Plant elemental composition and portable X-ray fluorescence (pXRF) spectroscopy: quantification under different analytical parameters

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Emergence of portable X-ray fluorescence (pXRF) systems presents new opportunities for rapid, low-cost plant analysis, both as a lab system and in situ system. Numerous studies have extolled the virtues of using pXRF for a wide range of plant applications, however, for many such applications, there is need for further assessment with regards to analytical parameters for plant analysis. While pXRF is a potential powerful research tool for elemental composition analysis, its successful use in plant analysis is made more likely by having an understanding of X-ray physics, calibration process, and ability to test a variety of homogenous and well-characterized materials for developing a matrix-specific calibration. Because potential pXRF users may often underestimate the complexity of proper analysis, this study aims at providing a technical background for plant analysis using pXRF. The focus is on elemental quantification under different analytical parameters and different methods of sample presentation: direct surface contact under vacuum, placement in a sample cup with prolene as a seal, and without the aid of a vacuum. Direct analysis on elemental quantification under different analytical parameters and different methods of sample presentation: direct surface contact under vacuum, placement in a sample cup with prolene as a seal, and without the aid of a vacuum. Direct analysis on the surface of a pXRF provided highest sensitivity and accuracy ($R^2 > 0.90$) for light elements (Mg to P). Sulfur, K, and Ca can be reliably measured without the aid of a vacuum ($R^2 > 0.99$, 0.97, and 0.93 respectively), although lower detection limits may be compromised. pXRF instruments provide plant data of sufficient accuracy for many applications and will reduce the overall time and budget compared with the use of conventional techniques. Sensitivity and accuracy are dependent on the instrument’s settings, make, and model. © 2015 The Authors. X-Ray Spectrometry published by John Wiley & Sons, Ltd.

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Introduction

Key plant nutrients, including potassium (K), phosphorus (P), and sulfur (S), play important roles in regulating processes such as photosynthesis, carbon respiration, and tissue building. Elemental analysis of plants is critical to plant studies and agriculture. However, despite the routine application of digestion-based analytical techniques in many laboratories, the slow and often hazardous sample digestion process can present a challenge in the analyses of plant nutrients, particularly where hundreds or even thousands of samples are to be analysed. The conventional plant elemental analyses based on plant extracts are cumbersome and of variable accuracy and present major limitations to exploiting the full potential of plant nutrient analysis. An ideal system for analysis would include accuracy, replicability, portability, and minimal sample preparation. The technology that currently provides the best option to meet these needs is energy-dispersive X-ray fluorescence (ED-XRF), particularly in the form of a portable instrument portable X-ray fluorescence (pXRF). These instruments are commonly used for scrap metal sourcing, geochemical provenience of obsidian, and soil analysis.

Traditionally, ED-XRF has been used to provide a rapid and non-destructive method for the analyses of trace and major elements in soil samples, the mobile fraction of soils, and aerosol particles. Energy dispersive X-ray fluorescence has also been useful in studies on elemental composition of plants and in identifying potentially hazardous trace metals in plants. Micro-ED-XRF, which can apply a spot size of only a few microns to provide cellular-level resolution, has been used to identify the deposition of elements within plant tissue. In a comparison study with inductively-coupled plasma-optical emission spectrometry (ICP-OES), data obtained through ED-XRF did not deviate more than 2 mg kg$^{-1}$ for either Fe or Zn in rice and pearl millet grain. Furthermore, XRF results were highly correlated with ICP-OES values for both Zn and Fe in both species ($R^2 = 0.79–0.98$). Similarly, Pattridge et al. used XRF for measuring Zn, Fe, and Se in whole grain wheat. They compared pXRF data to lab-based ICP-OES data and found strong correlations and low prediction errors ($\pm 2.2, 2.6, 1.5$ mg kg$^{-1}$ for Zn, Fe, and Se, respectively). McLaren et al. evaluated maize, cotton, soybean, and wheat samples via pXRF and compared them with standard acid digest laboratory analyses. They found significant linear
relationships for Ca, Co, Cr, Fe, K, Mn, Ni, P, S, Si, and Zn. Portable X-ray fluorescence analysis was also assessed as having merit for quantitative analysis of heterogenous plant samples for calcium and potassium and qualitative assessment for other elements, in particular manganese and sulfur.\textsuperscript{16}

Energy dispersive X-ray fluorescence is presently available as a portable instrument with a tube-based X-ray source, as opposed to a radioactive isotope source. More recently, new kinds of detectors have been designed for these systems: peltier-cooled silicon drift detectors (SDD), which effectively measure the energy of incoming photons via the ionization produced in the detector and achieve impressive resolution, able to detect elements as light as carbon.\textsuperscript{51} A portable system is capable of detection of low atomic number elements down to either Mg or Na, depending on the configuration of the system and/or type of matrix. The emergence of portable ED-XRF systems, henceforth referred to as pXRF, presents new opportunities for rapid, low-cost plant nutritional analysis, both as a traveling lab system and also as an in situ analyzer. For example, the Food and Drug Administration of the United States has adopted this technology in screening for toxic metals in consumer products due to its portability.\textsuperscript{17} Despite the portability of the systems, the data acquired have similar limits of detection as traditional laboratory systems.\textsuperscript{18} However, this relative increase in performance depends as much on the variability in lab systems as it does on the pXRF units in question. For example, the sensitivity of a pXRF instrument (i.e. net fluorescence intensity obtained per unit of analyte concentration) has been evaluated, and the detection limit estimated for light elements has been found within the 0.1 g kg\textsuperscript{-1} range for plant certified reference materials.\textsuperscript{11}

As Grave et al.,\textsuperscript{19} Speakman and Shackley\textsuperscript{3} and Frahm\textsuperscript{20} noted, applications of pXRF continue to be cautiously treated, principally because of a perceived lack of analytic rigor. According to Speakman and Shackley,\textsuperscript{3} ‘most pXRF users lack experience—whether it be X-ray physics, basic analytical chemistry, statistics, or the fundamentals of provenance studies’. Many users approach pXRF from a ‘black box’ perspective in which the inner workings of the XRF instrument are not understood by the user, nor does the user care to learn how and why the instrument functions—the only importance is that the sample is analysed and that numbers are generated.\textsuperscript{16,21,22} This has often resulted in users not recognizing the most basic problems that can and do occur with XRF analyses.\textsuperscript{21} In addition, because of the manner in which the capabilities of pXRF are (mis)represented by many manufacturers (e.g. promoting ideas of internal consistency, ability to measure low Z elements accurately, etc.), users tend to disregard conventional knowledge concerning XRF fundamentals, and consequently many pXRF-based studies are founded on poor science.\textsuperscript{3} However, as Spearman and Shackley\textsuperscript{3} note, the use of international standards can be used to produce valid and reliable data, enabling replicability in pXRF studies.

A pXRF unit can be used under either field or laboratory conditions, and considerations should be given to which approach will yield optimal results for the investigator. Numerous research studies e.g.\textsuperscript{11–16,23–28} have extolled the virtues of using XRF for wide range of plant and environmental science applications. For many such applications, the validity of the XRF method would be called into question without use of well-established analytical parameters and sample presentations to the instrument. While pXRF is a powerful research tool for plant elemental composition analysis, its successful application in plant tissue analysis requires an understanding of X-ray physics, the calibration process, and the ability to test a sufficient variety of homogenous and well-characterized reference materials for developing a matrix-specific calibration. This study aims at providing a technical background for the analysis of plant materials using pXRF with a particular focus on plant elemental quantification under different analytical parameters because pXRF users may underestimate the complexity of proper analysis. To the best of our knowledge, there are limited studies on the optimal pXRF scanning parameters for plant matrices.\textsuperscript{15,16} Plant sample analysis is characterized by the interplay of at least three domains of activity: sample preparation, sample introduction, and instrumentation. The present study thus examines the quantification of plant nutrients via pXRF using different sample presentations. These include direct surface contact under vacuum conditions, placement in a sample cup with prolene as a seal, and without the aid of a vacuum.

### The physics of XRF

In view of the aforementioned challenges, the use of methods such as pXRF, which is an extension of energy-dispersive ED-XRF, for direct and multi-elemental analysis of plants is of potential interest. Portable XRF techniques are characterized by a simple sample preparation, mainly sample homogenization, and fast and multi-elemental analysis over a large concentration range, which makes the procedure fast and cheap, and therefore suitable for application to a large number of samples. The method involves a process in which electrons of a given energy range interact with a metal anode (the X-ray source) to produce an X-ray spectrum. The configuration of pXRF devices, save for miniaturization, are not profoundly different than benchtop instruments. Photons are emitted from an X-ray tube using Rh, Ag, Mo, Cr, or W as an anode. These photons interact with the atoms within a sample, which in turn emit photons specific to each element. These photons then pass through an atmosphere to reach a detector, typically a peltier-cooled SDD. From here, photons are identified as counts within a particular channel, which can be converted in a computer into a spectrum of energy with elements clearly identifiable as peaks. The atmosphere in which these photons pass through includes dry air (N\(_2\), O\(_2\), and Ar), the 4 \(\mu\)m prolene window on the nose of the instrument and any barrier present in a sample cup used. These matrices contrive to the attenuation of photons, particularly those of lighter energies.

The range of elements currently analyzable using the pXRF method range from neon (10) to plutonium (94) and on to synthetic elements. Detection of lighter elements requires modification of atmospheric parameters, as the nitrogen (7) and oxygen (8) in the air readily absorb their relatively lower energy emissions. The depth of penetration can be calculated by the following equation:

\[
\frac{I}{I_0} = e^{-\left(\frac{\mu}{\rho}x\right)}
\]

where \(I\) is the quantity of photons returning from the sample, \(I_0\) is the quantity of photons entering the sample, \(\mu/\rho\) represents the mass attenuation coefficient of a given element for a particular matrix, and \(x\) represents the density of the object. Assuming, only 1% of photons return from a sample, the equation can be reduced to:

\[
\text{depth (cm)} = 4.36\left(\frac{\mu}{\rho}\right)\rho.
\]

The analytical depth of photons can thus be approximated using mass attenuation for X-rays. The measurement depth of common substrates in plants is featured in Table 1 using mass attenuation coefficients (\(\mu/\rho\)) obtained from the website of the National Institute of Standards and Technology (NIST). The depth of analysis for each element in plant matter can be high, with water and sugar...
Empirical quantification theory

Empirical calibration involves the use of reference standards with known concentrations, which can be used to build models for the purpose of quantifying photon intensities in XRF spectra. A set of standards, once produced, can be used to calibrate many instruments. This approach differs from fundamental parameters calibrations or standard-less calibration, which uses information about the geometry of XRF units and assumptions about the matrix to generate semi-quantitative estimates about the concentrations of materials. While the latter approach is widely used in commercial and industrial XRF instruments, it has caused considerable controversy when used in research contexts due to validity and reliability concerns.[3,20,29] When variance at the ppm or mg kg \(^{-1}\) is needed to evaluate a hypothesis, simple empirical calibrations are superior in that they can quantify measurement uncertainty. In addition, reference standards can be analysed on multiple instruments and thus provides a degree of reliability with different instruments and instrumentation.[30]

Simple empirical calibration follows the Lucas-Tooth and Price empirical calibration equation:

\[
C_i = r_0 + l_i (r_1 + \Sigma \epsilon_{in} + l_n)
\]

Where \(C_i\) represents the concentration of element \(i\), \(r_0\) is the intercept/empirical constant for element \(i\), \(r_i\) is the slope/empirical coefficient for intensity of element \(i\), \(\epsilon_{in}\) is the slope/empirical constant for effect of element \(n\) on element \(i\), \(l_i\) is the net intensity of element \(i\), and \(l_n\) is the net intensity of element \(n\). [31] Empirical calibrations following this algorithm will be accurate within the confines of the regression line (that is to say, minimum and maximum point). The accuracy of the algorithm is contingent upon the elemental variation captured by the empirical reference set and its appropriateness to the material being studied. For an empirical calibration to work, a number of assumptions must be met. First, the samples must be homogenous or, at the very least, sufficiently well mixed to be practically homogenous. Second, the reference set must consist of the same material. Third, every element present in the sample must also be present in the reference set. Fourth, the reference set must encapsulate the minimum and maximum of every element. In addition to these four principles, the data must be taken with the same parameters. These include the same energy in keV of the pXRF, current in A, filter of the pXRF instrument, and atmosphere (dry air, vacuum, etc.).

The Lucas-Tooth and Price method provides an empirical basis for determining concentrations from a sample through the use of simple linear models.[31] However, this comes with some limitations, including the selection of elements of interest prior to analysis.[2] An attractive feature of the Lucas-Tooth and Price method is that, in order to create a calibration equation for an analyte, one only needs samples with known concentrations.[3] For the calculation of the concentration from spectra, the process requires extraction of either the gross or net (fluorescence peak—background) intensities of elements within the analyte, typically with some form of normalization to Compton (inelastic) scattering, Rayleigh (elastic) scattering, background, time, or a region of interest. Following this preparation, simple linear, multi-linear, or nonlinear models can be developed from empirical reference standards to create calibration curves. Spectral overlaps and effects on fluorescence efficiency must be resolved, either using elements as a slope multiplier or constant correction to the concentration estimate of the analyte [31] or through the use of physical parameters such as K-alpha/K-beta ratios, fluorescence efficiency, or other known variables.[2]

Materials and methods

To study the applicability of pXRF for direct determination of elemental analysis of plant samples with varying matrices, we prepared different plant materials for use in empirical calibration that included brown and white cowpea (Vigna unguiculata) seeds, cowpea leaves, coroñ (Croton megalocarpus) leaves, mango (Mangifera indica) pulp and leaves, mahogany (Khaya senegalensis) saw dust, cyprus (Cupressus sp.) saw dust, maize (Zea mays) leaves and stem, prunus (Prunus africana) bark and leaves. All leaf samples were washed under running tap water and rinsed with deionized water to remove superficial dusts and contaminants before drying them in an oven at 60 °C for 3 days. Milling of the plant materials was done in a Thomas-Wiley Laboratory Mill, Model 4 (Thomas Scientific, USA) consisting of an all steel construction with four rotating cutting knives and later passed through a McCrone micronizing mill (McCrone, Westmont, USA) consisting of a 125 ml capacity polypropylene jar packed with an ordered array of 48 identical cylindrical Agate grinding elements that resulted in a fine (<53 μm) and non-fibrous powder to satisfy conditions of homogeneity. Reference values for these plant materials were measured in an external Lab (Rothamsted Research Ltd, Hertfordshire, UK) using an in-house method for conventional total acid dissolution ICP-OES analysis. The following certified reference materials (NIST standards) were also included and used to establish robust empirical calibrations: NIST 1515 apple leaves, NIST 1547 peach leaves, NIST 1568a rice flour, NIST 1573a tomato leaves, NIST 1575s pine needles, and NIST 1575a pine needles. Portable XRF data were obtained using a Bruker III Tracer SD (#T352731; Bruker Kennewick, WA, USA) with a 4W Rhodium anode and a Bruker Xflash SDD with 2048 channels. Spectra were acquired using a voltage of 15 keV, an anode current of 25 μA, and no filter for 180 s per assay (170 s live time). Spectra had a full width height maximum (FWHM) of 142 eV, with 20.026 eV per channel and a pulse density of 17,578 counts per second (cps). Directly measured samples had a FWHM of 141 eV. Three different forms of sample presentation were used: direct measurement.

| Atomic number | Energy (keV) | Depth in water (μm) | Depth in cellulose (μm) | Depth in fructose (μm) |
|---------------|-------------|---------------------|------------------------|-----------------------|
| Magnesium     | 12          | 1.25                | 20                     | 16                    |
| Phosphorus    | 15          | 2.01                | 80                     | 70                    |
| Sulfur        | 16          | 2.31                | 110                    | 100                   |
| Potassium     | 19          | 3.31                | 320                    | 280                   |
| Calcium       | 20          | 3.69                | 440                    | 380                   |
| Manganese     | 25          | 5.90                | 1930                   | 1710                  |
| Iron          | 26          | 6.41                | 2360                   | 2085                  |
| Molybdenum    | 42          | 17.48               | 40370                  | 34370                 |

Table 1. Estimated analytical measurement depth for different elements in different matrices, actual values for plants are contingent on presence/absence of cuticle, water content, and density being critical factors, providing a strong reason, for the time being, to limit analysis to dried plants.
(e.g. surface contact with powdered samples) of plant samples on
the nose of the instrument with a vacuum <5 torr, placement of
sample cups filled with plant powder using 4 μm prolene as a film
at the base of the cups on the nose of the instrument with
<5 torr vacuum, and placement of the plant powder in sample cups
using 4 μm prolene at the base with no vacuum (e.g. ambient air
conditions). In addition, total X-ray fluorescence (TXRF) data were
obtained with a benchtop Bruker S2 PICOFOX™ spectrometer
(Bruker AXS Microanalysis GmbH, Germany) for an independent
measurement of photon intensities, to identify potential contami-
nation of samples, and for additional checks of the calibrations;
samples were placed in sample cups with a 50 W molybdenum
tube (max power) with an XFlash SDD detector. Spectra were ac-
quired with a voltage of 20 keV, a current of 750 μA, a 9 μm molyb-
denum filter, and a dry air atmosphere for an average of 681 s (600 s
livetime). Spectra from the TXRF had a FWHM of 141 eV and
5.004 ev/ch with an average pulse density of 45 021 cps.

Data were processed in Bruker Spectra software (7.4.6.1) (Bruker
AXS Microanalysis GmbH, Germany) using Bayesian deconvolution
to correct for background counts, escape peaks, and simple ele-
mental overlaps to determine net count rates per second. These
data were compared with the given (reference) values of standards
and given concentrations indicate that light element detection
effects are evident for the two lightest elements, magnesium and
phosphorus, with different sample presentations. Sensitivity for light elements (Mg–S) for data collected using a vac-
uum and direct contact was highest while data collected with sam-
ple cups but without vacuum provided the worst sensitivity to light
elements (Fig. 1). Comparisons between net photon counts per sec-
ond and given concentrations indicate that light element detection

| Table 2. X-ray fluorescence validation following three sample treatments for portable X-ray fluorescence and total X-ray fluorescence |
|----------------|----------------|----------------|
| Slope | Intercept | R² | Slope p-value |
|----------------|----------------|----------------|----------------|
| Magnesium | | | | |
| No Prolene | 1.0 | −8.82E-13 | 0.98 | p < 0.001 |
| With Prolene | 1.0 | 8.82E-13 | 0.78 | p < 0.001 |
| No Vacuum | 1.0 | −4.41E-13 | 0.36 | p < 0.05 |
| Phosphorus | | | | |
| No Prolene | | −2.21E-13 | 0.95 | p < 0.001 |
| With Prolene | 1.0 | 2.21E-13 | 0.95 | p < 0.001 |
| No Vacuum | 1.0 | −1.54E-12 | 0.95 | p < 0.001 |
| Sulfur | | | | |
| No Prolene | 1.0 | 4.41E-13 | 0.99 | p < 0.001 |
| With Prolene | 1.0 | 4.41E-13 | 0.99 | p < 0.001 |
| No Vacuum | 1.0 | 0 | 0.99 | p < 0.001 |
| Potassium | | | | |
| No Prolene | 1.0 | 0 | 0.96 | p < 0.001 |
| With Prolene | 1.0 | 0 | 0.96 | p < 0.001 |
| No Vacuum | 1.0 | 3.53E-12 | 0.97 | p < 0.001 |
| Calcium | | | | |
| No Prolene | 1.0 | 5.29E-12 | 0.90 | p < 0.001 |
| With Prolene | 1.0 | 5.29E-12 | 0.91 | p < 0.001 |
| No Vacuum | 1.0 | −5.29E-12 | 0.93 | p < 0.001 |
| Manganese | | | | |
| No Prolene | | 0 | 0.91 | p < 0.001 |
| With Prolene | 1.0 | 2.76E-14 | 0.84 | p < 0.001 |
| No Vacuum | 1.0 | −2.76E-14 | 0.85 | p < 0.001 |
require direct measurement under vacuum conditions (Table 2, Fig. 2). Light elements showed the strongest correlations with their given concentrations with a direct measurement while relatively heavier elements, including K and Ca, are more easily measured within sample cups and even without vacuum conditions (Fig. 2). The absence of a vacuum made reliable analysis of either of the light elements much more difficult. This can be seen in the validation of the calibrations presented in Table 3, where only direct measurements of plant material on the nose of the instrument provided reliable quantification of Mg. The mean total elemental composition for the essential elements found in the dried plant tissues is shown in Table 4. The elemental concentrations will of course vary with nutrient availability, plant species, growing conditions, and time of sampling.

**Discussion**

Bayesian analysis of slopes for light elements indicate high unreliability in quantifying Mg and P without the aid of a vacuum as
Validation of calibrations taken under different analytical parameters against two standards

Table 3. Validation of calibrations taken under different analytical parameters against two standards

|                | Mg pXRF | Mg pXRF | P pXRF | P pXRF | S pXRF | S pXRF | K pXRF | K pXRF | Ca pXRF | Ca pXRF | Mn pXRF | Mn pXRF |
|----------------|---------|---------|--------|--------|--------|--------|--------|--------|---------|---------|---------|---------|
|                | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) | (mg kg⁻¹/C₀) |
| Direct measurement | 47.05  | 19.00  | 12.10  | 12.10  | 12.10  | 12.10  | 12.10  | 12.10  | 12.10  | 12.10  | 12.10  | 12.10  |
| Prunus Leaves | 2450   | 19.85  | 19.85  | 19.85  | 19.85  | 19.85  | 19.85  | 19.85  | 19.85  | 19.85  | 19.85  | 19.85  |
| White Cowpeas | 4430   | 18.95  | 18.95  | 18.95  | 18.95  | 18.95  | 18.95  | 18.95  | 18.95  | 18.95  | 18.95  | 18.95  |

Evidenced by the wider distribution of potential calibration curves (Fig. 3). For Mg in particular, it is difficult to see how it can be quantified unless it is directly presented on the nose of the instrument under vacuum conditions. Even the addition of 4 μm prolene in sample cups used adds variance to relationship between given and quantified concentrations. However, elements from S and up are resistant to changes in sample presentation (Fig. 3), primarily because of less absorption in the atmosphere or additional polymer thickness. Markov chain Monte Carlo regressions indicate that the most reliable analytical conditions for light elements are under internal vacuum conditions with no prolene (e.g. direct contact with the nose of the instrument). This leads to less variance in the quantification results. The prolene barrier increases the error in quantification for light elements; however, it may still be possible to obtain accurate results, depending upon the concentration level.

As illustrated in Figs. 1 and 2, the counts per second for each element are affected by sample presentation. This is particularly important with regards to P, a sample with 4500 mg kg⁻¹ has a net count rate of 50 photons per second when not using a vacuum, while it has a count rate of 200+ photons per second using direct measurement. While both sample presentation methods may result in a high R² value for its calibration curves (in this example 0.95, Table 2), only the latter offers reliability from measurement to measurement if one is concerned with smaller differences, such as that between 635 and 695 mg kg⁻¹ in the apple mango and mango leave standards, respectively. When measured with a direct sample presentation on the surface of the pXRF, those two standards have net counts of 24 and 26 ncp, respectively. When these samples were presented in sample cups with a 4 μm prolene barrier, they had net counts of 20 and 20 ncp, respectively. Even though the calibration curve for P using both sample preparations methods had an R² value of 0.95, the difference in counts makes a difference in differentiating the two standards. For reliable and accurate analysis at the mg kg⁻¹ level for light elements such as Mg and P, the conditions of analysis (sample presentation and atmosphere) are important factors that should be carefully considered with portable equipment. Even high-powered instruments such as a TXRF unit will not provide satisfactory light element data unless atmosphere is controlled, although it is still possible to obtain accurate values for higher ranges of light elements.

Heavier elements, such as sulfur and potassium, are not as dependent upon the presence of a vacuum as they experience less attenuation from the air. An important point worth consideration is the relative value of direct sample presentation versus sample cups. Because of the attenuation of photons for light elements, lower limits of detections are possible for elements like Mg and P if measurements are taken directly on the surface of the instrument. This presents the interesting possibility that in situ analysis can be more, not less accurate, than traditional homogenization and placement in a sample cup. At the very least, it does not present error above and beyond what would be expected from standard laboratory measuring conditions. Nonetheless, the water content of living plants at this time precludes accurate quantification with standards, as water content will attenuate photons and can vary in plants.

This raises a point typically not considered in laboratory analysis but important with in situ analysis, the inhomogeneity of samples. Analysis of plants in situ will not be accurate in the same sense that ground prepared samples are. The user will be exposed to increased variation because of exposure to different tissue types, which can differ in elemental composition. For a dried, homogenized plant sample there is a direct relationship between the elemental weight contribution to the sample and the spectral manifestation of atomic
excitation. In an unprepared analysis of a leaf, different tissues can have very different compositions. For example, the stomata on the photosynthetic surface of a leaf may have higher concentrations of \( K \), as they are used to regulate closure in response to water shortage.\(^{[33]}\) When scanning plant materials directly, consideration must be given to the density of the matrix because the density of wood/bark would not be the same as maize or bean leaves.\(^{[5]}\) As X-rays can easily penetrate thinner plant samples such as leaves, clips can be used to thicken the leaf surfaces e.g. perhaps from five to six leaf layers if elements such as \( Fe \) are to be quantified. Finally, the structure of plants may include layering of tissues relative to the beam. For example, a waxy cuticle may attenuate and reduce sensitivity to elements such as \( Mg \). In these cases, it may be preferable to use semi-quantitative methods, such as counts per second, as are common in the analysis of art and other heritage objects. As seen in Table 1, the depth of analysis for each element in plant matter can be high, with water and sugar content being critical factors and this provides a strong reason, for the time being, to limit analysis to dried plants.

## Conclusion

For best detection of light elements (Mg–P), direct analysis on the surface of a pXRF provides data of highest sensitivity and accuracy (\( R^2 > 0.90 \)). The sensitivity and accuracy are not only dependent on the settings but also on the instrument’s make and model. Elements such as \( S \), \( K \), and \( Ca \) can be reliably measured (\( R^2 > 0.99 \), 0.97, and 0.93, respectively) without the aid of a vacuum, although lower detection limits may be compromised. The calibrations held up across a diverse range and complex matrices of plants (e.g. from mango pulp to maize leaves). Given the present results on the use of pXRF as a lab-based instrument to analyse specimens through sample cups, which negatively affects the measurements, there is potential for bringing the pXRF instrument to measure such samples in situ. Portable XRF instruments provide plant chemical data of sufficient accuracy for many agronomic, environmental, and nutritional applications to support decision-making in the field and will reduce the overall time and budget compared with use of conventional XRF investigation techniques off-site. The results obtained in the present

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### Table 4. Elemental content (in mg kg\(^{-1}\)) of the plant materials measured by portable X-ray fluorescence

|        | Na  | Mg  | Al  | P   | S   | K   | Ca  | Mn  | Fe  |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Apple Mango | 30  | 490 | 25  | 695 | 330 | 13205 | 565 | 10  | 40  |
| Brown Cowpeas | 30  | 2160| 85  | 4510| 2055| 14880 | 885 | 20  | 100 |
| Cowpeas Leaves | 60  | 8040| 2245| 4390| 3445| 22745 | 28330 | 140 | 2350 |
| Croton Leaves | 20  | 3470| 180 | 2070| 2440| 28970 | 11285 | 805 | 240 |
| Cyprus Bark | 10  | 490 | 25  | 250 | 200 | 1815 | 1980 | 5   | 15  |
| Kent Mangoes | 5   | 480 | 15  | 600 | 280 | 8615 | 470 | 30  | 60  |
| Mahogany | <LLD | 415 | 30  | 20  | 170 | 220 | 3390 | <LLD | 10  |
| Maize Leaves | 85  | 1720| 315 | 1690| 2145| 17935 | 4655 | 105 | 320 |
| Maize Stem | 10  | 1425| 50  | 1070| 460 | 16400 | 1310 | 20  | 105 |
| Mango Leaves | 40  | 900 | 200 | 635 | 1730| 7605 | 17390 | 1140 | 185 |
| NIST 1515 | 25  | 2710| 290 | 1590| 1800| 16100 | 15260 | 55  | 80  |
| NIST 1547 | 30  | 4075| 210 | 1300| 1780| 23935 | 15035 | 95  | 175 |
| NIST 1568a | 10  | 465 | 10  | 1435| 1200| 1260 | 110 | 20  | 5   |
| NIST 1573a | 110 | 10130| 400 | 1985| 10345| 26355 | 52740 | 230 | 290 |
| NIST 1575 | 55  | 965 | 500 | 1040| 850 | 3965 | 2395 | 465 | 40  |
| Prunus Bark | 30  | 1510| 290 | 315 | 260 | 3015 | 25770 | 15  | 155 |
| Prunus Leaves* | 20  | 4430| 175 | 1210| 1215| 10045 | 16945 | 45  | 310 |
| White Cowpeas* | 15  | 1985| 205 | 3490| 1735| 13295 | 1185 | 65  | 270 |

<LLD = less than the lower limit of detection; * Used for validation

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Figure 3. Markov chain Monte Carlo simulations of calibration validation curves for different sample treatments. The x-axis shows the range of possible slopes given the error in each calibration model; the y-axis shows the density of the distribution. A slope of one indicates a 1:1 correspondence between X-ray fluorescence quantified and given values the width of the density indicates the range of possible results.
study for the various plant materials tested were within the typical range of element concentrations encountered in agronomic applications. Agronomists could take the instruments to the field and relate the range of element concentrations encountered in agronomic applications for the various plant materials tested were within the typical sources of nutrient deficiency or toxicity.

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