sufficiency value	Standard deviation
		Leaf concentration								
N (%)	2.20	2.35	1.80	2.10	1.90	2.40	2.10	2.20	2.04	0.23
P (%)	0.20	0.14	0.17	0.18	0.18	0.17	0.17	0.20	0.17	0.01
K (%)	1.45	1.37	1.38	1.38	1.63	1.36	0.95	1.45	1.37	0.15
Ca (%)	2.00	1.86	2.00	2.00	2.25	2.00	1.90	2.00	2.00	0.09
Mg (%)	0.53	0.58	0.53	0.53	0.58	0.53	0.69	0.53	0.54	0.05
S (%)	0.40	0.40	0.40	0.40	0.53	0.45	n. r.	0.40	0.41	0.04
Fe (mg kg ⁻¹)	125.0	91.0	125.0	125.0	120.0	125.0	99.0	125.0	119.0	12.00
Mn (mg kg ⁻¹)	265.0	240.0	265.0	265.0	257.0	265.0	134.0	265.0	250.0	39.00
Zn (mg kg ⁻¹)	60.0	27.0	90.0	90.0	n. r.	90.0	35.0	60.0	70.0	24.00
Cu (mg kg ⁻¹)	10.0	10.0	10.0	10.00	9.0	10.0	19.0	10.0	12.0	5.00
B (mg kg ⁻¹)	50.0	75.0	75.0	75.00	120.0	75.0	239.0	50.0	90.0	52.00

n. r.= not reported

Fertilization recommendation

The fertilization recommendation was made considering the I_{zi} indices and the order of nutritional limitation generated by the CND analysis. Likewise, the nutritional extractions reported in the literature (Table 2) for avocado were considered. These values reported in the literature were used to determine the extraction of nutrients to obtain a target yield of 10 t ha⁻¹, given that in the season prior to the samplings (July-December, 2018), there was a yield of 8 t ha⁻¹.

	g 100 kg ⁻¹	kg 1 t ⁻¹	kg 1 t ⁻¹	kg 1 t ⁻¹	kg 10 t ⁻¹	kg 20 t ⁻¹	
	References						
Element	Salazar- García and Lazcano- Ferrat, 2001	Rebolledo-Roa and Dorado- Guerra, 2007	Maldonado- Torres <i>et al.</i> , 2007	Avilan <i>et al.</i> , 1986	Lahav and Kadman. 1980	Salazar-García and Lazcano- Ferrat, 2001	
Ν	257.0	94.560	2.73000	3.1520	11.30	51.50	
Р	103.0	22.080	0.72000	0.7360	1.70	20.60	
Κ	469.0	105.900	4.00000	3.5300	19.50	93.80	
Ca	8.4	14.220	0.23000	0.5470	2.10	1.70	
Mg	29.5	5.490	0.46000	0.4740	5.00	5.90	
S	34.5	16.410	n. r.	0.1830	8.00	6.90	
Fe	0.6	0.222	0.00937	0.0074	0.09	0.12	
Mn	0.1	0.060	0.00154	0.0020	0.02	0.02	
Zn	0.4	0.135	0.00406	0.0045	0.04	0.08	
Cu	0.2	0.090	0.00246	0.0030	0.01	0.04	
В	0.4	0.111	0.00547	0.0037	0.04	0.08	

Table 2. Estimates of nutritional extraction of the avocado (*Persea americana* Mill.) crop for different yields

n. r.= not reported

The nutrient supply capacity of the soil (results of chemical analysis) and the efficiency in the use of chemical fertilizers are also considered (Baligar *et al.*, 2001; Cassman *et al.*, 2002; Castro-Luna *et al.*, 2006; Stewart, 2007). In the estimates for obtaining the total amount of source fertilizer for each deficient nutrient, the following data were taken into consideration:

Area covered by canopy = 19.63 m^2 . Population density = $294 \text{ trees ha}^{-1}$. Cultivated area = 25000 m^2 .

Results

Soil diagnosis

The results of the analyses indicate that the soil in which the orchard is established has a loamy texture, a moderately acidic pH, and a medium level of organic matter (Table 3).

D	Soil unit sampling number					
Determination	1	2	3			
рН	5.84	5.73	5.68			
Electrical conductivity (dS m ⁻¹)	0.29	0.24	0.35			
Organic matter (%)	6.50	7.78	8.62			
NO ₃ ⁻ (mg kg ⁻¹)	26.60	42.00	65.80			
NH4 ⁺ (mg kg ⁻¹)	28.35	26.60	29.40			
Total N (%)	0.28	0.26	0.29			
P (mg kg ⁻¹)	2.69	3.13	3.53			
K (cmol _c kg ⁻¹)	1.04	1.10	1.32			
Ca (cmol _c kg ⁻¹)	2.01	2.13	2.17			
Mg (cmol _c kg ⁻¹)	0.70	0.58	0.63			
Na (cmol _c kg ⁻¹)	1.03	1.40	0.55			
$Fe (mg kg^{-1})$	13.17	10.60	12.12			
Mn (mg kg ⁻¹)	2.46	3.07	4.49			
$Zn (mg kg^{-1})$	0.54	0.70	0.79			
Cu (mg kg ⁻¹)	0.79	1.06	0.74			
B (mg kg ⁻¹)	0.04	0.02	0.03			
SO_4^{2-} (mg kg ⁻¹)	10.59	9.94	11.43			
Bulk density (g cm ⁻³)	1.136	0.839	0.900			
Sand (%)	36.56	40.56	42.56			
Silt (%)	36.00	38.00	38.00			
Clay (%)	27.44	21.44	19.44			
Texture class	Loamy	Loamy	Loamy			

Table 3. Results of the soil analysis where the avocado (*Persea americana* Mill.) orchard is established

Leaf nutrient diagnosis

The results of the leaf chemical analysis are shown in Table 4.

Elamana	Leaf sampling unit number									
Element	1	2	3	4	5	6	7	8	9	
N (%)	2.55	2.79	2.62	2.65	2.94	2.72	2.40	2.26	2.42	
P (%)	0.08	0.09	0.08	0.07	0.08	0.07	0.06	0.06	0.06	
K (%)	0.42	0.49	0.46	0.39	0.35	0.33	0.36	0.34	0.31	
Ca (%)	1.24	1.21	1.20	1.17	1.37	1.32	1.33	1.08	1.13	
Mg (%)	0.48	0.46	0.46	0.35	0.45	0.43	0.37	0.33	0.37	
S (%)	0.18	0.19	0.18	0.15	0.18	0.17	0.14	0.13	0.15	
Fe (mg kg ⁻¹)	69.00	79.00	79.00	80.00	84.00	73.00	82.00	73.00	82.00	
Mn (mg kg ⁻¹)	121.00	134.00	166.00	208.00	235.00	239.00	294.00	319.00	346.00	
Zn (mg kg ⁻¹)	12.00	14.00	14.00	14.00	15.00	13.00	15.00	14.00	14.00	
Cu (mg kg ⁻¹)	6.00	7.00	7.00	6.00	7.00	5.00	6.00	5.00	5.00	
$B(mg kg^{-1})$	21.00	22.00	23.00	18.00	19.00	17.00	16.00	16.00	16.00	

Table 4.	Results	of the	leaf c	hemical	analysis
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Order of nutrient limitation

The I_{zi} values (CND indices) for each nutrient in the nine analyzed areas are shown in Table 5.

Element		Leaf sampling unit number							
Element	lement 1		3	4	5	6	7	8	9
Ν	1.67	1.71	1.61	1.76	1.76	1.78	1.55	1.59	1.62
Р	0.58	0.84	-1.87	-4.86	-2.43	-3.81	-8.17	-7.25	-6.14
K	-1.35	-1.11	-1.31	-1.57	-2.09	-2.06	-1.79	-1.77	-2.12
Ca	0.87	0.45	0.44	0.66	0.91	1.13	1.16	0.60	0.57
Mg	4.69	3.73	3.72	2.14	3.34	3.64	2.47	2.18	2.58
S	-1.58	-1.78	-1.95	-3.45	-2.37	-2.17	-4.22	-3.93	-3.26
Fe	47.02	71.38	71.57	100.60	81.43	62.39	106.54	94.46	113.29
Mn	-6.14	-1.98	21.74	54.81	56.96	68.45	91.49	109.12	112.42
Zn	-187.95	-178.46	-176.78	-156.95	-167.78	-172.40	-156.51	-149.91	-159.43
Cu	57.50	83.73	39.35	60.77	101.49	-54.78	45.56	-110.81	-79.87
В	-65.32	-66.59	-61.11	-77.02	-80.37	-79.41	-84.27	-81.61	-85.71

Table 5. Compositional nutrient diagnosis indices (I_{zi}) that indicate the magnitude of the nutritional limitation (values with a negative sign) or excess (values with a positive sign) of each sample of avocado (*Persea americana* Mill.)

Table 6 shows the interpretation ranges for the values of the I_{zi} index. All the sample means were lower than the reference mean, so the concentration of each element that was equal to or greater than its reference resulted in a relative nutritional excess. In the opposite case, the indices were used to observe their magnitude and based on that, decide the order of limitation presented in Table 7.

Table 6. Interpretation ranges for values of the I_{zi} index in avocado (*Persea americana* Mill.)

E suelise es in equalise	I _{zi} values					
Equality or inequality	Xi < xi	Xi = xi	Xi > xi			
g(X) < g(x)	a	>0	>0			
g(X) = g(x)	<0	0	>0			
g(X) > g(x)	<0	<0	a			

 $L_{zi} < 0$: relative nutritional deficiency, $L_{zi} = 0$: nutritional balance. $L_{zi} > 0$: relative nutritional excess. Xi, xi, and g (X), g (x); nutrient level Xi and the geometric mean of the compositional arrangement for the independent sample and the reference, respectively. The trend for "a" depends on the magnitude of inequality (Parent and Dafir, 1992)

Table 7. Order of	nutrient limitati	on of each leaf	sampling area	obtained from	the avocado (Persea	
<i>americana</i> Mill.) or	chard					

Area	Order of limitation
1	Zn > B > Mn > S > K
2	Zn > B > Mn > S > K
3	Zn > B > S > P > K
4	Zn > B > P > S > K
5	Zn > B > P > S > K
6	Zn > B > Cu > P > S > K
7	Zn > B > P > S > K
8	Zn > Cu > B > P > K
9	Zn > B > Cu > P > S > K

Discussion

Soil diagnosis

The determination of electrical conductivity indicated minimal effects of soil salinity, with values less than 1 dS m^{-1} (Table 3); this condition is suitable for avocado development due to its high sensitivity to salinity (Coria, 2008; Castro *et al.*, 2015).

Regarding the nutrients analyzed, both inorganic N $(NH_4^+ \text{ and } NO_3^-)$ and total N were very high, the latter as a result of the N reserves present in organic matter, since around 90% of the total N of the soil is found in complex organic forms. The other macronutrients (P, Ca, Mg, and S) showed low levels. K concentrations were found high in the soil.

Table 3 shows that the micronutrients Fe, Mn, and Cu presented adequate levels; Zn registered a marginal level; and B had a level classified as very low (DOF, 2002).

Exchangeable Na⁺ showed concentrations in soil comparable to those of exchangeable K⁺ in areas 1 and 2. Sodic soils are classified using the sodium adsorption ratio (SAR) and the percentage of exchangeable sodium (PES) as parameters. The SAR is based on the concentration of Na⁺, Ca²⁺ and Mg²⁺; while the PES is the proportion of the cation exchange capacity (CEC) occupied by Na⁺ and is expressed as a percentage. A sodic soil must have a PES > 15 or a SAR > 13 (Alcántar *et al.*, 2016). In this study, area 1 presented a PES = 21.62 and area 2 a PES = 26.82, indicating a soil sodicity problem (Table 3). Although, "Hass" is a hybrid between the Mexican and Guatemalan races, which present medium and high tolerance to salinity, respectively (Lemus *et al.*, 2010), the problem of high levels of Na⁺ in the soil lies in the negative impact it has on K⁺ absorption (Alcántar *et al.*, 2016).

Leaf nutrient diagnosis

According to the leaf nutrient reference values (Table 1), N was the only element in the plant tissue analysis that exceeded its optimal reference value in the nine subplots, indicative of a good supply of this element from the soil. The levels of P, Ca, Mg, and S were found to be below the nutritional optimum in the samples from all the subplots, which agrees with the low levels of these elements in the soil. Leaf K concentration also showed levels below the optimum, despite the fact that K levels were classified as high in the soil. None of the micronutrients analyzed (Fe, Mn, Zn, Cu, and B) reached the optimum leaf concentration of the respective reference, even though Fe, Mn, and Cu were found at adequate levels in the soil.

Order of nutrient limitation

When analyzing the order of limitation in all the areas studied, it was observed that the most deficient element was Zn, followed by B (Table 7). Deficiencies of these two nutrients are common in many subtropical areas where avocado is grown commercially, especially in acid soils with a sandy texture (Crowley *et al.*, 1996; Salazar-García *et al.*, 2014).

The highly important degree of limitation that Zn presents reflects its low level in the soil (Table 7), which can be explained by the high presence of the NO_3^- ion that promotes its solubility and therefore also its leaching (Alcántar *et al.*, 2016). Furthermore, the absorption of Zn and Fe is antagonistic (Sequi, 2004); therefore, the high levels of Fe observed could be exacerbating the situation of Zn deficiency.

B deficiency is also a reflection of its low level in the soil. Soil texture influences the availability of this nutrient for the plant, since clay soils adsorb much more B than loamy and sandy soils (Communar and Keren, 2006; Ahmad *et al.*, 2012). In our case study, the clay content in the soil exacerbates the problem of B deficiency. Moreover, organic matter plays a preponderant role in B adsorption. The absorption of B by plants is regulated by its level in the soil solution, more than by its total content in the soil, and the great affinity of organic matter for B hinders its release into the liquid phase and its availability to plants (Yermiyahu *et al.*, 2001). In fact, B associated with humic colloids in organic matter is the main source of B for plant growth in most agricultural

soils (Ahmad *et al.*, 2012). In this study, the relative deficiency of B (more negative I_{zi} values) increases as the organic matter content does.

The degree of B adsorption in soils is quite dependent on pH, increasing sharply as the pH increased from 7.0 to 9.2 (Communar and Keren, 2006). However, in this study no relationship was found between soil pH and the availability of B.

Mn ranked third in nutritional limitation only in areas 1 and 2, a situation that may be due to the presence of Mg, which showed the highest levels in the entire higher zone of the orchard (areas 1, 2 and 3). In general, Mn absorption is affected by the presence of Ca and Mg, due to the similarity in their chemical properties with these alkaline-earth elements (Mousavi *et al.*, 2011).

Similarly, the high presence of Fe in the soil can interfere with the absorption of Mn, antagonism due to the fact that the absorption of these two micronutrients occurs through common membrane transporters, as is the case of the family of transition metal transporters of natural resistance macrophage-associated proteins (Nramp) (Rietra *et al.*, 2017).

On the other hand, the capacity of the soil to retain ions of transition elements (such as Mn) at a certain pH is reinforced by the adsorption of phosphate on the surface of the oxides present; that is, phosphate adsorption has a positive effect on cation adsorption (Diaz-Barrientos *et al.*, 1990; Neilsen *et al.*, 1992). Therefore, it is possible that the presence of P increases Mn adsorption, causing a reduction in its concentration in the soil solution and therefore in its availability.

In fact, interactions between P and Mn are well documented (Barben *et al.*, 2010). In soybeans, the increase in P concentration decreased Mn accumulation and even mitigated the symptoms of Mn toxicity (Nogueira *et al.*, 2004). In wheat, the leaf concentration of Mn was reduced in soils with abundant P (Neilsen *et al.*, 1992). In potatoes and tomatoes, a reduction in the concentration of leaf P has been observed as the Mn supply increases (Gunes *et al.*, 1998; Sarkar *et al.*, 2004). In this research, it is observed that, only in the areas where P was found above the nutritional balance (1 and 2), was Mn deficient. The data indicate a negative relationship between these elements; as well as the influence of P on Mn absorption ($R^2 = 0.87$) (Figure 3a).

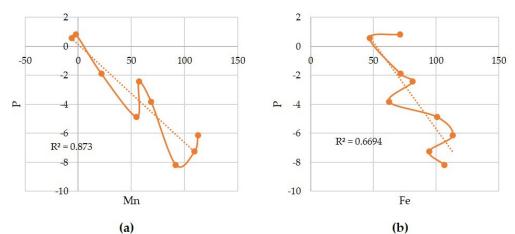


Figure 3. Relationship of the CND indices for P and Mn (a) and for P and Fe (b) in avocado (*Persea americana* Mill.)

On the other hand, the relative P deficiency observed in areas 3-9 may result from the high levels of Fe in the soil. Figure 3b indicates a negative relationship between the CND indices for P and Fe ($R^2 = 0.66$). In acid soils, the process of fixing P by Fe is as follows: 1) the highly reactive Fe is hydroxylated when wet conditions are encountered; 2) the acidic conditions of the soil cause a protonation of the hydroxyl, which confers it properties of Lewis acid; 3) Protonation weakens the Fe-OH bond and the electronegativity of O is conferred on H, making it very reactive and easily interchangeable with organic and inorganic anions in

solution, 4) protonation of the hydroxyl ion can trigger one of the two processes of P adsorption: a) protonated surfaces generate a positive electric field that attracts phosphate ions; and b) phosphate replaces protonated hydroxyl groups (Fink *et al.*, 2016). In this study, the soil pH is considered moderately acidic with values ranging between 5.63 and 5.84 (Table 3).

The indices corresponding to S indicated a relative deficiency in all sampled areas (Table 5). Although the spraying of calcium sulphide (calcium polysulphide) is an important practice in the orchard, the amount that is applied is insignificant to supply the crop requirement, since the objective is not the nutrition of S or Ca through the leaves, but phytosanitary control, in addition to the fact that, in practice, no importance has been given to the application of S to the soil in this cultivation land.

Despite the high exchangeable K⁺ concentration in the soil, the I_{zi} values derived from the leaf analysis (Table 5) placed it as a deficient element, although in the last place in the order of limitation, just below the nutritional balance. These results may be due to the inhibition in K⁺ absorption caused by the high concentration of Na⁺ in the soil; particularly in the soils of areas 1 and 2, where the K⁺:Na⁺ ratios in the exchange complex were 1 and 0.79, respectively (Table 3). Given the similarity in the physical and chemical properties between Na⁺ and K⁺, both compete in the absorption process, in particular through high-affinity potassium transporters (HKT) and non-selective cation channels (NSCC). Furthermore, the depolarization of the membrane caused by Na⁺ hinders the absorption of K⁺ by the inward rectifier channels (KIR) and increases cellular K⁺ leakage when the K⁺ outward rectifier channels (KOR) are activated (Villa-Castorena *et al.*, 2006; Zhang *et al.*, 2010; Wakeel, 2013).

Cu deficiencies were found in areas 6, 8, and 9, and it even displaced B as the second most limiting element in area 8 (Table 5). This could be caused by the content of organic matter, since most Cu is found forming very stable complexes with it and only a small fraction is found as exchangeable Cu²⁺ (Alcántar *et al.*, 2016).

The elements that were found in relative excess in all samples were N, Ca, Mg, and Fe. It was expected that N was not part of the group of deficient elements (order of limitation), since it exceeded the value of reference in each of the samples. This was not so in the case of Ca, Mg, and Fe, but due to the multivariate relationships, they were positioned as non-limiting elements.

Fertilization recommendation

The total amount of Zn required to achieve an abundant harvest of quality fruits does not represent a problem. The real difficulty lies in ensuring that the tree receives the necessary amount of this element to satisfy its physiological requirements and produce profitable crops (Salazar-García *et al.*, 2014).

A common practice to correct micronutrient deficiencies is foliar fertilization. Zn deficiency in 'Hass' avocado can be corrected spraying the leaves with 2 g ZnSO₄ (36% Zn) L⁻¹ (Salazar-García, 2002). However, other studies indicate that Zn foliar sprays are not effective in avocado trees because the mature leaves show a limited capacity to absorb and transport the salts applied this way (Salazar-García *et al.*, 2014).

In a study carried out in alkaline soils, in California, USA, it was concluded that less than 1% of the Zn is absorbed after a foliar application, and of this, only between 5 and 8% is translocated towards demanding tissues. The application of 3.2 kg ZnSO_4 (36% Zn) tree⁻¹ year⁻¹ turned out to be the best method to raise the leaf concentration to the nutritional optimum (Crowley *et al.*, 1996).

Soil application of $ZnSO_4$ appears to be effective only when large amounts are used. Calabrese (1992) suggests using at least 2 kg $ZnSO_4$ (36% Zn) tree⁻¹ year⁻¹. Avilán *et al.* (1986) and Villaseñor (1999) recommend 1.5 kg for trees older than five years. Lahav and Whiley (2002) indicate that 200 kg ha⁻¹ is sufficient, while Abercrombie (2011) recommends applications between 5 and 10 g $ZnSO_4$ m⁻², spread under the tree canopy.

In a study carried out with acid and loamy soils in Nayarit, Mexico, foliar sprays did not increase leaf Zn levels to the optimum (Salazar-García *et al.*, 2008). On the other hand, two applications of 0.750 kg ZnSO₄ (36% Zn) tree⁻¹ year⁻¹ (total 540 g of Zn) resulted in an increase in the leaf Zn content (24.9 mg kg⁻¹; classified

as normal) (Salazar-García *et al.*, 2008). This recommendation seems to be the most appropriate to correct the Zn deficiency problem.

Since the entire orchard under evaluation presented the Zn deficiency problem, the calculation to obtain the amount of fertilizer to apply is as follows:

$$\frac{0.540 \text{ kg Zn tree}^{-1}}{0.36} = 1.5 \text{ kg ZnSO}_4 \text{ tree}^{-1}$$

1.5 kg ZnSO₄ x 294 trees = 441 kg ZnSO₄ ha⁻¹

Leaf applications of B in the flowering season can be beneficial for fruit set (Jaganath and Lovatt, 1999), which is reflected in increases in production of up to 26% when applied in orchards without deficiency of this nutrient (Lovatt, 1999). However, care must be taken with leaf applications of B since the gap between deficiency and toxicity is small, and they should only be carried out during floral flows, which is when they have the highest B absorption capacity (Joubert, 2016). A foliar spray of 2 g B tree⁻¹ by means of Solubor (20.5% B) at 0.1% concentration should be sufficient for each tree (Bender, 2004).

Similar to Zn, applications of B to the soil are usually more effective than foliar sprays (Salazar-García *et al.*, 2014). For California, the application recommendations for this element fluctuate between 50 g B tree⁻¹ from Borax (11% B) (Embleton and Jones, 1966), and 3 g B m⁻² using Boronat (30% B) (Joubert, 2016). Other recommendations are: for Hawaii, 69 g B tree⁻¹ (Miyasaka *et al.*, 1992), for Australia, 41 g B tree⁻¹ (Smith *et al.*, 1997), and for South Africa, the recommended dose was 31 g B tree⁻¹, divided into three applications (Bard and Wolstenholme, 1998).

The proportion of fruit in prime and super extra sizes increases the following year after having applied two sprays of 0.5 g B L⁻¹ (Salazar-García *et al.*, 2014), although the leaf levels of this element were below the optimal value (28.3 mg kg⁻¹) and yield did not increase. Similar results were obtained in other studies (Coetzer *et al.*, 1993; Lovatt, 1999), since spraying the leaves with B only increased the yield in some orchards and in some years. These findings indicate that foliar fertilization with B is not effective in correcting deficiencies in the short term and is not profitable. On the other hand, two annual applications of 0.75 g B m⁻² (29.5 g Boronat tree⁻¹ application⁻¹) increased the leaf concentration of B (34.9 mg kg⁻¹) until the fourth year of treatment. That is, the B deficiency was controlled four years after the first applications. Nevertheless, fruit production increased two years later by more than 40 kg tree⁻¹, as well as the proportion of prime, extra, and super extra fruit (Salazar-García *et al.*, 2014).

In this study, the recommendation is to apply 39 g B tree⁻¹ (2 g B m⁻²), divided into two applications. This amount is intermediate between those established in previous studies (Salazar-García *et al.*, 2014; Joubert, 2016). The calculation to obtain the amount of fertilizer is as follows:

$$\frac{39 \text{ g B}}{0.30} = 130 \text{ g Boronat tree}^{-1}$$

130 g Boronat x 294 trees = 38.22 kg Boronat ha⁻¹

P levels in trees can be improved by two methods. The first is by applying 46 L of H_3PO_4 per hectare through the irrigation system, followed by an annual application of 28 L ha⁻¹. The second is by applying dry fertilizer at a rate of 1.13 kg P₂O₅ tree⁻¹ for three years (Bender, 2004). Phosphate fertilization for 7-year-old and older trees should be between 500 and 1500 g P₂O₅ tree⁻¹ year⁻¹ (Avilán *et al.*, 1986). It is also recommended to correct this deficiency with applications of simple superphosphate (SSP, 10% P) at a rate of 50 g m⁻² in the dripping area, or of monoammonium phosphate (MAP, 27% P), using a dose of 10-20 g m⁻² (Abercrombie, 2011).

A yield of 10 t ha⁻¹ needs to extract 6.64 kg of P from the soil (Table 2), for which it is important to consider that phosphorous fertilizers show an efficiency of 15% in use (Stewart, 2007). In this case, the use of triple superphosphate (TSP) (00-46-00 + 14 Ca, 20% P) is considered, as seen below:

$$6.64 \text{ kg P} = 15.21 \text{ kg P}_2\text{O}_5$$
$$\frac{15.21 \text{ kg P}_2\text{O}_5}{0.15} = 101.4 \text{ kg P}_2\text{O}_5$$
$$\frac{101.4 \text{ kg P}_2\text{O}_5}{0.46} = 220 \text{ kg TSP ha}^{-1}$$

The application is expected to raise the concentration of P in the soil to 10 mg kg⁻¹. The application of 220 kg TSP adds 30 kg Ca ha⁻¹.

Little is known about K deficiency in avocado, making it difficult to recommend a management procedure. The leaf K concentration can be increased by means of foliar sprays of K_2SO_4 (00-00-52 + 18 S; 43% K) at a 3% concentration, but it should not replace soil fertilization, which should be between 150 and 300 kg K_2O ha⁻¹.

In California, an application of 1.8 to 3.6 kg K tree⁻¹ is recommended (Bender, 2004), although KNO₃ or K_2SO_4 has also been applied at a dose of 10 g m⁻² (Joubert, 2016). Potassium fertilization for trees 7 years of age and older should be between 600 and 1400 g K_2O tree⁻¹ year⁻¹ (Avilán, 1986).

In this study, the high concentration of K observed in the soil suggests little response by the crop to soil fertilization. However, to increase the K absorption capacity, it is necessary to solve the problem of soil sodicity and lower the Na levels, for which the use of agricultural gypsum (CaSO₄ $2H_2O$) is recommended. This amendment provides Ca which can be exchanged with Na, thus leading to flocculation of soil particles and a better development of the general soil structure (Chen and Dick, 2011). In this sense, the projected objective is to obtain a PES = 10 in the entire study area.

The levels of Mn in soil observed in this study are low, and at a soil pH lower than 6, an amount of 4.8 kg Mn ha⁻¹ would have to be added to the soil through $MnSO_4$ (32% Mn), to correct the nutritional deficiency.

Mn is an abundant element in agricultural soils of acid pH. In deficient conditions, this element can be supplied through the application of 320 to 640 g Mn tree⁻¹ incorporated into the soil, under the layer of organic matter (Bender, 2004). Applications of 2 and up to 6 kg Mn ha⁻¹ are also recommended, or foliar sprays of MnSO₄ (32% Mn) at 0.5% (Yamada, 2004), which in this case is convenient because the problem is not the Mn content in the soil but its absorption through the roots.

In general, spraying cupric fungicides included in the phytosanitary management is sufficient to cover the Cu need of the avocado crop. For soil applications, 3 kg Cu ha⁻¹ from CuSO₄ (25% Cu) is recommended. However, since there is an adequate Cu content in the soil, the use of foliar sprays based on CuSO₄ (25% Cu) is desirable, as long as they do not exceed 0.1% concentration because it can cause burns on the leaves (Bennet, 1993; Bender, 2004; Alcántar *et al.*, 2016).

S deficiency is not common in avocado orchards. Due to its inclusion as a SO_4^{2-} anion in various fertilizer sources, it is not directly taken into consideration in fertilization programs, but rather its application is a result derived from the application of other nutrients (Abercrombie, 2011). With applications of 1 to 3 t ha⁻¹ of agricultural gypsum (CaSO₄), it has been possible to correct S deficiency successfully, as well as with applications of 10 g K₂SO₄ m⁻² (Joubert, 2016). In this study, the S needs are covered with the supply of ZnSO₄ and CaSO₄ to the soil.

Conclusions

This is the first work in which the Compositional Nutritional Diagnosis (CND) method is used to assess the nutritional status of avocado orchards. This method allowed a better interpretation of the leaf analyses, since all the evaluated nutrients, except N, were below the norm, which would be a greater problem if we considered each nutrient independently. With the CND, the concentration of each particular nutrient influences all the others, causing a relative deficiency or excess response, which helps determine the focus of nutritional correction in the fertilization plan, in order to be efficient, increase productivity, and lower costs.

Authors' Contributions

Conceptualization, L.I.T.-T.; methodology, R.G.-V. and L.C.-B.; validation, L.I.T.-T. and F.C.G.-M.; formal analysis, R.G.-V. and L.C.-B.; investigation, R.G.-V.; resources, L.I.T.-T.; writing—original draft preparation, R.G.-V. and J.S.-E.; writing—review and editing, F.C.G.-M. and J.S.-E.; supervision, L.I.T.-T.; project administration, L.I.T.-T.; funding acquisition, L.I.T.-T. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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