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Note

Simple procedure for nutrient analysis of coffee plant with energy dispersive X-ray fluorescence spectrometry (EDXRF)

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ABSTRACT: Nutrient analysis is used to estimate nutrient content of crop plants to manage fertilizer application for sustained crop production. Direct solid analysis of agricultural and environmental samples by energy dispersive X-ray fluorescence spectrometry (EDXRF) was chosen as alternative technique to evaluate the simultaneous multielemental quantification of the most important essential elements in coffee (*Coffea arabica* L.) plants. Inductively coupled plasma atomic emission spectrometry and certified reference materials made from leaves were used to calibrate and check the trueness of EDXRF method for the determination of the concentration of several nutrients in coffee leaves and branches. Fluorescence spectrometry proved to be advantageous and presented low cost as loose powder samples could be used. Samples collected from a field experiment where coffee plants were treated with excess of Ni and Zn were used to verify the practical application of the method. Good relationships were achieved between certified values and data obtained by EDXRF, with recoveries ranging from 82 to 117 %.

Keywords: *Coffea arabica*, metal toxicity, mineral nutrition, analytical procedure

Introduction

The nutritional status of crops is governed by the geochemical features of the soil, the capacity of plants to accumulate elements (nutrients), environmental pollution and fertilization (Queralt et al., 2005). The quantification of the concentrations of nutrients have been carried out by several methods including colorimetry, flame emission photometry, flame atomic absorption spectrometry (FAAS), inductively coupled plasma atomic emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) (Fifield and Haines, 1997). ICP-MS has been mainly used for trace elements. However, these methods require complete sample matrix destruction by dry ashing (combustion of the sample) or wet ashing (digestion with strong acids). Furthermore, sample dissolution is usually a tedious and time-consuming step that sometimes may limit application of the analytical procedure and requires caution to avoid the explosion of the acids on heating (Noda et al., 2006).

Studies on the suitability of other methods for direct and multi-elemental analysis of plant samples have been increased and X-ray fluorescence (XRF) is a promising technique for nutrient plant analysis (Clark et al., 1992; Frank et al., 1992; Miah et al., 1999). Most X-ray fluorescence (XRF) techniques comply with desired features for analysis of plant specimens, including: (i) possibility of performing analysis directly on solid samples; (ii) multi-element capability; (iii) possibility of performing qualitative, semi-quantitative and quantitative determinations; (iv) a wide dynamic range; (v) high throughput; and (vi) low cost per determination (Marguí et al., 2009).

XRF has been successfully applied in studies on the elemental composition of plants (Chuparina and Gunicheva, 2003; Marguí et al., 2009), soil contamination (Orescanin et al., 2003; Aslan et al., 2004; Marguí et al., 2005; Necemer et al., 2008) and agriculture (Noda et al., 2006; Oliveira et al., 2006; Gratão et al., 2008; Maity et al., 2010), which most of them applied pressed powder pellet to perform EDXRF analysis.

This study aimed to describe and calibrate the application of an expeditious EDXRF (Energy dispersive X-ray fluorescence) method to determine the levels of P, K, Ca, Mg, Cu, Fe, Mn, Ni and Zn in loose powder obtained from leaves and branches of coffee plants.

Materials and Methods

Sample collections for method calibration: Starting from the branch apex, 45 samples of leaves of third pair were collected from *Coffea arabica* L. trees growing in several locations in the states of Bahia (8°43′00″ to 18°23′00″ S, 46°22′00″ to 37°21′00″ W), Minas Gerais (14°17′00″ to 22°56′00″ S, 51°00′00″ to 39°50′00″ W) and São Paulo (19°49′00″ to 25°18′00″ S, 53°04′00″ to 44°08′00″ W). The leaves were washed thoroughly with deionized water to remove dust, dried at 60 °C to complete dryness, powdered in a ball mill to pass a 500 µm sieve, and then stored in capped polypropylene flasks in desiccators until analysis in ICP-OES and EDXRF.

ICP-OES analysis: The contents of P, K, Ca, Mg, Cu, Fe, Zn, Mn and Ni in coffee leaves from several plants were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; JobinYvon, JY50P Longjumeau, France). The material was digested with 4 mL HNO_3 (65 %, Sigma-Aldrich, puriss. p.a.) and 2 mL $HClO_4$ (70 %, Merck, p.a.) concentrated acids on a digestion block heated gradually to 203 °C.

EDXRF analysis: The same coffee samples used for ICP-OES were used for EDXRF analysis and were prepared as loose powder. One gram of ground leaves was packed into a polyethylene cup of 20 mm internal diameter and covered with 6-μm-thick polypropylene film (Mylar®). The samples were irradiated in triplicate for 300 s under vacuum using an energy dispersive X-ray fluorescence spectrometer Shimadzu EDX-720. The samples were irradiated using an Rh X-ray tube operated at 15 kV (Na to Sc) and 50 kV (Ti to U). The current was automatically adjusted (maximum of 1 mA). A 10 mm collimator was chosen. The detection was carried out using the Si (Li) detector cooled with liquid nitrogen. Certified reference materials (CRMs) were analyzed using the same method as described above in order to verify trueness and precision. The CRMs used were apple leaves (NIST 1515), peach leaves (NIST 1547) and tomato leaves (NIST 1573a). The intensity of element Kα counts per second (cps/μA) was obtained from the sample X-ray spectrum deconvolution using the EDX Shimadzu software package.

Detection limits (LD): LD of each nutrient was estimated from the standards sample materials collected. As blank samples were not available, the detection limits were calculated as

$$LD = \frac{3}{Si}\sqrt{\frac{I_{i(BG)}}{t}}$$

where "$I_{i(BG)}$" is the background intensity of nutrient i (cps μA^{-1}); "t" = acquisition time; S_i = analytical sensitivities to analyte *i* (cps μg g^{-1}) (De Vries and Vrebos, 2002).

Practical application: Seven years old coffee plants (*C. arabica* L. cv. Obatã) growing in an experimental station in Piracicaba (22°42′16′′S, 47°37′20′′ W), state of São Paulo, Brazil, were treated separately with $NiSO_4$ and $ZnSO_4.7H_2O$ to reach 300 and 450 mg dm^{-3} in the soil as free Ni and Zn, respectively. Healthy plants were available as control. The metal applications were split in two rounds, the first half of the salts were applied on Dec 18, 2008, and the second on Feb. 19, 2009. The salts were dissolved in water and the solution slowly spread around the plant to allow good infiltration in the soil in five plants for each treatment. Harvesting was performed on Dec 18 (prior to application) and on Apr 24, 2009 (128 days after) and the third leaf pairs and the branches they belong to were harvested and processed as described previously for EDXRF analysis.

The precision and trueness of the EDXRF method was checked by analyzing certified reference materials made from leaves (NIST 1515 "Apple leaves", NIST 1547 "Peach leaves" and NIST 1573a "Tomato leaves" from National Institute of Standards and Technology), therefore expected to have a similar matrix composition of coffee leaf samples.

To calibrate the data obtained for coffee leaves with the EDXRF method, a comparison with the data obtained with ICP-OES using the same samples was performed. Linear regression analyses were carried out with these data and R^2, intercept (A) and slope (B) were obtained. If the results from the different methods were equal these parameters should be $R^2 = 1$, $B = 1$ and $A = 0$.

Statistical data analysis: Linear regression analysis was used to compare the performance of the ICP-OES and EDXRF methods. Regression coefficients were tested in the level of significance by the F test. Standard deviation was used to analyze the data from the practical application analysis. Sigma Plot 2000 software were used for the statistical analyses.

Results and Discussion

Good relationships were achieved between certified values and data obtained by EDXRF, with recoveries ranging from 82 to 117 % (Table 1). However, Cu and Ni of certified reference materials were below the detection

Table 1 – Nutrient concentration (μg g^{-1} DW; n = 5) of certified reference material (± standard deviation) determined by EDXRF.

		P	K	Ca	Mg	Cu	Fe	Mn	Ni	Zn
NIST 1515 – Apple leaves	This work	1750 ± 30	18700 ± 100	14900 ± 110	2500 ± 160	ND	81.3 ± 0.9	60 ± 2	ND	13.7 ± 1.2
	Certified value	1590 ± 11	16100 ± 20	15260 ± 15	2710 ± 8	5.6 ± 0.2	83 ± 5	54 ± 3	0.91 ± 0.12	12.5 ± 0.3
	Recovery (%)	110	116	98	94	-	98	112	-	110
NIST 1547 – Peach leaves	This work	1420 ± 30	25000 ± 40	14300 ± 30	4500 ± 120	ND	191.1 ± 1.3	103.9 ± 1.5	ND	20.6 ± 0.6
	Certified value	1370 ± 7	24300 ± 30	15600 ± 20	4320 ± 8	3.7 ± 0.4	218 ± 14	98 ± 3	0.7 ± 0.1	17.9 ± 0.4
	Recovery (%)	103	103	92	104	-	88	106	-	115
NIST 1573a – Tomato leaves	This work	2490 ± 50	22200 ± 40	50100 ± 140	11900 ± 300	ND	318 ± 2	245 ± 3	ND	36.2 ± 0.8
	Certified value	2160 ± 4	27000 ± 50	50500 ± 90	12000*	4.70 ± 0.14	368 ± 7	246 ± 8	1.59 ± 0.07	30.9 ± 0.7
	Recovery (%)	116	82	99	100	-	86	100	-	117

*Not certified; ND – not detected.

limits. The detection limits (in µg g^{-1}) in this study were: P (40.2), K (27.5), Ca (27.6), Mg (547.0), Cu (5.8), Fe (2.3), Mn (2.6), Ni (4.0) and Zn (2.1). Detection limits for P, K, Ca and Mg (light elements) were higher than those for Cu, Fe, Mn, Ni and Zn (metallic nutrients), but they were low enough considering that all these elements are present at very high concentration in plant tissues and therefore, their ranges are appropriate for routine analysis of plant materials. The ranges for diagnosis of plant analysis were (in µg g^{-1}): P: 800-13,000, K: 5,000-80,000, Ca: 700-50,000, Mg: 1,000-12,000, Cu: 2-100, Fe: 5-1000, Mn: 5-2000 and Zn: 5-250 (van Raij et al., 1996). These results indicated that the method is appropriate for nutritional plant analysis.

The LDs of the proposed approach have the same order of magnitude compared to those observed in other reports. Small differences in the limit of detection considering different approaches might be due to different equipment condition analysis and sample preparation, as we used loose powder instead pellet samples. For a comparison, the detection limits reported by Margui et al. (2005) for plant tissues were as follows: (in µg g^{-1}) K: 10, Ca: 20, Mn: 4, Fe: 3.1, Cu: 1.2 and Zn: 1.2. However, our results clearly suggest that loose powder could be used for plant diagnosis analysis decreasing costs and saving time, as it does not need sample pellet preparation.

EDXRF method and ICP-OES were very similar (Table 2). However, Ca, Fe and Mg showed the lowest R^2 value when compared to other nutrients. To carry out a practical test we harvested leaves from a field experiment where plants were exposed to high doses of Zn and Ni (Figure 1 and 2). As expected from plants grown in the field, a large variation was observed for all analyzed nutrients as also previously reported by Lima Filho and Malavolta (2003).

As samples were collected at the beginning of the metal applications (in Dec.) and then collected after 128 days (in Apr.) at least part of the detected differences might be a consequence of the developmental stage of the leaves and the branches. Therefore, this might explain the reduction observed in P and K contents even in the control plants. On the other hand, Mg, Mn, Fe and Cu remained stable in all treatments. Interestingly the Cu data clearly showed that this metal is found in higher concentration in the branches than in the leaves. High concentration of Cu has already been reported in the literature suggesting that branch is a strong sink for heavy metals accumulation

Table 2 – Range of elements concentration (µg g^{-1}) determined in coffee leaves and regression analyzes parameters between EDXRF and ICP-OES determinations.

Element	Range	Parameters for LR analyses		
		R^2	A	*B*
P	660-2010	0.9327	-22.6711^{ns}	1.0215**
K	12240-24990	0.7934	2746.8513*	0.8783**
Ca	7650-20400	0.8322	3851.8327**	0.6793**
Mg	1600-4700	0.7582	-981.4349*	1.4989**
Cu	4.2-556	0.9853	0.7443^{ns}	0.9330**
Fe	48.3-1183	0.9515	50.6917**	0.7410**
Mn	46.4-556	0.9891	-3.2915^{ns}	1.0371**
Ni	11.6-52.3	0.9875	2.9151^{ns}	0.8856**
Zn	6-80.4	0.9658	3.56**	0.8617**

Regression analysis: LR model, EDXRF = *A* + *B**ICP method (*A*: intercept, *B*: slope).*$p < 0.05$; **$p < 0.01$; ns: non-significant.

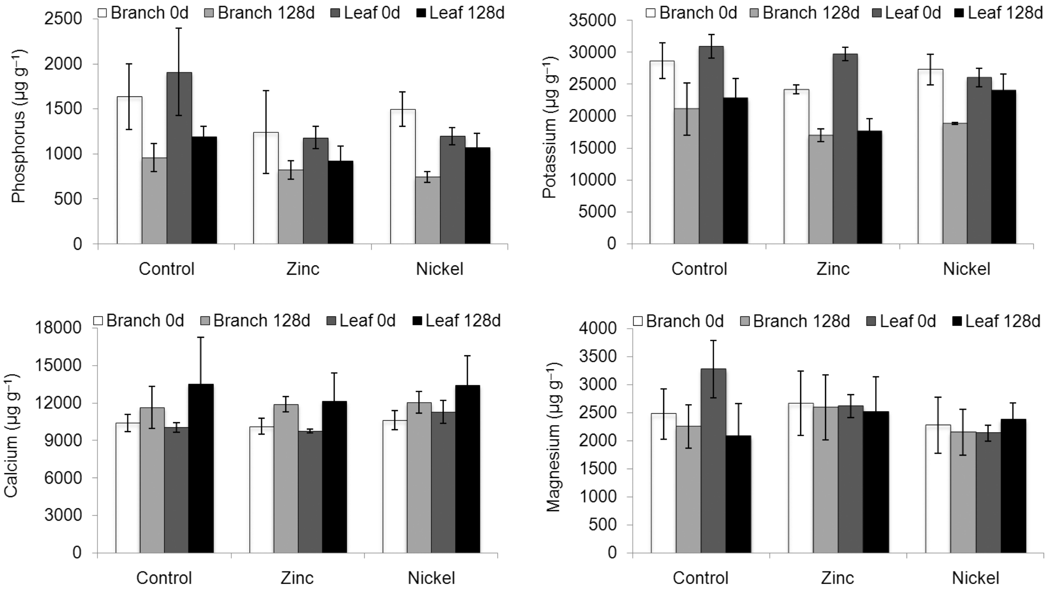

Figure 1 – EDXRF P, K, Ca and Mg contents of coffee plants exposed to excess of Zn and Ni, at application and 128 days after metal application (n = 5).

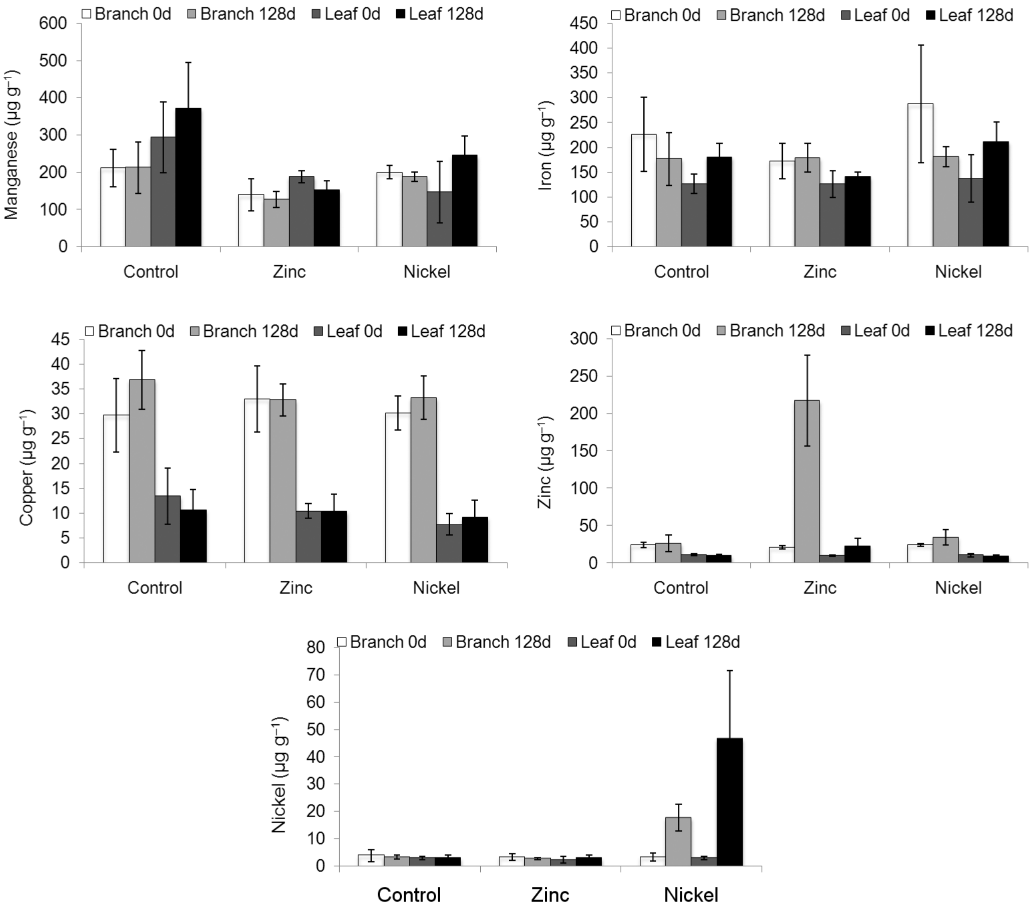

Figure 2 – EDXRF Cu, Fe, Mn, Ni and Zn contents of coffee plants exposed to excess of Zn and Ni, at application and 128 days after metal application (n = 5).

(Malavolta et al., 2002). This preferential accumulation was also observed for Zn. When coffee plants received Zn application an increase in the analyzed tissues was verified, but again, a much higher accumulation was observed in the branches (Figure 2). It was not possible to identify a preferential accumulation of Ni in the analyzed tissues, but plants subjected to Ni exposure exhibited a higher accumulation in the leaves than in the branches.

Conclusions

X-ray fluorescence method (EDXRF) uses loose powder, which decreases time and analysis costs. Samples collected from plants fertilized with an excess of Zn and Ni were used as a practical application and as expected exhibited an increase of these metals contents.

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