



Article

Advancing Leaf Nutritional Characterization of Blueberry Varieties Adapted to Warm Climates Enhanced by Proximal Sensing

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Abstract: Blueberries offer multiple health benefits, and their cultivation has expanded to warm tropical regions. However, references for foliar nutritional content are lacking in the literature. Proximal sensing may enhance nutritional characterization to optimize blueberry production. We aimed (i) to characterize the nutrient contents of healthy plants of three blueberry varieties adapted to warm climates (Emerald, Jewel, and Biloxi) using a reference method for foliar analysis (inductively coupled plasma (ICP)) and a portable X-ray fluorescence (pXRF) spectrometer on fresh and dry leaves and (ii) to differentiate blueberry varieties based on their nutrient composition. Nutrient content was statistically compared per leaf moisture condition (fresh or dry) with ICP results and used to differentiate the varieties via the random forest algorithm. P and Zn contents (ICP) in leaves were different among varieties. Dry leaf results (pXRF) were strongly correlated with ICP results. Most nutrients determined using ICP presented good correlation with pXRF data (R^2 from 0.66 to 0.93). The three varieties were accurately differentiated by pXRF results (accuracy: 87%; kappa: 0.80). Predictions of nutrient contents based on dry leaves analyzed by pXRF outperformed those based on fresh leaves. This approach can also be applied to other crops to facilitate nutrient assessment in leaves.

Keywords: berries; pXRF; foliar analysis; elemental composition



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1. Introduction

The worldwide consumption of blueberries (*Vaccinium* sp.) is increasing due to the several benefits they provide to human health [1,2]. Blueberries are rich in vitamins, mineral nutrients (e.g., Ca, K, and Fe), and flavonoids, such as anthocyanins, including bioactive components related to antioxidant and anti-inflammatory processes [3–5]. Recently, their production and consumption have increased even in countries that were not familiar with this fruit, such as Brazil [6].

Blueberries are native to cold climate regions, which limits their production in warm tropical areas [6]. Recently, varieties adapted to warm climates have been developed, such as the southern highbush Emerald, Jewel, and Biloxi [7,8], which require fewer hours below a temperature of 7.2 °C (low-chill areas). The introduction of adapted varieties is increasing blueberry cultivation in the tropics, such as in warm regions of Brazil [7–10]. In addition to climate conditions, blueberries require well-drained soils that have high sand content, low pH, and high porosity [11,12]. Blueberries grown in fertigated containers filled with well-drained and highly porous substrates (e.g., rice husk, pine tree bark, and sand) have been successful in overcoming these limiting factors, contributing to the expansion of cultivation areas [8].

Most studies focus on the assessments of blueberry fruit properties (e.g., anthocyanin content, firmness, number of fruits per plant, etc.) [9,13–16], but very little can be found in the literature about the nutritional diagnosis of blueberry leaves. Foliar analysis is extremely helpful due to its relation to plant health and yield, helping optimize crop management and fertilizer applications [17,18]. Reports of reference nutrient contents for blueberry leaves are scarce, especially for new varieties cultivated in warm regions. This information could help expand blueberry cultivation in the tropics by providing a better understanding of the nutrient requirements under different cultivation conditions for optimizing yields and reducing the costs of production systems.

The evaluation of the nutritional status of plants can be facilitated by proximal sensors such as the portable X-ray fluorescence (pXRF) spectrometer [19]. This equipment can conduct non-destructive analysis in situ, simultaneously detecting the content of several chemical elements in a sample in a few seconds and without using chemical reagents [20]. However, only a few studies have explored the use of pXRF to evaluate the nutritional status of plants, despite the promising initial results for some crops and native species [19,21,22].

Although it is a promising tool, it is well known that pXRF analysis is influenced by the moisture content of samples, causing the underestimation of some elements [22–24]. This poses some questions about the efficacy of pXRF for the analysis of fresh and dry leaves for nutritional evaluation. Moreover, the conventional method to determine the nutritional status of plants involves the acid digestion of leaves followed by elemental quantification using ICP [22]. It is not known how accurate pXRF analysis is compared to the conventional ICP method when applied to assess the chemical contents of blueberry leaves. If results from both methods are correlated, pXRF could contribute to reducing time and costs of nutritional diagnoses of plants, especially for blueberries cultivated in such fertigation-dependent systems.

This study was motivated by the benefits of blueberries for human health and the recent expansion of blueberry cultivation in tropical regions. Despite the growing interest for blueberry cultivation, there is a lack of information about the nutritional requirements of warm-climate blueberry varieties and about the potential of pXRF analysis of fresh and dry leaves to facilitate nutritional assessment of plants. The objectives were to (a) characterize the foliar nutrient content of three warm-resistant (no-chill) varieties of blueberries (Emerald, Jewel, and Biloxi) cultivated in Brazil to provide a reference for future research and for new producers, (b) test the accuracy of pXRF analysis to assess the nutritional status of fresh and dry blueberry leaves compared to a reference method (acid digestion followed by ICP), and (c) attempt to differentiate the three blueberry varieties based on the nutritional status of their leaves determined by pXRF analysis. Our hypotheses are that pXRF and ICP analysis will present a stronger correlation for dry leaves compared to fresh leaves and that the three blueberry varieties investigated herein will have contrasting elemental concentrations in their leaves, allowing pXRF to differentiate each variety even though they were cultivated under the same conditions.

2. Materials and Methods

2.1. Study Area and Sampling

The study area is located on São José Farm, in the municipality of Lavras, Minas Gerais state, Brazil, at latitude 21°15'52.57" S and longitude 45°01'35.99" W, Zone 23K, datum SIRGAS 2000 (Figure 1). The climate of the region is Cwa according to the Köppen classification, characterized by hot and rainy summers and cold and dry winters, with mean annual temperature and rainfall of 19 °C and 1530 mm, respectively [25]. The native vegetation is semi-perennial tropical forest.

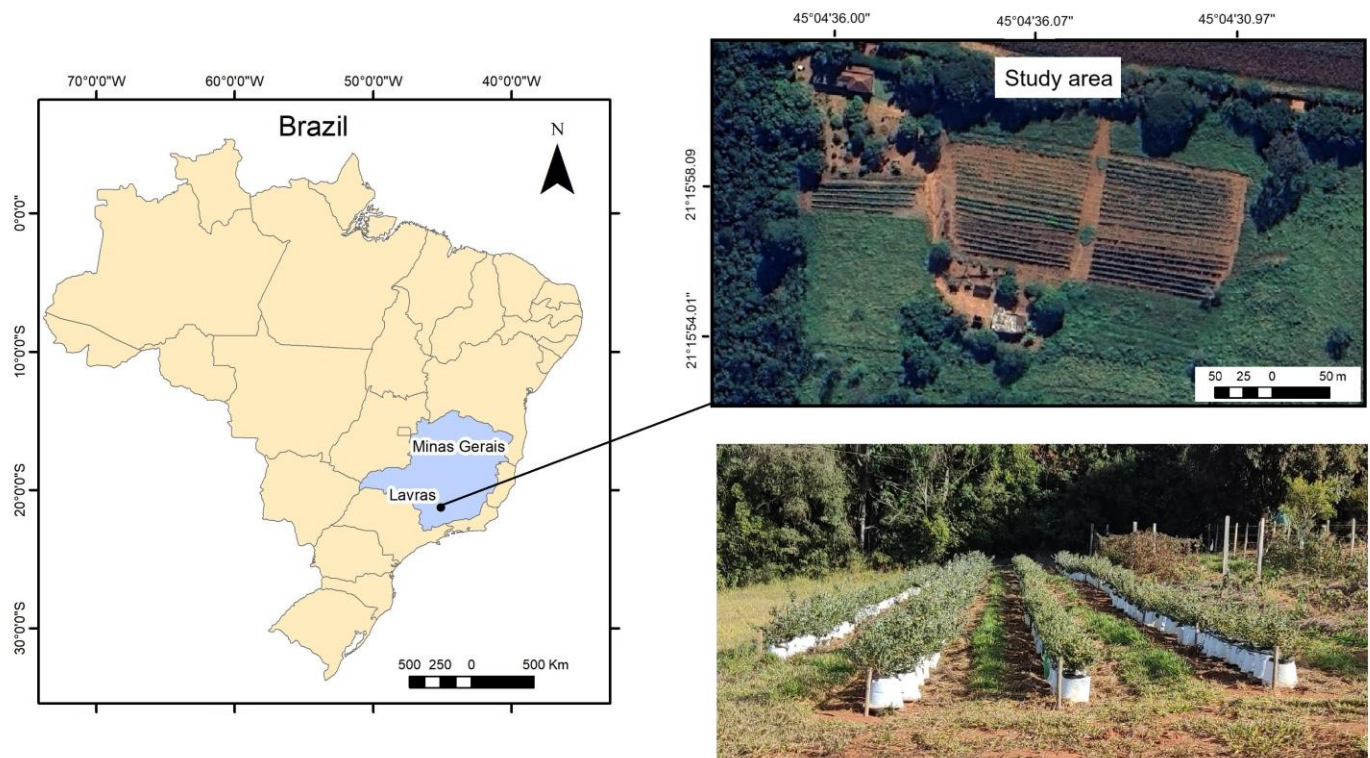


Figure 1. Location of the blueberry plantation in the municipality of Lavras, Minas Gerais, Brazil, and picture of part of the plantation (**bottom right**).

Three varieties of southern highbush blueberry plants were cultivated: Biloxi, Emerald, and Jewel. All the three varieties are resistant to areas with mild winter and require few hours per year below 7.2 °C (<200 h for Biloxi and between 200 and 300 h for Emerald and Jewel) [8]. The plants were cultivated for 1 year and 10 months in plastic containers filled with rice husk (substrate) mostly composed of SiO₂ (approximately 91%), presenting bulk density of 0.22 g cm⁻³, total, macro-, and microporosity, respectively, of 89%, 63%, and 26%, and water-holding capacity of 0.586 cm³ cm⁻³ (1 kPa). Rice husk was the substrate of choice because it allows adequate aeration and water-holding capacity. The containers had 70 cm of diameter and 60 cm of height, totalizing a volume of 20 dm³. They were perforated in the bottom to drain the excess solution. The plants were fertigated three times per day, totalizing approximately 1.5 L per plant, with a nutritional solution containing macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (B, Cu, Fe, Mn, Mo, Ni, and Zn) calibrated by the producer to reach pH and electrical conductivity of 5.5 and 1.1 mS cm⁻¹, respectively. The same solution was provided to all the plants via an irrigation system containing four drip emitters surrounding each plant.

2.2. Laboratory Analyses

Five leaves of five plants per blueberry variety were collected at the third pair of leaves counting from the top of the branch, making up a total of 25 leaves per variety. All 25 fresh leaves were washed with distilled water and dried at room temperature. Next, samples were analyzed (2 h after sample collection) via a Tracer 5 g portable X-ray fluorescence (pXRF) spectrometer (Bruker Analytical Instrumentation, Billerica, MA, USA). This pXRF model contains a 50 keV and a 100 µA Rh X-ray tube. Samples were scanned in triplicates, 60 s per scan, using plant mode through the inbuilt Geochem software (730.0225). To check the accuracy of the equipment, certified reference materials were scanned. The utilized materials were CS-P, provided by the manufacturer, and samples 1573a and 1547 (for tomato and peach leaves, respectively), certified by the National Institute of Standards and Technology (NIST). The recovery values per element were calculated as follows: recovery

value (%) = content determined by pXRF/certified content $\times$ 100. The elements chosen for this study were the ones detected by pXRF for all the samples and their recovery values (CS-P/1573a/1547) are found in Table 1.

Table 1. Recovery values (%) between the element contents determined by pXRF and the contents reported in three certified reference materials (CRMs).

CRM	Al	Ca	Cl	Cu	Fe	K	Mn	P	S	Si	Ti	Zn
-----%												
CS-P	–	87	–	–	–	87	102	81	82	–	–	100
1573a	136	73	72	137	138	72	121	56	60	–	–	197
1547	506	80	190	198	181	85	135	78	64	–	–	147

Dashes (–) indicate either the element has no certified content in the reference material or it was not detected using the pXRF spectrometer.

After scanning all fresh leaf samples, samples were dried for three days at 60 °C, ground, and sieved at 50 μ m. Dried samples were analyzed again via pXRF in triplicates (dry leaves). Next, 15 samples were selected from the dried and ground samples (5 per blueberry variety). Selected samples were prepared for nitri-perchloric acid digestion and determination of 10 macro- and micronutrients (B, Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn) [26,27]. These 15 samples were weighed (0.5 g) and transferred to a glass digestion tube of 50 mL and mixed with 6 mL of a solution composed of HNO₃:HClO₄ 2:1 *v/v*, with purity degree of 65 and 70, respectively (Sigma Aldrich, Schnellendorf, Germany). For the digestion process, this solution was heated in a microwave (CEM MARS-5, CEM Corp., Matthews, NC, USA) up to 180 °C and under a pressure of 448 kPa for 10 min. Then, samples were passed through a Whatman paper filter number 40 and the volume was increased up to 50 mL with ultrapure water. The chemical elements in the resulting solution were quantified through inductively coupled plasma optical emission spectrometry (ICP-OES), using Spectro Blue equipment (Spectro Analytical Instruments, Kleve, Germany). The elements quantified using a multielement calibration curve and their wavelengths (nm) were: B (249.677), Ca (315.887), Cu (324.754), Fe (373.486), K (769.896), Mg (285.213), Mn (403.076), P (178.287), S (182.034), and Zn (213.856).

2.3. Statistical Analyses

After the characterization of fresh and dry leaves via pXRF, and the digestion/ICP-OES quantification of elements, the results were analyzed using statistical methods. Descriptive statistics and Pearson correlation coefficients were calculated using R software (version 4.3.1) [28], and a correlogram was plotted through the package “corrplot” [29]. Then, results of fresh and dry leaf analysis by pXRF and ICP-OES were compared per element via the Scott–Knott mean test at a significance level of 5% via the software Sisvar (version 5.6) [30]. Since there are very few studies on southern highbush blueberries, especially when cultivated in Brazil, ICP-OES results of nutrients in plant leaves were reported to demonstrate the elemental composition of the three blueberry varieties. This report may also serve as a reference to future works on the nutritional status of these three blueberry varieties.

A principal component analysis (PCA) was conducted to assess the differences of results per variety and per condition (fresh or dry leaves) based on pXRF results using the R package “factoextra” [31]. No rotation method, such as varimax or others, was applied to the PCA results. Finally, a random forest model was calibrated using the R package “caret” [32] in attempt to separate the leaves from the three blueberry varieties based on their elemental contents determined by pXRF using fresh and dry/ground leaves. For that, a leave-one-out cross-validation was used to assess the accuracy of these predictions per element and per leaf condition (fresh or dry). The accuracy of the predictions was assessed

by the calculation of overall accuracy (number of correctly classified samples/total number of samples) and kappa index (Equation (1)).

$$\text{Kappa} = (\text{Po} - \text{Pe}) / (1 - \text{Pe})$$

(1)

where Po is the proportion of correctly classified samples according to their varieties, and Pe is the probability of random agreement. The kappa values range from −1 to 1, indicating greater accuracy as the values become closer to 1 [33].

3. Results and Discussion

3.1. Characterization of Nutritional Differences of Blueberry Varieties

The three blueberry varieties presented different means and standard deviations of nutrient contents in leaves (Table 2) despite being under the same management. Biloxi accumulated a greater amount of P, while Jewel had greater contents of Zn. Ref. [34] reported that little is known about the mineral composition of blueberry fruits of these varieties, as also shown by [35] in the Himalayas, but there is even less information on the nutrient contents in leaves of healthy plants. According to these authors, nutrient contents in blueberry fruits are dependent on the variety, and the same seems to happen for leaves. Emerald presented the lowest contents of Ca, Cu, K, and Mg and the highest contents of Fe and Mn among the three varieties. Biloxi presented the greatest accumulation of Ca, P, and S.

Table 2. Mean and standard deviation (SD) of elemental contents determined by Inductively Coupled Plasma (ICP) in leaves of three varieties of blueberry cultivated in warm climate, in Brazil.

Variety	Statistics	B	Ca	Cu	Fe	K	Mg	Mn	P	S	Zn
		mg kg ^{−1}									
Emerald	Mean	269.92	5725.50	3.27	3343.39	8296.18	1572.52	534.08	1862.20	2993.91	17.94
	SD	58	1012	1	6946	2870	344	74	399	470	4
Jewel	Mean	263.60	6787.21	5.34	2123.23	10,278.41	2109.30	426.94	1775.18	2564.49	28.24
	SD	54	2644	3	3194	808	499	141	452	673	12
Biloxi	Mean	255.75	7627.59	5.14	119.88	9050.80	2108.72	480.19	2491.53	3441.49	16.13
	SD	80	1429	3	30	1793	508	46	443	652	5

Ref. [36] is one of the rare studies that investigated nutrient contents in the leaves of the Jewel and Emerald varieties. The authors evaluated the contents of P, K, Ca, and Mg and reported the following contents for Jewel and Emerald leaves, respectively: P 1400 and 1200 mg kg^{−1}, K 3700 and 4800, Ca 5800 and 9200, and Mg 1600 and 2400 mg kg^{−1}. Compared to the results obtained herein, Ca and Mg had the same range, while for P and K the contents reported by [36] were lower. This may be a consequence of multiple factors, including different nutrient concentrations in fertigation and the cultivation directly in soils (performed in their study) compared to cultivation in bags in our case. Compared with results of the northern highbush varieties Draper and Bluecrop, Ca contents of our three varieties were within the range of contents reported by [37]. Ref. [38] also reported Zn contents in leaves of the Patriot variety that were similar to those found for the three varieties evaluated herein, but contents of Fe were around 50 mg kg^{−1}. For varieties different from the ones evaluated here (including southern highbush, rabbiteye, northern highbush), ref. [39] found lower contents of Cu, Fe, and Mn in leaves, while Zn contents were similar. Ref. [40] found macro- and micronutrient contents in the leaves of the Bluecrop variety grown in Poland that were mostly different from those found here, but the authors state it is common to find a wide range of nutrients in leaves, as they depend on several other conditions besides variety. Given the lack of literature on the contents of nutrients in leaves for Emerald, Jewel, and Biloxi, other studies are encouraged to detail such information and help blueberry production. Meanwhile, the chemical characterization

presented in our study (Table 2) can be used to support decision making and to monitor the nutritional status of blueberry crops.

3.2. Effects of Moisture on pXRF Results

Analysis via pXRF under different leaf conditions (fresh and dry) demonstrated differences not only among elemental contents but also in the number of elements that were found below the limit of detection. Thirteen elements were detected for most samples and were used herein (Table 3). However, in the 15 studied plants, Ti could not be detected in any fresh leaf, while Cl, Ni, and S were not detected in seven, four, and three fresh leaves, respectively, indicating they were more sensitive to moisture changes in blueberry leaves. The other seven elements were detected both in fresh and dry leaves. The non-detection of some elements for the same sample analyzed under different moisture conditions is commonly reported for other materials as well [22,41].

Table 3. Mean and standard deviation (SD) of elemental contents detected by pXRF in fresh and dry leaf conditions (Cond.) of three varieties of blueberries.

Variety	Cond.	Stats	Al	Si	P	S	Cl	K	Ca	Ti	Cr	Mn	Fe	Cu	Zn
-----mg kg ⁻¹ -----															
Emerald	Fresh	Mean	695 aA	2874 aA	137 bB	143 bA	116 aA	1839 b	1140 bA	0 bA	3 aB	287 bA	150 bA	9 aB	13 bA
		SD	434.8	1239.0	7.9	48.8	112.2	632.7	254.7	0.3	0.8	102.5	30.7	1.0	1.0
	Dry	Mean	522 aA	2023 aA	799 aB	1356 aA	164 aA	8418 aA	6925 aB	3 aA	3 aA	663 aA	278 aA	7 bB	24 aA
		SD	227.3	441.1	50.0	84.5	54.3	1636.0	607.3	1.0	1.2	194.7	75.8	1.1	2.2
Jewel	Fresh	Mean	799 aA	2176 aA	119 bB	11 bC	26 bA	1623 b	973 bA	0 bA	4 aA	195 bA	126 bA	10 aA	14 bA
		SD	418.2	877.4	18.5	20.1	41.7	331.4	128.6	0.0	0.3	47.6	36.3	1.0	2.0
	Dry	Mean	320 bB	1721 aA	620 aB	856 aB	270 aA	7449 aA	6879 aB	4 aA	4 aA	495 aA	258 aA	10 aA	26 aA
		SD	140.8	246.6	128.1	251.4	125.6	1456.1	1063.9	1.8	1.6	105.2	66.6	2.6	4.1
Biloxi	Fresh	Mean	586 aA	3301 aA	171 bA	74 bB	62 bA	2478 b	1218 bA	0 bA	4 aA	229 bA	134 bA	10 aA	12 bA
		SD	311.5	536.2	26.1	17.4	137.7	2133.3	85.5	0.0	0.3	32.7	23.0	0.4	1.5
	Dry	Mean	149 bB	1697 bA	1000 aA	1385 aA	243 aA	7918 aA	7962 aA	1 aB	3 aA	640 aA	249 aA	10 aA	23 aA
		SD	160.9	488.9	204.0	197.2	88.5	1805.0	443.5	1.0	1.1	97.0	49.6	2.0	3.0

Mean values followed by the same lowercase letter indicate no significant difference between values of the same element per blueberry variety. Mean values followed by the same uppercase letter indicate no significant difference between values of the same element when comparing the blueberry varieties.

The effect of leaf moisture on pXRF results was remarkable for most elements, similar to what was reported by previous studies [22,42]. Except for Cr (for all varieties), Cu (for Jewel and Biloxi), Al (for Emerald), and Cl (for Emerald and Jewel), all the results presented a significant difference between fresh and dry conditions, i.e., 77% of the cases presented a significant difference (Table 3). More importantly, except for Cr, all elemental contents of dry samples were greater than those of fresh samples. This corroborates previous studies indicating the effects of analyzing fresh and dry leaves and other matrices, such as soils [22,43].

Not only did the values vary per condition but also per blueberry variety (Table 3), especially for dry leaves. For dry leaves, Al, P, S, Ca, Ti, and Cu presented significant differences among the three blueberry varieties, while in fresh leaves, a statistical difference was only found for P, S, Cr, and Cu. This demonstrates that differences among varieties can be noticed especially when analyzing dry leaves.

The PCA based on pXRF results of fresh and dry leaves and the factor loadings of pXRF variables (Figure 2) were able to separate dry and fresh leaves, explaining 69% of data variability. Dry samples of all varieties were concentrated to the right of the PCA plot, on the opposite side from fresh samples. This demonstrates the contrasting effect of moisture conditions for pXRF analyses. Moreover, the dry leaf data were less dispersed

than fresh leaf data. The factor loading plot (Figure 2B) demonstrated that Al, Si, Cr, and Cu were negatively correlated with PC1, with Al and Si being the key variables for the segregation of fresh leaves. On the other hand, all other pXRF variables showed a positive correlation with PC1, highlighting Ca, Mn, P, S, Sr, and Zn as the most important elements for the segregation of dry samples. This observation agrees with the difference in elemental contents of dry and fresh leaves shown in Table 3, demonstrating the effect of moisture on pXRF results.

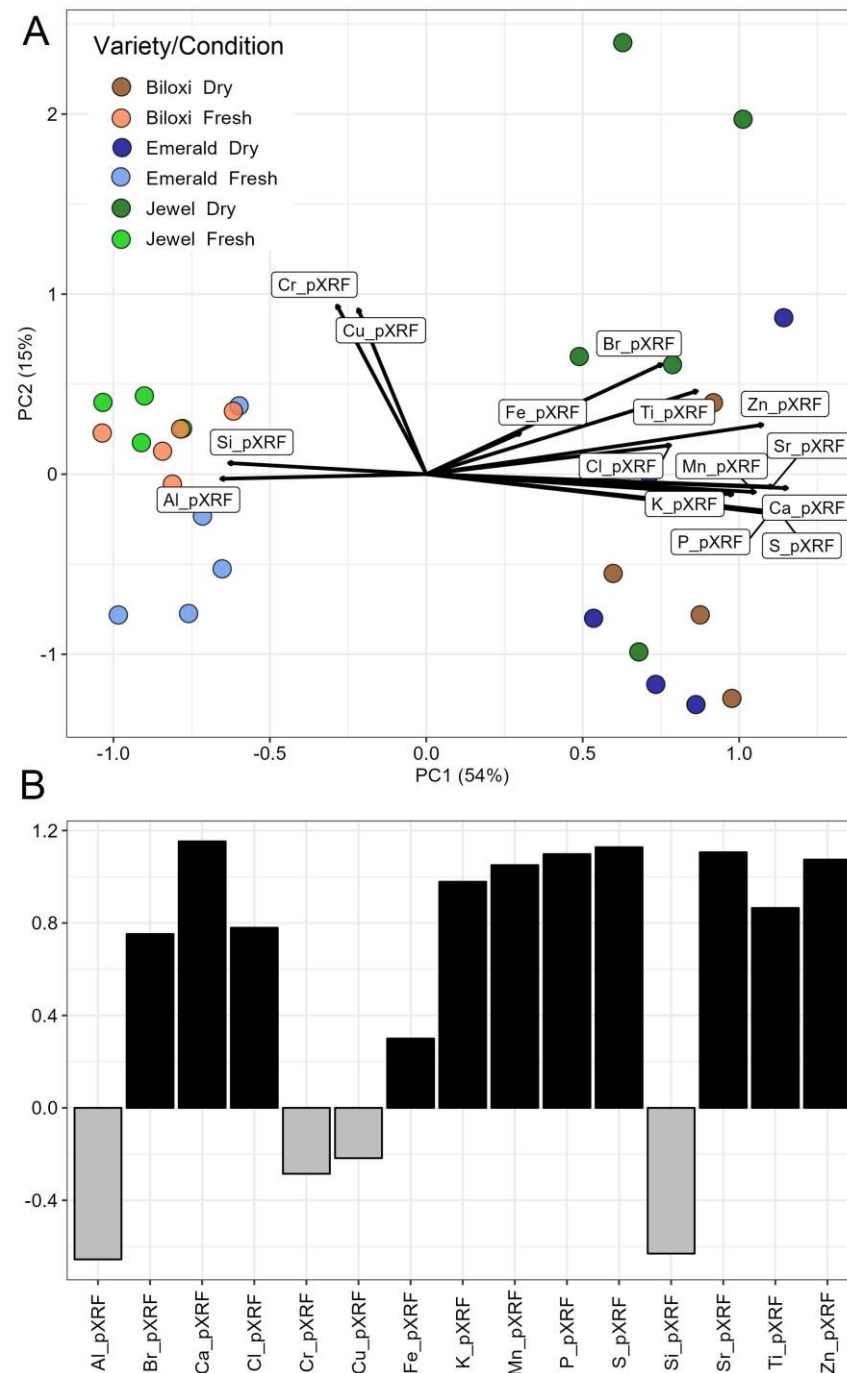


Figure 2. Principal component analysis (PCA) of pXRF data separating results of fresh and dry leaves (A) and factor loading plot of each pXRF variable in the PC1 plane (B).

3.3. Correlations between pXRF and ICP Results

Although comparisons between pXRF and ICP results were also dependent on the leaf conditions (fresh and dry) (Tables 2 and 3), it is important to notice that they vary compared with ICP results (Table 4). Moreover, these comparisons vary according to the element and not all elements presented the same trend. For instance, ICP results for P and S were always greater than pXRF results (both fresh and dry). For Ca and Mn, ICP results were smaller than pXRF_dry and greater than pXRF_fresh results. For Cu, ICP results were the smallest. For the other elements, results were variable. Thus, caution is needed to compare pXRF results with those of ICP, especially when evaluated per blueberry variety (discussed later in this section). However, correlations between ICP and pXRF results are important to demonstrate if such variation can be depicted using both methods (Figures 3 and 4).

Table 4. Summary of inductively coupled plasma (ICP) and portable X-ray fluorescence (pXRF) spectrometer analysis results of elemental contents of blueberry leaves demonstrating the differences caused by varying techniques (ICP and pXRF) and moisture/condition of the samples (dry and fresh) when analyzed by pXRF.

Variety	Condition	Ca	Cu	Fe	K	Mn	P	S	Zn
		-----mg kg ⁻¹ -----							
Emerald	ICP	5726	3	3343	8296	534	1862	2994	18
	Dry	6925	7	278	8418	663	799	1356	24
	Fresh	1140	9	150	1839	287	137	143	13
Jewel	ICP	6787	5	2123	10,278	427	1775	2564	28
	Dry	6879	10	258	7449	495	620	856	26
	Fresh	973	10	126	1623	195	119	11	14
Biloxi	ICP	7628	5	120	9051	480	2492	3441	16
	Dry	7962	10	249	7918	640	1000	1385	23
	Fresh	1218	10	134	2478	229	171	74	12

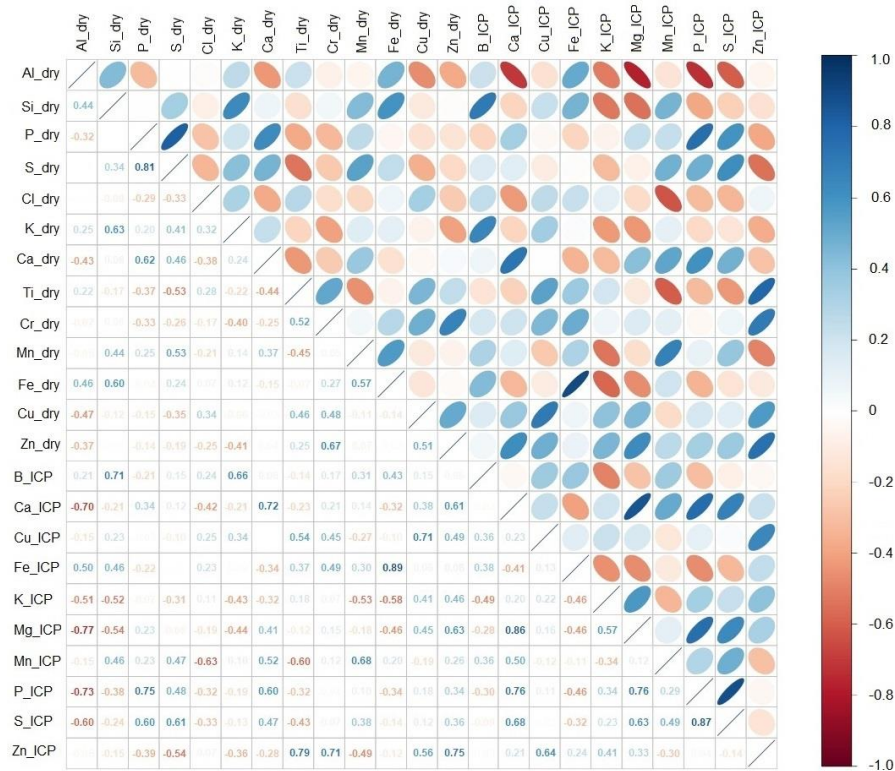


Figure 3. Correlations between inductively coupled plasma (ICP) and portable X-ray fluorescence (pXRF) spectrometer analysis of dry leaves of three blueberry varieties.

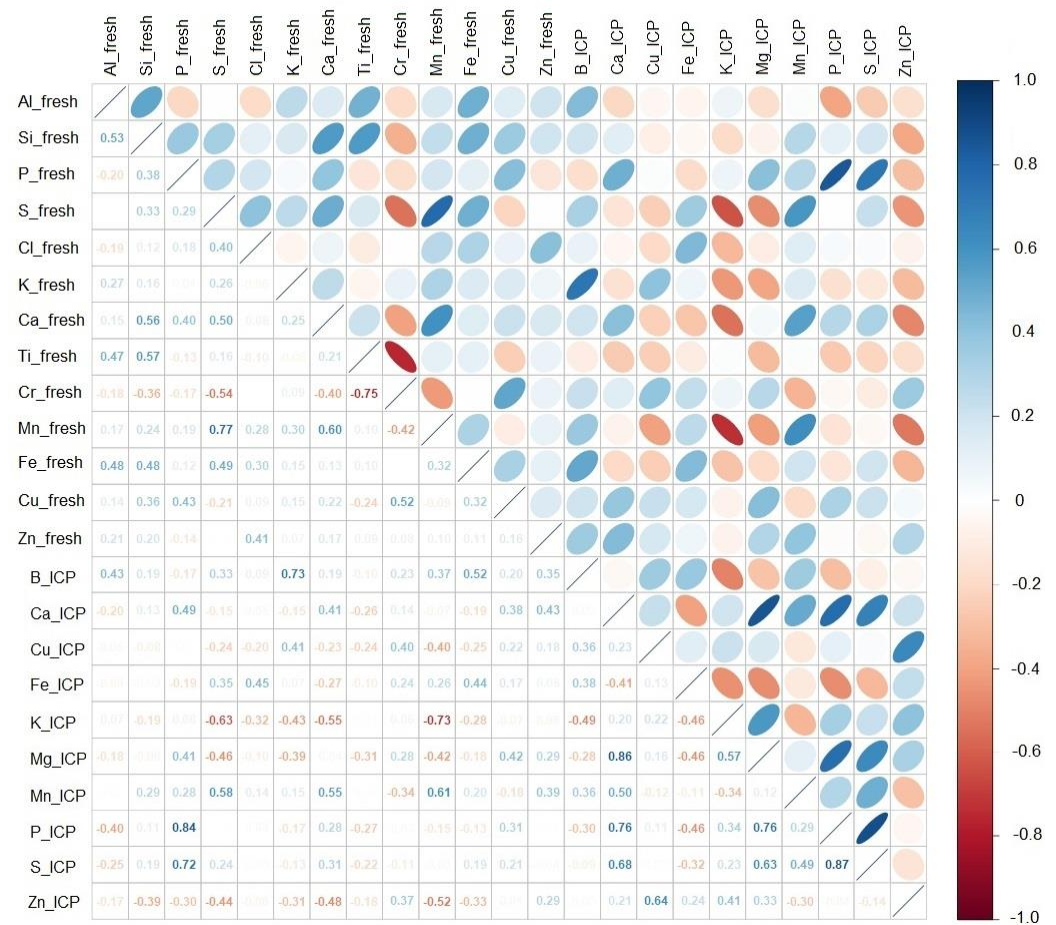


Figure 4. Correlations between inductively coupled plasma (ICP) and portable X-ray fluorescence (pXRF) spectrometer analysis of fresh leaves of three blueberry varieties.

Comparing the correlation coefficients among chemical contents of fresh and dry leaves determined by pXRF and ICP, correlations were stronger when using dry leaves for most elements. In dry leaves, results between pXRF and ICP produced very strong correlation for Fe (0.89) and strong correlations for P and Zn (0.75), Ca (0.72), Cu (0.71), Mn (0.68), and S (0.61) (Figure 4). Comparatively, for fresh leaves (Figure 5), results were worse, except for P, which presented a slightly stronger correlation with ICP results than when analyzed in dry leaves (0.84 in fresh leaves and 0.75 in dry leaves). The best correlations between fresh leaves and ICP results were found for: P (0.84) and Mn (0.61). Thus, for relating pXRF results with ICP results, dry leaves are a better option. Using dry leaves, refs. [19,21] achieved strong correlations between pXRF and ICP results of nine nutrients evaluated in this study for eucalyptus and several other crops, like citrus, coffee, corn, and soybean. Ref. [22] evaluated such correlations (pXRF and ICP) for both dry and fresh leaves in addition to other plant tissues and also noted higher correlations in dry samples for most elements. This first report for blueberry leaves agrees with those studies, confirming that pXRF is also helpful in rapidly detecting nutrients in leaves.

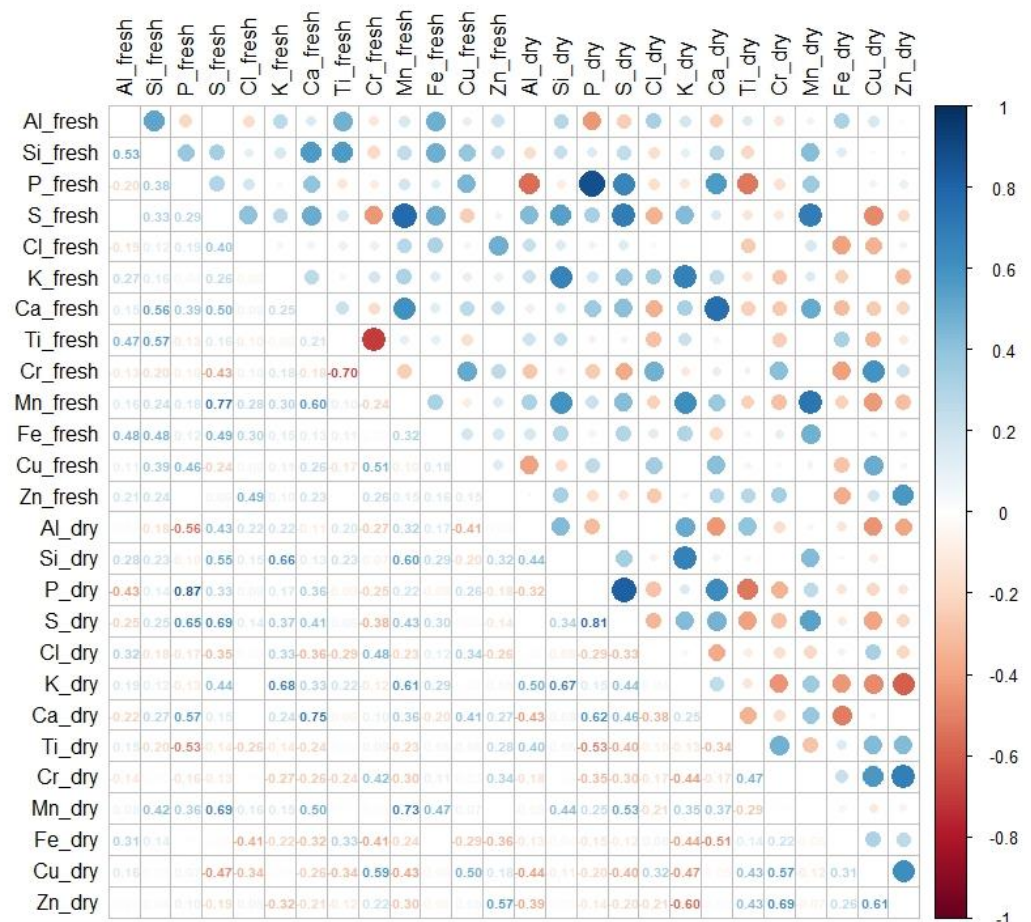


Figure 5. Correlations of portable X-ray fluorescence (pXRF) spectrometer analysis of fresh and dry leaves of three blueberry varieties.

Correlations were found between fresh and dry pXRF data, demonstrating that results from fresh leaf analysis, which is easier and faster to perform compared to dry leaf analysis, can be somewhat converted to dry leaf results. This was observed for P ($r = 0.87$), Ca (0.75), Mn (0.73), S (0.69), K (0.68), and Zn (0.57). Also, correlations were found between different elements, e.g., between S and P (0.81) and Ca and P (0.62), both in dry leaves, which is related to their biochemical behavior in physiological processes. For instance, S and P are related to the synthesis of amino acids, energy production, photosynthesis, and several other essential metabolic processes for plants [44,45].

Blueberry variety-specific correlations between pXRF and ICP results were calculated and compared with those generated from the whole dataset (all varieties together). Table 5 demonstrates that some elements present better correlations when data from only one variety is used. First, it was noticed that dry leaf data presented overall better correlation than fresh leaves when comparing ICP and pXRF analysis, even per variety, confirming what was already shown for the whole dataset by correlograms (Figures 3 and 4). Second, correlations were stronger in several cases when calculated per variety, surpassing those obtained for the whole dataset, even for fresh leaves in a few cases, which can drastically accelerate the in situ diagnosis of nutrient contents when using pXRF.

Table 5. Correlations between inductively coupled plasma (ICP) and portable X-ray fluorescence (pXRF) spectrometer data, with pXRF analyzing fresh and dry blueberry leaves, calculated per variety separately (Emerald, Jewel, and Biloxi) and in combination (General, all varieties).

Variety	Emerald		Jewel		Biloxi		General	
	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh
Ca	0.34	0.22	0.87	0.89	0.44	0.67	0.72	0.41
Cu	0.66	−0.38	0.88	0.33	0.29	−0.33	0.71	0.22
Fe	−0.34	0.57	0.69	−0.27	0.87	0.60	0.71	0.22
K	−0.89	−0.96	−0.82	−0.21	−0.56	−0.44	−0.73	−0.43
Mn	0.73	0.65	0.88	0.81	0.15	−0.44	0.68	0.61
P	0.85	0.27	0.86	0.86	0.48	0.90	0.75	0.84
S	0.16	−0.45	0.88	0.76	−0.03	0.12	0.61	0.24
Zn	0.84	−0.39	0.66	−0.01	0.92	0.82	0.75	0.29

Strong ($r > 0.60$) and very strong ($r > 0.80$) correlations were obtained for all varieties. In leaves of the Emerald variety, strong correlations were achieved for Cu and Mn in dry leaves, while very strong correlations were found for K (both fresh and dry leaves), P, and Zn (dry leaves). Nutrient contents in Jewel leaves presented strong correlations for Fe, and Zn (dry leaves) and very strong correlations for Ca (both fresh and dry), Cu, K, Mn, and S. Nutrient contents of dry Jewel leaves presented correlations above 0.60 for all elements evaluated herein. In the leaves of the Biloxi variety, the best correlations were achieved using fresh leaves. Correlations were strong for Ca, and Fe (all fresh) and very strong for P (fresh), Zn (both fresh and dry), and Fe (dry). General correlations (including all varieties) were strong for most elements in dry conditions (Ca, Cu, Fe, K, Mn, P, S, and Zn), except for Mn, which presented strong correlation in fresh leaves. Only one very strong correlation was found for P in fresh conditions.

Equations ($R^2 > 0.60$) were calculated to estimate contents determined by ICP based on pXRF data from the analysis of fresh and dry blueberry leaves (Table 6). Only elements that showed stronger correlations were used. Most models were based on results from dry leaf analysis. Six elements that presented strong correlations were chosen for the Jewel variety (Ca, Cu, K, Mn, P, and S), while for Emerald and Biloxi, equations were built for three elements (Emerald: K, P, and Zn; Biloxi: Fe, P, and Zn). P was the only element with adequate equations both for individual varieties and for the combined data with all three varieties (general). Other elements, such as Cu, Fe, and S, only presented adequate models for one variety (Jewel—Cu and S; Biloxi—Fe). Interestingly, the best fits were obtained for different moisture conditions even for the same element, e.g., for Emerald, P content determined by ICP presented a better fit when using dry leaf data, while for Biloxi, the best fit for P was obtained using data from fresh leaves. For Jewel, equations using data from both fresh and dry leaves presented an adequate fit. These results highlight how the specificities of each variety affect the relationship between pXRF and ICP results, demonstrating the need for testing models per variety, when necessary. For eucalyptus, there was no need for clone-specific model calibration to predict nutrient contents in leaves based on pXRF data [21], contrary to our findings for blueberries. For other crops, results for different nutrients were also promising [46–50]. These results are very promising to accelerate the determination of nutrient contents in leaves via pXRF, providing results comparable to ICP.

Table 6. Best fits between inductively coupled plasma (ICP) and portable X-ray fluorescence (pXRF) spectrometer results from analyses of fresh and dry leaves of three varieties of blueberries. Equations were built individually for each variety and from data of all varieties combined (general).

Element	Variety	Condition	Equation *	R ²
Ca	Jewel	Dry	$Ca_{ICP} = 2.1622x - 8087$	0.76
Ca	Jewel	Fresh	$Ca_{ICP} = 18.284x - 11,012$	0.79
Cu	Jewel	Dry	$Cu_{ICP} = 1.065x - 5.741$	0.78
Fe	Biloxi	Dry	$Fe_{ICP} = 0.534x - 12.967$	0.76
K	Emerald	Dry	$K_{ICP} = -1.5603x + 21,431$	0.79
K	Emerald	Fresh	$K_{ICP} = -4.3644x + 16,320$	0.93
K	Jewel	Dry	$K_{ICP} = -0.4552x + 13,669$	0.67
Mn	Jewel	Dry	$Mn_{ICP} = 1.1794x - 156.93$	0.78
Mn	Jewel	Fresh	$Mn_{ICP} = 2.3995x - 39.814$	0.66
P	Emerald	Dry	$P_{ICP} = 6.803x - 3576.1$	0.73
P	Jewel	Dry	$P_{ICP} = 3.0482x - 113.36$	0.75
P	Jewel	Fresh	$P_{ICP} = 20.877x - 709.14$	0.73
P	Biloxi	Fresh	$P_{ICP} = 15.226x - 111.56$	0.80
P	General	Fresh	$P_{ICP} = 15.371x - 147.12$	0.71
S	Jewel	Dry	$S_{ICP} = 2.367x + 539.29$	0.78
Zn	Emerald	Dry	$Zn_{ICP} = 1.4748x - 17.258$	0.70
Zn	Biloxi	Dry	$Zn_{ICP} = 1.4984x - 18.634$	0.85
Zn	Biloxi	Fresh	$Zn_{ICP} = 2.6286x - 16.255$	0.67

* x refers to the content of that element detected by pXRF under a moisture condition.

Despite the adequate results for most nutrients, it is important to mention that we encourage further studies for this crop using a larger number of samples. Future investigations could also use a greater number of blueberry varieties to assess whether a larger dataset could deliver more powerful models capable of converting pXRF into ICP results, independently of variety. By adopting a greater number of samples, more robust models, such as machine learning algorithms, could be used, as they have proven to be successful in predicting nutrient contents in leaves from pXRF results [21,46], which could also be achieved for blueberries. As such, the adoption of pXRF for characterization of nutrient contents in leaves would be more practical since it would avoid the need for calibration of models per variety. This pXRF-ICP approach can drastically accelerate and reduce the costs for the determination of nutrient contents in leaves and, thus, we encourage future works on this topic for blueberries and other crops, specifically involving the most representative varieties and a larger dataset than the one used herein.

3.4. Prediction of Blueberry Varieties Based on the Elemental Contents of Leaves

Given the differences in the chemical composition of the three blueberry varieties detected by pXRF and ICP, their differentiation based on the elemental contents of their leaves determined via ICP and pXRF (fresh and dry conditions) were tested using the random forest algorithm. Results showed that blueberry varieties can be accurately differentiated and predicted using random forest, especially using dry leaves (Figure 6).

In this condition, OA and kappa index reached the highest values: 0.87 and 0.80, respectively, corresponding to an outstanding classification per [33]. ICP results also achieved reasonably accurate predictions (OA = 0.73, kappa = 0.60), while the least accurate results were observed for fresh leaves (OA = 0.67, kappa = 0.50). These results demonstrate the effectiveness of pXRF to detect chemical differences even within varieties of the same crop under the same management practices. Similar results were achieved for differentiation of eucalyptus clones [21] and for detecting the origin of soybeans [51]. Differentiation of charcoal from native and exotic trees [52] has been successful using a similar approach based on elemental data. Future work will focus on the differentiation of varieties based on pXRF analysis of fruits.

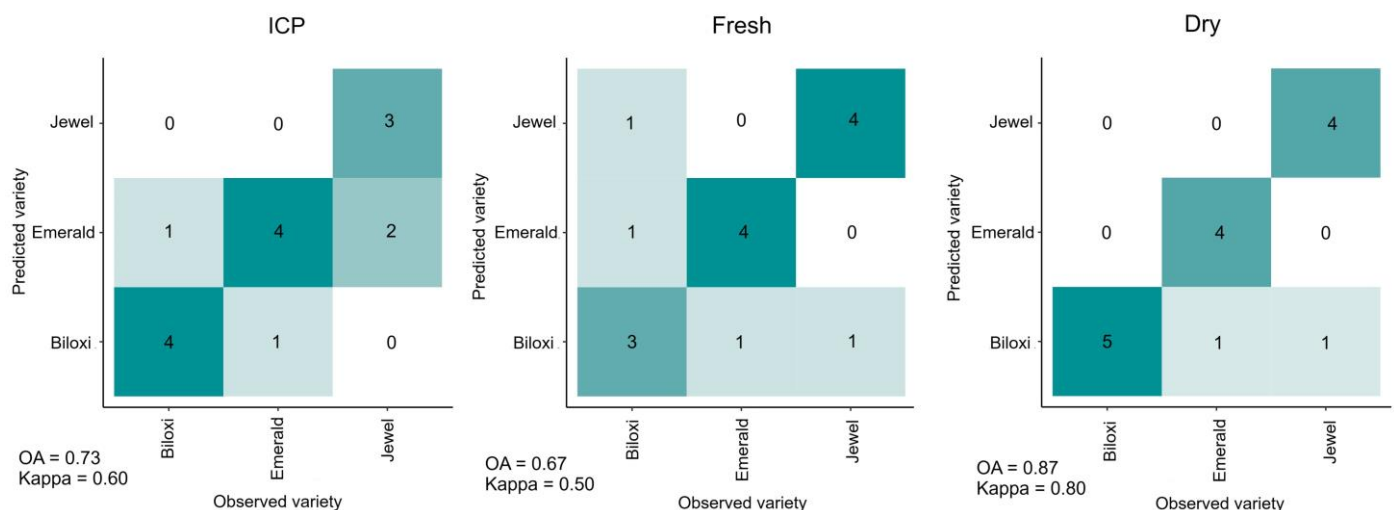


Figure 6. Prediction of blueberry varieties based on elemental contents of their leaves determined by inductively coupled plasma (ICP) and portable X-ray fluorescence (pXRF) spectrometer (fresh and dry leaves). OA—overall accuracy.

4. Conclusions

The chemical characterization via ICP-OES of nutrients in the leaves of three blueberry varieties (Emerald, Jewel, and Biloxi) adapted to warm climates can be used as a reference for producers and future works, given the lack of nutritional information for these plants in the literature. Nutritional data can help define fertigation management practices and optimize the use of fertilizers, especially for places with emerging and newly established blueberry plantations.

Elemental data of fresh and dry blueberry leaves obtained by pXRF analysis were strongly correlated with ICP results (r ranging from 0.61 to 0.90) for the eight nutrients evaluated herein (Ca, Cu, Fe, K, Mn, P, S, Zn). The highest correlations were obtained for dry leaf analysis. However, strong correlations were also found between pXRF and ICP results for Ca, K, P, Mn, and Zn even with fresh leaf data. Being able to convert pXRF data into ICP data can drastically facilitate the diagnosis of the nutritional status of blueberry plants, as it would make possible the in situ analysis of plant leaves with a pXRF unit.

Regression models created to predict ICP data from pXRF data performed better when using data of each variety individually. Models created with data from all varieties combined presented poorer performance. Random forest models were able to use pXRF data to accurately differentiate the three blueberry varieties, reaching overall accuracy of 0.87 and kappa index of 0.80. These findings corroborate the usefulness of pXRF to facilitate the diagnosis of nutrients in leaves with adequate accuracy and stimulate future works on the relationships between pXRF and ICP results. More importantly, this approach can be applied to other crops after an appropriate evaluation per crop and/or variety, which motivates future efforts on this topic.

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