Boundary-Line Approach micro-nutrient optimum concentrations and sufficiency ranges for *Opuntia ficus-indica* (L.) Miller fruiting

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ABSTRACT

Optimum concentrations and/or sufficiency ranges of nutrients are useful for correct diagnosis and improvements of nutrient status of cultivated plants. However, *Opuntia ficus-indica* nutrient storage at plant and cladode levels and best fit plant requirements for fruiting remain practically unknown. Then, this research work aimed to identify the Boundary-Line Approach (B-LA) micronutrient optimum concentrations linked to maximum fruit yield per one-year-old fructification cladode and sufficiency ranges at 90% maximum fruit yield for *O. ficus-indica* variety 'Rojo Pelón'. Four years (2012 – 2015) data of fruit yield per cladode and micronutrient concentrations (B, Cu, Fe, Mn, and Zn) were used for the elaboration of scatter diagrams (n = 228) and selection of 7 to 10 points to estimate the B-LA quadratic functions. Then, the vertices allowed estimation of the optimum micronutrient concentrations: B = 33.62 mg kg^{-1}, Cu =11.74 mg kg^{-1}, Fe = 108.51 mg kg^{-1}, Mn = 149.33 mg kg^{-1} and Zn = 414.91 mg kg^{-1} as linked to estimated maximum yield per cladode; and the sufficiency ranges at 90% maximum fruit yield: B = 24.44 to 46.25 mg kg^{-1}, Cu = 8.68 – 15.87 mg kg^{-1}, Fe = 73.73 – 143.3 mg kg^{-1}, Mn = 114.89 – 183.77 mg kg^{-1} and Zn = 334.31 – 514.95 mg kg^{-1}. The linked estimated maximum fruit yield per cladode varies from 1999.48 g to 2139.59 g. The proposed B-LA standards can be used to perform reliable micronutrient diagnosis and proper fertilization recommendations.

Keywords: *Boron, Copper, Iron, Manganese, Zinc, Fruit.*

INTRODUCTION

Plant analysis can be an useful tool for estimating plant nutrient status, maximizing crop yield, and evaluating fertilizer requirements. Then, using the plant analysis as a diagnostic criterion requires knowledge of the relationships between yield and plant nutrient concentrations (Reis Junior and

Monnerat, 2003). Therefore, good relationships between crop performance and plant nutrient status (Dow and Roberts, 1982) are expected when the involved nutrient is a limiting factor (Blanco-Macías et al., 2010). Thus, optimum concentrations and/or sufficiently ranges of nutrients may be useful for correct diagnosis and improvements of nutrient status of cultivated plants (Blanco-Macías et al., 2009, 2010).

Traditionally, optimum concentrations and sufficiency ranges have being estimated through the Critical Values Technique. Moreover, the determination of critical nutrient values and nutrient balances in plant-diagnostic models (Walworth et al., 1986) has being carried out by using the principle of the Boundary-Line Approach (B-LA) as described by Webb (1972). In addition, the B-LA has been used to describe the relationship between soil nutrient concentrations and crop yields (e.g. Evanylo, 1990).

B-L A standards have been developed for several crops. For instance, miscanthus, reed canary grass and triticale (Lewandowski and Schmidt, 2006), sugar maple (Vizcayno-Soto and Côté, 2004), white spruce (Quesnel et al., 2006), maize (Walworth et al., 1986), areca nut (Bhat and Sujatha, 2013), wild lowbush blueberry (Lafond, 2013), mango (Ali, 2018), and *Opuntia ficus-indica* (Blanco-Macías et al., 2009; 2010). However, the B-LA nutrient standards for the *O. ficus-indica* case were developed under the basis of relationships between one-year-old cladode macronutrient concentrations and produced yearly cladode fresh matter as yield (Blanco-Macías et al., 2009, 2010). This information on *O. ficus-indica* allows for discarding the prevailing common opinion about cactus crop needs low inputs to give high yields. In addition, it is reinforced by a few studies that have documented the macro- and micro-element concentrations in cladodes. Several of them have been focused on the mineral contents of cladodes to demonstrate their forage potential, and of tender pads, one-year-old fruiting cladodes and fruits to determine their nutritional contribution to the human diet.

For instance, micro-element concentrations in one-year old fruiting cladodes of *Opuntia dillenii* are as follows (Kalegowda et al., 2015): Cu = 4.5 mg kg^{-1}, Fe = 21.9 mg kg^{-1}, Mn = 25.9 mg kg^{-1} and Zn = 14.1 mg kg^{-1}. In addition, estimated micro-element contents in one-year old cladodes of *Opuntia ficus-indica* were: B = 129.17 mg kg^{-1}, Cu = 1.83 mg kg^{-1}, Fe = 93.33 mg kg^{-1}, Mn = 36.33 mg kg^{-1} and Zn = 17.67 mg kg^{-1} when trees were growing under field conditions (Mayer and Cushman, 2019); whereas the concentrations were: B = 67.33 mg kg^{-1}, Cu = 22.17 mg kg^{-1}, Fe = 140.33 mg kg^{-1}, Mn = 705.0 mg kg^{-1} and Zn = 67.17 mg kg^{-1} when plants were growing under greenhouse conditions (Mayer and Cushman, 2019).

Other investigations have been focused on the effect of soil fertilization on fruit production. For example, nitrogen fertilization (0, 60, 120 kg ha^{-1}) did not affect flower bud formation in *O. ficus-indica* (Nerd and Mizrahi, 1994). No *O. ficus-indica* fruit response was evidenced in the case of fertilizer application even comparing application rates of 100 kg ha^{-1} N, 50 kg ha^{-1} P, 100 kg ha^{-1} K, 50 kg ha^{-1} Mg with the control that had never been fertilized (Karim et al., 1997; Galizzi et al., 2004). Nitrogen and phosphorus fertilization (0-0, 0-80, 40-40, 60-0, and 60-80 kg ha^{-1} N-P_2O_5) did not have any effect on fruit yielding in the first year, however, the doses 60 kg ha^{-1} N or 80 kg ha^{-1} N-P_2O_5 alone increased the fruit yield by +3 and +6.1 kg $plant^{-1}$, respectively, compared with the control (Arba et al., 2017). Those results suggest that fertilizer/response is difficult due to the high moisture content of cladodes and the large mass of *Opuntia'*s buffers nutrient changes (Felker and Bunch, 2009). In other words, these research works did not relate cladode nutrient concentrations with yield or quality of yield in terms of cladode biomass or fruit (prickly pear) using statistical trends or functions. This means that nutrient storage at plant and cladode levels, and best fit plant requirements for fruiting remain practically unknown (Inglese et al. 1995), mainly when related to growth or fruit yield. Then, this research work aimed to identify the B-LA micronutrient optimum concentrations related to maximum fruit yields per one-year-old fructification cladode, and sufficiency ranges at 90% maximum fruit yield for *O. ficus-indica* variety "Rojo Pelón".

MATERIAL AND METHODS

Experimental plot

The experimental orchard was established in 19th June 2006 at the Centro Regional Universitario Centro Norte of the Universidad Autónoma Chapingo (22°44'49.6''N; 102◦46'28.2''W; 2296 masl), near the city of Zacatecas, Mexico. The orchard was established to propagate *O. ficus-indica* variety 'Rojo Pelón'. For that, twenty mother cladodes were used. The basic statistics of their attributes were as follows (mean ± standard deviation): 548.6 ± 190 g, fresh matter; 28.5 ± 5.4 cm, cladode length; and 16.3 ± 2.2 cm, cladode width. Thus, 20 naturally vase-shaped trees were growing. A density of 625 plants ha^{-1} was used within the experimental plot. After the orchard establishment, weeds were removed each year in late spring and summer by low-intensity tillage, but fertilization, irrigation, and other agronomic practices were not performed.

The climate of the region is classified as BS1kw (w), with a yearly average precipitation of 472 mm and mean annual temperature ranging between 12°C and 18°C. Most of the precipitation (65%) occurs from June to August. The soil at the experimental orchard is a clay loam of calcareous origin with a pH of 7.5 and organic matter content of 3.2%.

Data

One-year-old fruiting cladodes and their fruits were collected during the years 2012, 2013, 2014, and 2015. The collection was performed as follows: 2012, 60 cladodes and 480 fruits; 2013, 52 cladodes and 364 fruits; 2014, 56 cladodes and 420 fruits and 2015, 60 cladodes and 480 fruits. In summary, 228 one-year-old fruiting cladodes and their 1744 fruits of *O. ficus-indica* variety 'Rojo Pelón' were involved in this study.

All fruiting cladodes were selected from the uppermost part of the trees to ensure they were one-year-old. We selected cladodes having from one to 15 fruits to include representative variability in fruit yield per cladode or cladode load (Valdez-Cepeda et al., 2013). Four cladodes having each of these numbers of fruits were selected from different plant orientations (north, south, east, and west) as pointed out by Valdez-Cepeda et al. (2013). All 1744 fruits were harvested when most of them showed peel coloration change indicating the beginning of fruit ripeness. All harvested fruits and fruiting cladodes were weighted; previously, cladodes were cleaned with distilled water. Afterward, all cladodes were cut into slices and dehydrated to constant dry weight in an oven at

75°C for 36 h to measure their dry weights. The estimation of micro-nutrient concentrations (B, Cu, Fe, Mg, and Zn) in all 228 fruiting cladodes was performed through spectrophotometry once acid digestion of the dry tissue samples was carried out.

Statistical analyses

Concentration values of Fe and Mn were normally distributed. Variables that were not normally distributed were transformed: fruit yield per cladode to square root (SQRT) and B, Cu, and Zn to the natural logarithm (ln). Data of fruit yield per cladode and each micronutrient concentration was used for the elaboration of bivariate scatter diagrams (n = 228). Later, the B-LA was applied as in *Blanco-Macías* et al. (2010) to describe the relationships between fruit yield per cladode versus each micronutrient concentration (B, Cu, Fe, Mn, or Zn) taking into account normally-distributed expressions.

The Boundary-Line (B-L) is created when all values for two variables are plotted and a line enclosing most of these points is established (Michael et al., 1985; Blanco-Macías et al., 2010). The line represents the limiting effect of the independent variable on the dependent variable (Webb, 1972; Lark, 1997). Thus, it is assumed that all values below the line result from the influence of another independent variable or a combination of variables that are limiting the dependent one (Webb, 1972; Hinkley et al., 1978).

Data selected for estimating the B-L was used to estimate quadratic functions using Microsoft® Excel for Mac, Version 15.13.3 (Microsoft, 2015). Then, vertices were calculated. Later, lower and higher values of sufficiency ranges at 90% maximum fruit yield per one-year-old fruiting cladode were solved through the estimated quadratic functions.

RESULTS

In this research work, the relationships between fruit yield per cladode and each micronutrient concentration in one-year-old fruiting cladodes of *O. ficus-indica* variety 'Rojo Pelón' were considered to estimate B-LA nutrient reference values. The main basic statistics of the variables are summarized in Table 1. The concentration of B, Cu, and Fe and the fruit yield per cladode showed high variability, whereas those of Mn and Zn showed moderately high variability. Variability is an important aspect to get our objective; thus, this database can be used to identify the load per cladode dependence on each micronutrient concentration.

Fruit yield per cladode versus B concentration

Most of the data points are cluster at the bottom of the plot (Figure 1), that is, at low fruit yields. Clearly, maximum yield order is appreciated as follows: four years > 2014 > 2015 = 2013 > 2012. In addition, the overall scatter diagram allows us to appreciate that maximum yield (SQRT = 44.537, i. e. 1983.544 g) is strongly link to the optimum ln B = 3.515 (33.625 mg kg^{-1}); this optimum concentration was almost the same to the four years. On the other hand, the estimated sufficiency range at 90% of maximum fruit yield per fructification cladode (SQRT = 40.083, i. e. 1606.65 g) is determinate by B concentrations of 24.445 (ln B = 3.196) and 46.253 mg kg^{-1} (ln B = 3.834).

Table 1. Basic statistics (n = 228) for fruit yield per cladode and micronutrient concentrations in one-year-old fruiting cladodes of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón'.

Variable	Mean	Standard deviation	Coefficient of variation	Minimum	Maximum
B (mg kg^{-1})	39.711	12.43	31.290	17.090	75.970
Cu (mg kg^{-1})	11.743	3.465	29.51	5.00	22.00
Fe (mg kg^{-1})	103.63	25.88	24.970	47.00	158.00
Mn (mg kg^{-1})	145.97	30.23	20.710	85.00	231.00
Zn (mg kg^{-1})	402.16	75.14	18.680	246.00	624.00
Fruit yield per cladode (g)	804.8	465.3	57.82	80.00	2186.00

Fruit yield per cladode versus Fe concentration

The scatter plot shows that the range of the Fe concentration was wide (Figure 3). The maximum fruit yield per cladode (SQRT = 44.767, i. e. 2004.315 g) is associated with an optimum concentration of Fe = 108.514 mg kg^{-1}. This concentration and fruit yield per cladode were similar to the estimated 2015 optimum Fe concentration and maximum income. Moreover, the estimated sufficiency range at 90% maximum fruit yield per fructification cladode (SQRT = 40.293, i. e. 1623.495 g) involves Fe concentrations between 73.729 and 143.298 mg kg^{-1}.

Fruit yield per cladode versus Mn concentration

Most of the data points are grouped at the bottom of the scatter plot (Figure 4). The diagram shows that maximum yield (SQRT = 46.256, i. e. 2139.593 g) is strongly link to a concentration of Mn = 149.333 kg^{-1}. Notably, the 2014 estimated optimum Mn concentration was higher than the overall estimated optimum Mn concentration. In addition, the estimated sufficiency range at 90% maximum fruit yield per fructification cladode (SQRT = 20.293, i. e. 1623.495 g) was determined by Mn concentrations between 114.894 and 183.772 mg kg^{-1}.

Fruit yield per cladode versus Zn concentration

Most of the data are agglomerate at bottom of the plot (Figure 5). The diagram indicates that maximum yield (SQRT = 44.716, i. e. 1999.477 g) is a markedly tied to optimum concentration of ln Zn= 6.028 (414.913 mg kg^{-1}). The estimated 2015 maximum yield per cladode and optimum Zn concentration were slightly higher than the overall estimated maximum yield and optimum Zn concentration. In addition, the estimated Zn sufficiency range at 90% maximum fruit yield per fructification cladode (SQRT = 40.244, i. e. 1619.576 g) is limited by the Zn concentrations of 334.308 mg kg^{-1} (ln Zn = 5.812) and 514.953 (ln Zn = 6.244).

DISCUSSION

A few studies have documented the micro-element concentrations in cladodes. Several of them have been focused on the mineral contents of cladodes to demonstrate their forage potential, and tender pads, 1-year old fruiting cladodes and fruits to determine their nutritional contribution to human diet. For instance, micro element concentrations in one-year old fruiting cladodes of *Opuntia dillenii* were as follows (Kalegowda et al., 2015): Cu = 4.5 mg kg^{-1}, Fe = 21.9 mg kg^{-1}, Mn

= 25.9 mg kg^{-1} and Zn = 14.1 mg kg^{-1}. In addition, 1-year old cladodes of *Opuntia ficus-indica* were B = 129.17 mg kg^{-1} and 67.33 mg kg^{-1}, Cu = 1.83 mg kg^{-1} and 22.17 mg kg^{-1}, Fe = 93.33 mg kg^{-1} and 140.33 mg kg^{-1}, Mn = 36.33 mg kg^{-1} and 705 mg kg^{-1}, and Zn = 17.67 mg kg^{-1} and 67.17 mg kg^{-1} when trees were growing under field and greenhouse conditions, respectively (Mayer and Cushman, 2019).

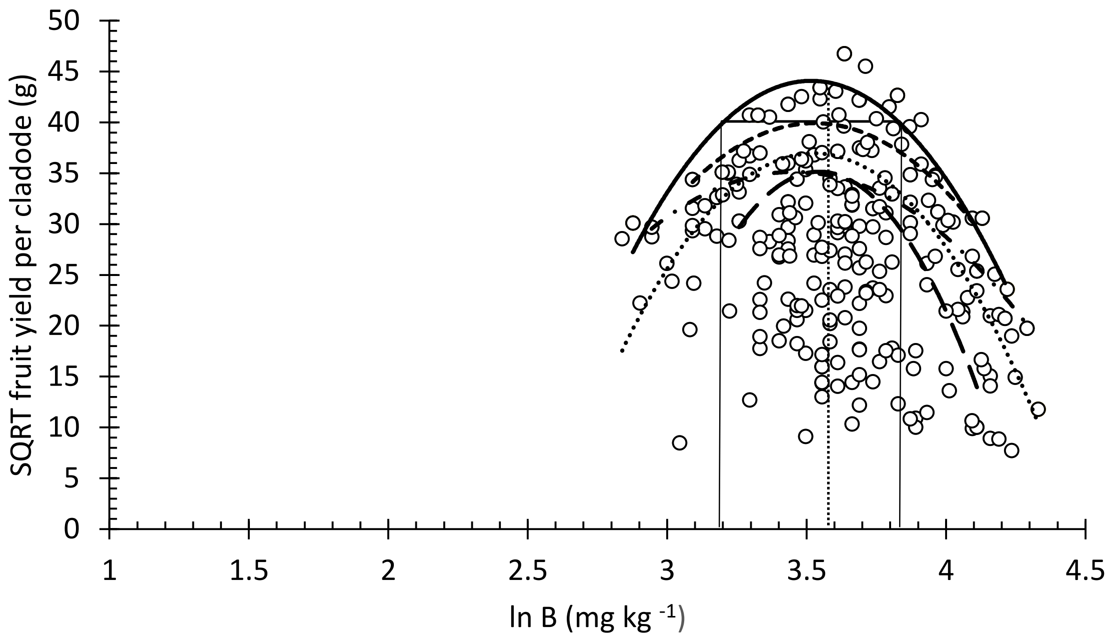

Figure 1. Relationships between squared root of fruit yield per cladode (g) and ln B (mg kg^{-1}) in 1-year old fructification cladodes of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' for the years 2012, 2013, 2014 and 2015. The lines represent quadratic function boundary-lines for each year and the four years: – – – 2012, – · − 2013, - - - 2014, ··· 2015 and — the four years. The estimated quadratic functions are: $y = -66.195x^2 + 469.33x - 796.74$, $R^2 = 0.9046$, and vertex defined by ln B = 3.545 (34.640 mg kg^{-1}) and squared root of fruit yield per cladode = 35.161 (1236.296 g) for 2012; $y = -21.494x^2 + 148.48x - 221.32$, $R^2 = 0.8956$, and vertex defined by ln B = 3.454 (34.34 mg kg^{-1}) and squared root of fruit yield per cladode = 35.104 (1232.291 g) for 2013; $y = -30.25x^2 + 213.47x - 336.68$, $R^2 = 0.7465$, and vertex defined by ln B = 3.530 (34.124 mg kg^{-1}) and squared root of fruit yield per cladode = 39.927 (1594.167 g) for 2014; $y = -41.206x^2 + 289.79x - 465.43$, $R^2 = 0.8736$, and vertex defined by ln B = 3.517 (36.683 mg kg^{-1}) and squared root of fruit yield per cladode = 44.072 (1942.341 g) for 2015; $y = -43.806x^2 + 307.98x - 496.78$, $R^2 = 0.9233$, and vertex defined by ln B = 3.515 (33.616 mg kg^{-1}) and squared root of fruit yield per cladode = 44.537 (1983.544 g) for the four years. The vertical dotted central line defines the vertex. The continuous horizontal line and the two continuous vertical lines intercepting it define the sufficiency range at 90% (ln B= 3.197 to ln B = 3.834, i. e. 24.445 to 46.253 mg kg^{-1}) of maximum fruit yield per cladode (squared root = 40.83, i. e. 1667.09 g).

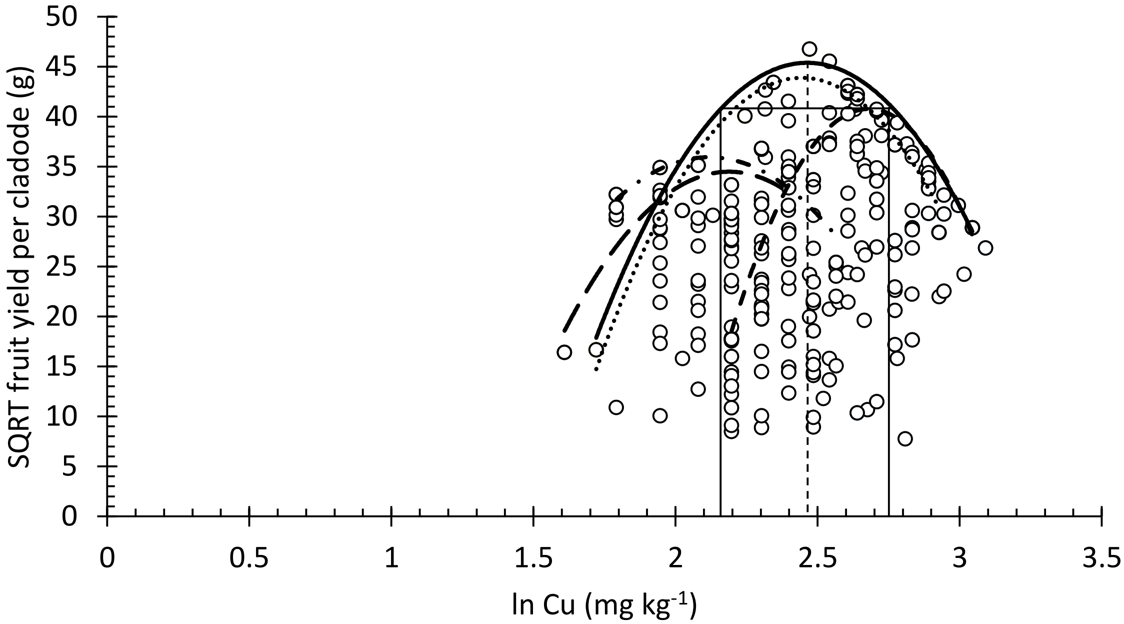

Figure 2. Relationships between squared root of fruit yield per cladode (g) and ln Cu (mg kg^{-1}) in 1-year old fructification cladodes of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' for the years 2012, 2013, 2014 and 2015. The lines represent quadratic function boundary-lines for each year and the four years: – – – 2012, – · − 2013, - - - 2014, ··· 2015 and — the four years. The estimated quadratic functions are: $y = -47.453x^2 + 207.7x - 192.8$, $R^2 = 0.7951$, and vertex defined by ln Cu = 2.188 (8.921 mg kg^{-1}) and squared root of fruit yield per cladode = 34.474 (1188.458 g) for 2012; $y = -39.584x^2 + 167.7x - 141.68$, $R^2 = 0.6505$, and vertex defined by ln Cu = 2.118 (8.319 mg kg^{-1}) and squared root of fruit yield per cladode = 35.938 (1291.524 g) for 2013; $y = -95.477x^2 + 511.75x - 644.98$, $R^2 = 0.9641$, and vertex defined by ln Cu = 2.678 (14.584 mg kg^{-1}) and squared root of fruit yield per cladode = 40.756 (1661.051 g) for 2014; $y = -55.766x^2 + 272.55x - 289.15$, $R^2 = 0.9486$, and vertex defined by ln Cu = 2.444 (11.515 mg kg^{-1}) and squared root of fruit yield per cladode = 43.864 (1924.077 g) for 2015; y = -49.925x2 + 245.94x - 257.52, $R^2 = 0.9675$, and vertex defined by ln Cu = 2.463 (11.741 mg kg^{-1}) and squared root of fruit yield per cladode = 45.367 (2058.142 g) for the four years. The vertical dotted central line defines the vertex. The continuous horizontal line and the two continuous vertical lines intercepting it define the sufficiency range at 90% (ln Cu= 2.162 to ln B = 2.765, i. e. 8.685 to 15.872 mg kg^{-1}) of maximum fruit yield per cladode (squared root = 40.83, i. e. 1667.095 g).

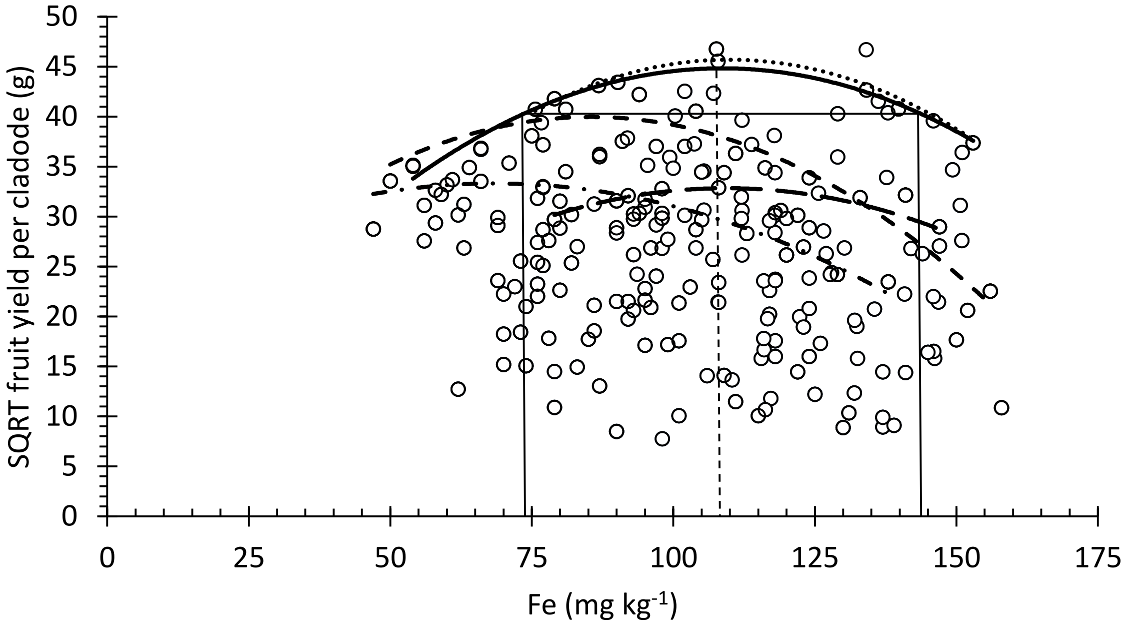

Figure 3. Relationships between squared root of fruit yield per cladode (g) and Fe (mg kg^{-1}) in 1-year old fructification cladodes of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' for the years 2012, 2013, 2014 and 2015. The lines represent quadratic function boundary-lines for each year and the four years: – – – 2012, – · – 2013, - - - 2014, ··· 2015 and — the four years. The estimated quadratic functions are: $y = -0.0029x^2 + 0.6333x - 1.8072$, $R^2 = 0.5776$, and vertex defined by Fe = 109.190 mg kg^{-1} and squared root of fruit yield per cladode = 33.494 (1121.860 g) for 2012; $y = -0.0023x^2 + 0.3115x + 22.646$, $R^2 = 0.7511$, and vertex defined by Fe = 67.718 mg kg^{-1} and squared root of fruit yield per cladode = 33.193 (1101.774 g) for 2013; $y = -0.0038x^2 + 0.6445x + 12.411$, $R^2 = 0.7332$, and vertex defined by Fe = 84.803 mg kg^{-1} and squared root of fruit yield per cladode = 39.739 (1579.160 g) for 2014; $y = -0.0043x^2 + 0.9534x - 6.7453$, $R^2 = 0.5959$, and vertex defined by Fe = 110.860 mg kg^{-1} and squared root of fruit yield per cladode = 46.102 (2125.384 g) for 2015; $y = -0.0037x^2 + 0.803x + 1.2014$, $R^2 = 0.8814$, and vertex defined by Fe = 108.514 mg kg^{-1} and squared root of fruit yield per cladode = 44.770 (2004.315 g) for the four years. The vertical dotted central line defines the vertex. The continuous horizontal line and the two continuous vertical lines intercepting it define the sufficiency range at 90% (Fe = 73.723 to Fe = 143.299 mg kg^{-1}) of maximum fruit yield per cladode (squared root = 40.293, i. e. 1623.495 g).

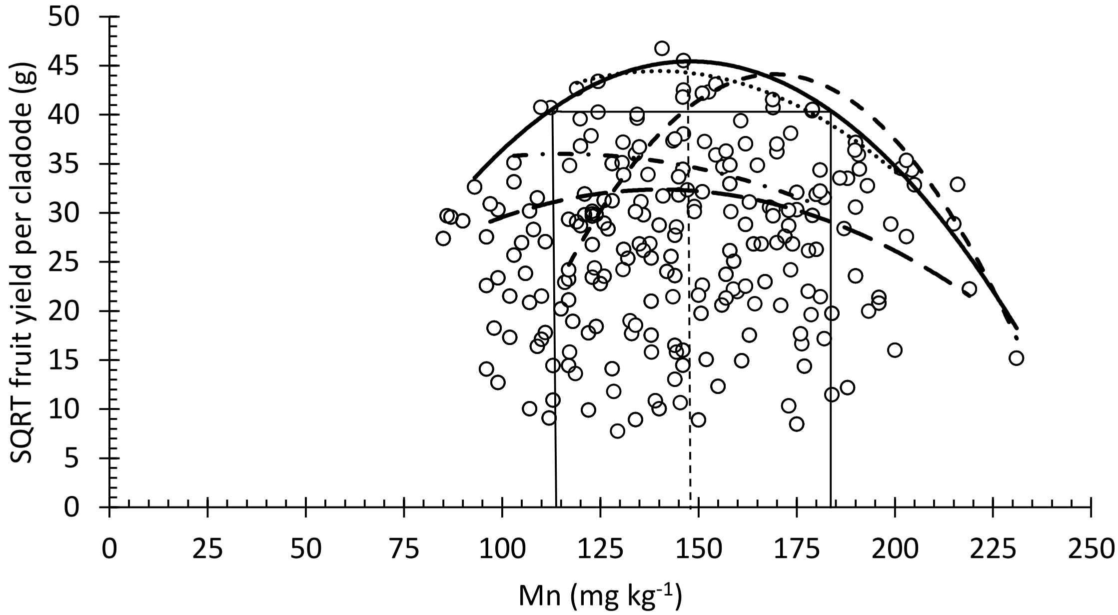

Figure 4. Relationships between squared root of fruit yield per cladode (g) and Mn (mg kg^{-1}) in 1-year old fructification cladodes of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' for the years 2012, 2013, 2014 and 2015. The lines represent quadratic function boundary-lines for each year and the four years: – – – 2012, – · − 2013, - - - 2014, ··· 2015 and — the four years. The estimated quadratic functions are: $y = -0.0017x^2 + 0.4894x - 1.9448$, $R^2 = 0.6541$, and vertex defined by Mn = 143.941 mg kg^{-1} and squared root of fruit yield per cladode = 33.278 (1107.310 g) for 2012; $y = -0.0012x^2 + 0.2862x + 19.482$, $R^2 = 0.6078$, and vertex defined by Mn = 119.250 mg kg^{-1} and squared root of fruit yield per cladode = 36.547 (1335.659 g) for 2013; $y = -0.0071x^2 + 2.3993x - 159.05$, $R^2 = 0.9456$, and vertex defined by Mn = 168.965 mg kg^{-1} and squared root of fruit yield per cladode = 43.649 (1905.201 g) for 2014; $y = -0.0028x^2 + 0.7861x - 10.46$, $R^2 = 0.9232$,and vertex defined by Mn = 140.375 mg kg^{-1} and squared root of fruit yield per cladode = 44.714 (1999.377 g) for 2015; $y = -0.0039x^2 + 1.1648x - 40.716$, $R^2 = 0.9294$, and vertex defined by Mn = 149.333 mg kg^{-1} and squared root of fruit yield per cladode = 46.256 (2139.593 g) for the four years. The vertical dotted central line define the vertex. The continuous horizontal line and the two continuous vertical lines intercepting it define the sufficiency range at 90% (Mn = 114.894 to Mn = 183.772 mg kg^{-1}) of maximum fruit yield per cladode (squared root = 40.293, i. e. 1623.495 g).

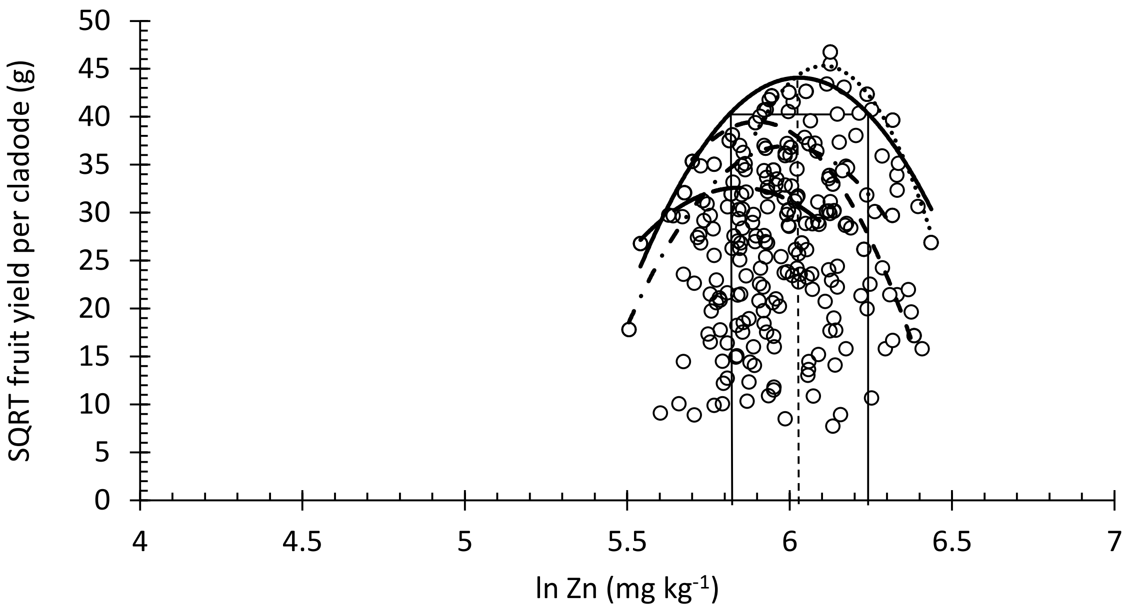

Figure 5. Relationships between squared root of fruit yield per cladode (g) and ln Zn (mg kg-1) in 1-year old fructification cladodes of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' for the years 2012, 2013, 2014 and 2015. The lines represent quadratic function boundary-lines for each year and the four years: – – – 2012, – · − 2013, - - - 2014, ··· 2015 and — the four years. The estimated quadratic functions are: $y = -58.137x^2 + 679.88x - 1955.1$, $R^2 = 0.4925$, and vertex defined by ln Zn = 5.847 (346.271 mg kg^{-1}) and squared root of fruit yield per cladode = 32.605 (1063.084 g) for 2012; $y = -78.585x^2 + 941.3x - 2781.8$, $R^2 = 0.957$, and vertex defined by ln Zn = 5.989 (399.038 mg kg^{-1}) and squared root of fruit yield per cladode = 36.945 (1365.260 g) for 2013; $y = -100.31x^2 + 1183.4x - 3450.9$, $R^2 = 0.9579$, and vertex defined by ln Zn = 5.899 (364.568 mg kg^{-1}) and squared root of fruit yield per cladode = 39.369 (1549.924 g) for 2014; $y = -160.14x^2 + 1955.4x - 5924.1$, $R^2 = 0.9466$, and vertex defined by ln Zn = 6.105 (448.219 mg kg^{-1}) and squared root of fruit yield per cladode = 45.035 (2028.157 g) for 2015; $y = -95.835x^2 + 1155.4x - 3437.7$, $R^2 = 0.9072$, and vertex defined by ln Zn = 6.028 (414.913 mg kg^{-1}) and squared root of fruit yield per cladode = 44.716 (1999.476 g) for the four years. The vertical dotted central line defines the vertex. The continuous horizontal line and the two continuous vertical lines intercepting it define the sufficiency range at 90% (ln Zn = 5.812 to ln Zn = 6.244, i. e. 334.308 to 514.953 mg kg^{-1}) of maximum fruit yield per cladode (squared root = 40.244, i. e. 1619.576 g).

Other efforts have been focused on fertilization effects on flower bud formation or fruit production (e.g. Nerd and Mizrahi, 1994; Karim et al., 1997; Galizzi et al., 2004; Arba et al., 2017) in *O. ficus-indica*; however, no clear associations were evidenced. In other words, the results of those research works did not link nutrient concentrations with yield or quality of yield in terms of cladode biomass or fruit (prickly pear) using statistical relationships. Therefore, we focused our effort on the identification of the best fit plant micro-nutrient requirements for fruiting, because they are not available as pointed out by Inglese et al. (1995).

Results from these prior research work differ from the estimated optimum micro-element concentrations as proposed by us. These discrepancies may be attributed to the diversity of involved species or cultivars and environmental factors such as soil and atmospheric conditions. Another explanation could be the different aims, mainly because in the former works nutrient concentrations were not related with yield through statistical trends or functions. Strongly, we found that virtually all five micro-nutrients when distributed normally can be related to fruit yield per cladode. Therefore, it was easy choosing points to estimate the boundary lines and then the estimation of their corresponding vertices.

This is the first study to our knowledge to estimate the micro-nutrient optimum concentrations linked to maximum fruit yields per 1-year-old fructification cladode and sufficiency ranges at 90% maximum fruit yield for *O. ficus-indica* variety 'Rojo Pelón'. According to the estimated optimum micro-element contents, the order of nutrient requirement is as follows: Zn > Mn > Fe > B > Cu, i.e. 414.913 > 149.333 > 108.514 > 33.616 > 11.741 mg kg^{-1}, respectively. These results suggest that *O. ficus-indica* variety Rojo Pelón' plants tend to concentrate much more Zn than the other micro-nutrients in their 1-year-old fruiting cladodes. A reason may be Zn plays an important role in reducing losses of water that could have implications for the survival of the dry season (Dimkpa and Bindraban, 2016). In addition, these estimated standards satisfactorily surpass the requirements of Zn, Mn, Fe, and Cu for beef cattle in gestation and lactation and do not exceed the maximum tolerable levels as pointed out by the National Academies of Sciences of the United States of America (2016). Then, 1-year-old cladodes of *O. ficus-indica* variety 'Rojo Pelón' can be used as a supplement for feed ruminant species.

Our results may be useful information for the maximization of fruit production of *O. ficus-indica* variety "Rojo Pelón" through fertilization practice. Of course, growers and technicians should use the micro-nutrient optimum concentrations and sufficiency ranges as a reference to carry out nutrient diagnosis and the proper fertilization recommendation taking in mind getting balanced nutrition although they are required in trace amounts. There is widely known that elements adequate supply improves nutrient availability and positively affects cell physiology that reflects in yield (Hao et al., 2007).

However, it is important to point out these estimated optimum micro-nutrient concentrations and sufficiency ranges may be valid only at local and regional levels, and specifically for the *O. ficus-indica* variety "Rojo Pelón". Therefore, such reference values can be used with caution in other *O. ficus-indica* cultivars to validate them. For instance, in a surprising experience, Arba et al. (2017) pointed out that they successfully used nutrient standards for *O. ficus-indica* fresh matter production as developed by Valdez-Cepeda et al. (2013) to identify the best soil fertilization dosage for *O. ficus-indica* cv. 'Moussa' fruit yield and fruit size improvements. In this way, we suggest that this kind of work should be developed in other horticultural regions where *O. ficus-indica* is an important crop whatever be the involved varieties or cultivars. In addition, it deserves to be mentioned that future research works should be considering the B-LA and/or other techniques such as the Diagnosis Recommendation-Integrated System and the Compositional Nutrient Diagnosis approach, among others to identify macro- and micro-nutrient standards at the local or regional level. Of course, each of these techniques has its advantages and disadvantages.

CONCLUSIONS

We propose the Boundary-Line Approach standards for *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' as follows: B = 33.616 mg kg^{-1}, Cu = 11.741 mg kg^{-1}, Fe = 108.514 mg kg^{-1}, Mn = 149.333 mg kg^{-1} and Zn = 414.913 mg kg^{-1} as optimum concentrations for maximization of fruit yield per 1-year old fructification cladode, and B = 24.445 – 46.253 mg kg^{-1}, Cu = 8.685 – 15.875 mg kg^{-1}, Fe=73.729 – 143.298 mg kg^{-1}, Mn = 114.894 – 183.772 mg kg^{-1} and Zn = 334.308 – 514.953 mg kg^{-1} as sufficiency ranges at 90% maximum yield, respectively. It deserves be noted that involved trees were growing under specific environmental conditions (clay loam soil of calcareous origin with a pH of 7.5 and organic matter content of 3.2%, and BS1kw (w) climate).

These micronutrient standards as novel knowledge may be important because what prevails in the mind of growers and technicians is the common opinion about cactus crop needs low inputs to give high yields; such an opinion is under ideas misconceived because of the limited scientific information (Felker and Bunch, 2009) on specific *Opuntia* species and/or cultivar nutrient requirements. Thus, the proposed B-LA standards can be used to perform reliable micro-nutrient diagnosis and proper fertilization recommendations. Great differences in *O. ficus-indica* fruit yield within and among regions persist as a result of poor orchard management. Therefore, correct micro-nutrient diagnoses may allow the design of compelling fertilization practices for improving fruit yield.

ETHICS STATEMENT

Not apply.

CONSENT FOR PUBLICATION

Not apply.

AVAILABILITY OF SUPPORTING DATA

Data might be available upon request addressed to RD V-C.

COMPETING INTERESTS

The research has no financial or commercial purpose that must be interpreted as a potential conflict of interest in the future.

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AUTHOR CONTRIBUTIONS

Conceptualization, T.S. A.-J., R.D. V.-C., and F.B.-M.; Project administration, fieldwork and data registration, T.S. A.-J., R.D. V.-C., and F. B.-M.; data organization and statistical analyses, T.S. A.-J., R.D. V.-C., F.B.-M., and C. L.-O.; writing and reviewing of the original draft, T.S. A.-J., R.D. V.-C., F.B.-M., and C. L.-O.; review and editing of the last manuscript, T.S. A.-J., R.D. V.-C., F.B.-M., C. L.-O., S. B.-L, and J.L. L.-M. All authors have read and agree to approve the final version of the manuscript.

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