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Assessment of monochromatic X-ray fluorescence spectrometry as a reliable analytical technique for cadmium quantification in cacao systems

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ABSTRACT

Cadmium in cacao is a key barrier to Colombian exports. Therefore, emergent technologies need to improve Cd testing capacity for cacao. Monochromatic X-ray fluorescence (MXRF) is a cost-effective alternative to conventional techniques such as inductively coupled plasma (ICP-OES/MS) for Cd quantification. MXRF can be applied to solid or liquid samples of cacao farming systems. Although this new technique has demonstrated high accuracy, it has not been validated against major analytical parameters to date. Therefore, the aim of this study was to demonstrate MXRF suitability for cacao and liquid culture samples. The method was validated using soil, soil litter, root, stem, leaf, pod, nib, shell, and Luria-Bertani microbial culture medium. The robustness, limit of quantification (LOQ), trueness, repeatability, intermediate precision, and reproducibility of Cd testing were assessed. Cd correlations between MXRF and ICP was performed using 363 field samples from cacao. The statistical analysis showed a Pearson correlation coefficient between 0.96 and 0.99 and a recovery percentage between 74.8 and 129.9 % for MXRF in comparison to ICP. The robustness parameter identified appropriate laboratory and field pretreatments. Likewise, the choice of measurement time of 60 and 200 s was critical. The LOQ ranged from 0.124 to 0.148 mg kg^{-1}. For the parameter trueness, the % R ranged from 90.7 % to 109.0 %. The Z'-scores for reproducibility ranged from −0.50 to 0.62. The validation demonstrated the suitability of MXRF for Cd testing in cacao systems and liquid media. The impact of this new technology on cacao and food safety is discussed.

1. Introduction

Cadmium is a highly toxic element for all living systems [1] except for tolerant bacteria [2]. The presence of this element is closely related to the species, and cacao (*Theobroma cacao* L.) has shown a high predisposition for Cd bioaccumulation [3]. International regulations set maximum permissible concentrations of Cd in cacao products and derivatives between 0.10 and 0.80 mg kg^{-1} [4]. There is no clear regulation for maximum cadmium contents in agricultural soils in Colombia, probably due to its natural occurrence. However, in previous studies carried out in soils from different departments of Colombia, mean values between 0.40 and 2.83 mg kg^{-1} were found [5].

The need to implement alternative sensitive techniques for Cd testing in solid and especially in liquid samples is highlighted. These comprise samples from the cacao system (e.g., cacao growing soils, soil-litter [6], root, stem, leaf, pod, shell, nibs, as the main) or liquid media to culture cadmium-tolerant bacteria (CdtB); Using inductively coupled plasma (ICP) techniques requires an extensive digestion process for liquid samples. This occurs for both solid samples and complex selective culture media, such as Schlegel and Mergaey, commonly used to isolate oxalotrophic and cadmium-tolerant bacteria, respectively [7,8]. Even though the binding capacity of oligo-elements present in these culture media with Cd is not well explored, the pursuit of immobilising Cd with ICP techniques for quantification is challenging.

Therefore, new techniques are needed that can compete with the sensitivity of conventional techniques, such as inductively coupled plasma-mass spectrometry (ICP-MS). Nonetheless, the most used reagents for ICP analysis are ultra-pure gases such as argon. This gas is used to generate plasma in ICP analysis, a non-renewable resource [9]. Interestingly, a laboratory performing cadmium analyses may consume, on average, 164 L of liquid argon per month, suggesting a significant amount of non-renewable resource economy when quantifying using

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MXRF [10]. Therefore, argon consumption has been reported several times as a challenge faced by ICP techniques [11], and as a serious disadvantage of ICP techniques compared to other analytical techniques, such as MXRF [12].

In this regard, monochromatic Energy-Dispersive (ED) X-ray fluorescence (MXRF) is a cheaper analytical technique than ICP, based on the cost of the instrument, reagents, and consumables. MXRF has shown good performance in terms of precision, limits of quantification, and correlation with ICP-MS in soil and cacao nibs [13,14]. However, validation of the analytical method is required to demonstrate the reliability of the results.

The physical nature of X-ray fluorescence (XRF) allows for exceptional advantages over conventional Cd analysis techniques, such as its portability [15] and non-destructive characteristics. Currently, MXRF-based technologies are increasing in popularity due to their versatility and advantages in terms of speed, cost, user-friendliness, and portability [16]. Moreover, another advantage found in the MXRF technique over conventional techniques is the increase in environmental sustainability represented by the elimination of acid digestion in the quantification process. Advances in optics and X-ray technology have allowed the signal-to-noise ratio in fluorescence spectra to increase significantly [17]. This has led to increased sensitivity and lower quantification limits.

The use of portable XRF instruments enables measurements with minimal sample pretreatment, as simple as grinding with a mortar and pestle, obtaining data in real-time as an alternative to the acid digestion process that involves ICP quantification [16]. However, to generate reliable measurements when using portable technologies in the cacao system, i.e., in the field, the quality of the results must be assured. For this, it is necessary to ensure that the applied pretreatments generate samples with adequate homogeneity and that the methodology applied is robust and suitable for all sample types involved.

The Emax equipment uses monochromatic X-ray fluorescence (XRF) technology to offer certified simultaneous measurement of other heavy metals, such as arsenic (As), lead (Pb), copper (Cu) and nickel (Ni). However, quantifying transition metals such as Fe, Zn, and Mn may requires adjusting the original calibration of the equipment. In addition, the quantification limits reported by the supplier for each analyte of interest should be thoroughly reviewed and validated with the main metrological parameters [14]. Interestingly, a recent study [18] have demonstrated the feasibility of the multi-element quantification of Pb, Cd, Hg, As, Mn, Ni, Cu, and Zn in food samples using an ED-XRF (Energy Dispersive X-Ray Fluorescence) single device. The quantified values have been found to align well with those reported for a certified reference material.

To date, there is some literature [13] in regards of the development and validation of a method to quantify Cd in soil and cacao using MXRF. However, there are no publications performing an in-deep study quantifying Cd with MXRF taking into account soils, all parts of the cacao system (soil litter, roots, stems, leaves, pod, shell and nib), and integrating a liquid culture medium for cadmium tolerant bacteria, as part of the system. Therefore, the current study aimed to evaluate, through analytical validation, the performance of MXRF for measuring Cd in soil, soil litter, root, stem, leaf, pod, shell, nibs, and microbial culture medium. This work contributes to implementing low-cost techniques in the process of monitoring Cd levels in the cacao system, providing the cacao sector with a decision-making tool to mitigate the presence of this contaminant in their products.

2. Methods

2.1. Soil and cacao plant tissue samples

All soil samples and cacao plant tissue samples used in this work were of Colombian origin, and except for those used in the robustness study, all samples were extracted from a bank of samples held in custody between 2022 and 2024 by the analytical chemistry laboratory of the Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA. 335 samples of the department of Arauca were selected, including the municipalities of Arauca, Arauquita, Fortul, Saravena, and Tame. The sample selection criterion was the Cd concentration previously quantified by ICP-MS or ICP-OES (inductively coupled plasma-optical emission spectrometry). In addition, 44 samples were selected from other departments, including Antioquia, Boyacá, Casanare, Córdoba, Cundinamarca, Guaviare, Huila, Norte de Santander, Putumayo, and Santander. The aim of selecting samples from Arauca and other departments was to assemble a sample set that included all possible cacao plant tissue covering a wide Cd concentration range. Furthermore, it is worth mentioning that root samples were collected at the C.I. La Suiza Research Centre of AGROSAVIA in Santander. Those samples coming from a seedling experiment performed there. Moreover, these samples were strategically selected for their high Cd content to guarantee the reliability of the MXRF analytical measurement over a wide range of concentrations.

2.2. Certified reference materials (CRM)

The certified reference materials (CRM) SRM® 1570a (spinach leaves), SRM® 1573a (tomato leaves), SRM® 2384 (baking chocolate), SRM® 2709a (San Joaquin soil), SRM® 2710a (Montana I soil), and SRM® 2586 (soil) were obtained from the National Institute of Standards and Technology (NIST, USA). Additionally, the CRMs BCR® 679 (white cabbage), BD513 (cacao), ERM®-BD514 (cacao), and ERM®-BD515 (cacao) were acquired from the Institute for Reference Materials and Measurements of the Joint Research Centre of the European Commission (IRMM-JRC, Belgium). For LB liquid bacterial culture media samples, the standard of Cd CRM-NIST was applied. The CRM standard of Cd for LB used was a NIST- based on $Cd(NO_3)_2$ diluted in HNO_3 3 % *w/w* (Certipur® SRM, Merck, Darmstadt, Germany). The starting concentration was 1000 mg L^{-1}. This CRM is accredited by the norm ISO 17034:2016.

2.3. Microbial culture medium samples

Liquid culture is important for analysing the cadmium immobilisation capacity of cadmium-tolerant bacteria (CdtB). Thus, in this study, we used the Luria-Bertani (LB) liquid medium [23] as the primary medium for Cd testing to analyse the Cd immobilisation capacity of CdtB. To do so, we spiked the LB with three concentrations of Cd, using the standard NIST-traceable (Certipur® SRM). The starting concentration was 1000 mg L^{-1}. Then, three concentrations of Cd were spiked into separate liquid solutions to achieve the wide concentration range required for this study. The LB medium was prepared in 1 L of distilled water with sodium chloride (5 g), yeast extract (5 g), and tryptone (10 g) [19]. Using the starting concentration, the LB was spiked with 1, 30 and 100 μg L^{-1} for Cd testing using MXRF, labelled as M1 – M3, respectively. Unlike the solid samples of the cacao system, the culture medium samples were not subjected to any pretreatment prior to analysis by MXRF or ICP.

2.4. Pretreatment of soil and cacao samples

The samples used from the sample bank of the analytical chemistry laboratory underwent the following full lab processing pretreatment: Soil samples were dried at 39 ± 1 °C (Binder FED 720, Germany) for 48 h and subsequent grinding (Condux, 1167, Germany) and sieving to a diameter of 0.5 mm. All other samples of the cacao system underwent a drying process at 70 ± 5 °C (Tecnal TE-394/5 MP, Brazil) for 48 h, grinding (KitchenAid, USA, for nibs and Retsch SM 300, Germany, for other plant samples), and sieving to a diameter of 0.5 mm.

2.5. MXRF Cd quantification

A *Z*-spec MXRF system (*E*-max, USA) with a 30 keV excitation beam was used [18]. The system uses an X-ray tube at 40 W, as the primary radiation source, which is monochromate in a doubly curved crystal (DCC) before irradiating the sample. The resulting fluorescence is collected in a silicon drift detector [20]. The assembly of the system is covered by several patents registered by the manufacturer [21,22]. Cd concentrations were recorded automatically from the HD data viewer software (version 2e14f5f) from the default calibrations in the instrument, which were set by the supplier using CRM. The calibration of the instrument in 'plant mode' ($y = 2012.02\ x + 40.09$) was used for all plant material samples (soil litter, root, stem, leaf, pod, shell and cacao nibs). The 'soil mode' calibration ($y = 2813.13\ x + 35.59$) was used for soil samples, and the' water mode' calibration ($y = 3157.76\ x + 156.9$) was used for liquid samples. In these cases, y represents the counts per second (CPS), and x denotes the concentration. These equations stem from the linear relationship between the instrumental response (CPS) and the concentrations of the CRM quantified by the supplier. The resulting calibration curves for each type of sample are then generated and stored accordingly. It is worth to mention, that Cd results for plant material samples were expressed on a fresh basis (dry samples were corrected for the percentage of moisture removed at 70 °C). This was done with the aim of aligning with the guidelines established by the Food and Agriculture Organization of the United Nations [23], which state that: 'Maximum limits should in general preferably be expressed as a level of the contaminant related to the product as it is, on a fresh weight basis'. The soil results were expressed on a dry basis (105 °C). The samples were introduced into the equipment using a sample holder sealed with a disposable 12 μm polypropylene film. For solid samples, a sample amount < 1 g was placed in the sample holder and compressed manually with the help of a plunger [14,20]. A ~ 2 mL sample holder was used for liquid samples, allowing the insertion of a syringe through which the liquid sample was injected into the sample holder.

2.6. ICP-OES and ICP-MS Cd quantification

For sample digestion, 65 % *w*/w HNO_3, 30 % w/w H_2O_2, and 37 % w/w HCl (Merck, Germany) were used. The water employed was treated in a Milli-Q water deioniser (Merck Millipore, USA), and the HNO_3 was purified in an acid distillation system (Berghof, Germany). Microwave-assisted digestion (Speedwave Xpert, Berghof, Germany) of plant material samples (0.4 g) was carried out with HNO_3:H_2O_2 (6:3) according to the study of [24]. Briefly, i. in 10 min, the temperature of the container rose to 160 °C with a pressure of 30 bar; the conditions were maintained for 15 min, ii. in 5 min, the temperature increased to 180 °C with a pressure of 35 bar; the conditions were supported for 15 min, iii. in 1 min, the temperature decreased to 50 °C with a pressure of 25 bar; the conditions were provided for 10 min. For soil, (0.5 g) was digested with HNO_3:HCl (6:2) and the digestion ramp suggested by the manufacturer [25]. Briefly, i. in 2 min, the temperature of the container rose to 180 °C with a pressure of 30 bar; the conditions were maintained for 25 min, ii. in 1 min, the temperature decreased to 50 °C with a pressure of 25 bar. The conditions were provided for 30 min. The LB culture medium (2 mL) was digested with 6 mL of HNO_3 on a heating plate at five successive temperatures: T1 = 80 °C for 30 min, T2 = 120 °C for 90 min, T3 = 150 °C for 10 min, T4 = 160 °C for 10 min, and T5 = 170 °C to until white fumes were observed. The digested samples were brought to a final volume of 25 mL. The conditions of use and tuning of the ICP-OES (Thermo Scientific iCAP 6500, USA) and ICP-MS (Agilent Technologies 7900, USA) equipment are described in the work of [26]. Forty-five soil samples plus all plant tissue samples were quantified by ICP-MS (Table S1). The remaining soil samples were quantified by ICP-OES. All ICP methods applied were previously developed in accordance with the guidelines established by ISO/IEC 17025:2017. The lower-bound precision approach [30] was used to calculate the limits of quantification (LOQ) for the ICP-OES / MS methods. To this, twelve blanks were digested and analysed. The blank for the soil samples were HNO_3:HCl (6:2) without the sample. Likewise, the blank for the plant sample type was HNO_3:H_2O_2 (6:3) without the sample. The blank for the LB culture media was HNO_3 without the sample. The LOQ was expressed as 10 × SD, where SD is the standard deviation of the 12 measurements.

2.7. Method validation

To demonstrate the suitability of the analytical method to quantify Cd in the cacao system by MXRF, an analytical validation was performed according to the ICH Q2 (R2) guideline [27]. This internationally accepted guideline provides uniform indications on how to perform analytical validations and includes decisions of the European Community, US regulations, and Japanese technical regulations [27]. According to the ICH Q2 (R2) guide, the validation parameters applied to the proposed analytical method and were studied during this research were i. robustness, ii. limits of detection and quantification, iii. Repeatability and intermediate precision, iv. reproducibility, and v. trueness.

2.8. Robustness

Robustness was measured through the influence of sample pretreatment and measurement time. The measurement times considered were 60 s, 200 s, and 600 s The experimental design was applied independently for fresh soil, fresh cacao nib, and microbial culture media. Three soil samples (So1–So3), four cacao nib samples (Cn1–Cn4), and three microbial culture liquid media (M1–M3) were used for this assay. For the solid samples (soil and nibs), a 3^2 factorial completely randomised experimental design without blocks was conducted. Nine experiments were performed randomly and in triplicate, with different 'sample pretreatment' factor levels for soil and cacao nib samples.

Three levels were selected for the 'sample pretreatment' factor for the soil samples. The levels consisted of i. full lab processing pretreatment, as described above, with oven drying (39 ± 1 °C) for 48 h, grinding and sieving until a diameter of 0.5 mm, ii. field pretreatment where samples were dried at room temperature for five days, crushed with a mortar and pestle and were not sieved, iii. in situ pretreatment where samples were not dried, only manually disaggregated, and were not sieved.

Three levels were selected for the 'sample pretreatment' factor for the cacao nibs samples. The levels consisted of i. full lab processing pretreatment, as described above, with oven drying (70 ± 5 °C) for 48 h, grinding and sieving to a diameter of 0.5 mm, ii. partial processing, where samples were frozen with liquid nitrogen and ground in that state, and no sieving was performed, and iii. Field pretreatment, where samples were not dried or frozen; these were crushed with a mortar and pestle, and no sieving was performed.

Three levels were selected for the microbial liquid culture media samples, using a completely randomised design 3^1 without blocks for the 'measurement time' factor. The levels consisted of i. 60 s, ii. 200 s, and iii. 600 s. These same levels were applied to the 'measurement time' factor in the soil and nib samples.

2.9. Limits of detection (LOD) and limits of quantification (LOQ)

The lower-bound precision approach [27] was used; 10 replicates of low Cd concentration in soil, nibs, and microbial liquid culture media samples were quantified for this approach. The LOD and LOQ were estimated as 3 × SD and 10 × SD, respectively, where SD is the standard deviation. The LOQ reported for each sample type was subsequently verified by measuring ($n = 10$) a sample of each type close to the estimated LOQ concentration. The coefficient of variation (CV = 100 × SD / mean) and the percentage recovery (% R = 100 × quantified value/ reference value) with respect to the ICP-MS were used to evaluate the verified LOQ [28].

2.10. Repeatability and intermediate precision

The precision of the method was studied under repeatability and intermediate precision conditions. Three samples of increasing concentration were selected for soil (So4, So5, and So6), nibs (Cn5, Cn6, and Cn7), and microbial culture media samples (M4, M5, and M6). Samples S6 and C7 corresponded to the highest Cd levels quantified in the analytical chemistry laboratory of AGROSAVIA for each sample type. The repeatability of the method was evaluated by measuring 10 replicates of the samples under the same analytical conditions, i.e., the same day and by the same analyst. Intermediate precision was assessed by measuring 10 replicates of the samples under three intermediate precision conditions (total, $n = 30$). The results were evaluated in terms of CV [28].

2.11. Reproducibility

Reproducibility-level precision was assessed through two proficiency tests (PTs) accredited by the regulation ISO 17043:2010. i. For soil, participation was in round 2024.2 of the PT titled 'International Soil-Analytical Exchange (ISE)'. This PT was obtained from the provider WEPAL (Wageningen Evaluation Programs for Analytical Laboratories, Wageningen, Netherlands) and consisted of two samples labelled 'ISE 849' and 'ISE 856'. ii. For cacao, participation was in round 341 of the PT titled 'CT-718'. This PT was obtained from the provider LGC Standards (London, UK) and implied a single sample of cocoa powder. The *Z*-score (Z = (Measured value – Assigned value) / Total error) was the evaluation criterion used by both providers to assess the performance of the PT results. A |Z| score < 2.00 indicated satisfactory performance in the proficiency test.

2.12. Trueness and uncertainty

The trueness in soil and plant tissue was assessed using CRM of soil, cocoa powder, and leaves specified previously in the CRM section. These materials were quantified ($n = 6$ replicates) by MXRF. Trueness was assessed as a function of percentage of recovery (% R) [28]. For liquid samples, three samples of microbial culture media of known Cd concentration (M7, M8, and M9) were spiked (n = 6 replicates) with Cd standards of similar concentration. In addition, twelve liquid standards ($n = 3$ replicates) of known concentration (0.2, 0.5, 1, 5, 10, 25, 50, 100, 250, 500, 750 and 1000 mg L^{-1}) were quantified. In both cases, the % R was assessed.

The bottom-up approach combined the contributions of individual sources of uncertainty through the law of propagation of uncertainty [29]. According to this approach, combining individual sources was performed using the Equation 1interpreted from [32]'s procedure as follows:

$$U(x) = \sqrt{u(x)_{CRM} + u(x)_{moisture} + u(x)_{precision}} \times k$$

Where, $u(x)_{CRM}$ represents the uncertainty of the bias in the CRM,

$u(x)_{moisture}$ represents the uncertainty of the correction of the result for moisture, and,

$u(x)_{precision}$ corresponds to the uncertainty of the precision method. The expanded uncertainty was expressed with a coverage factor of k = 2.

2.13. Statistical analysis

To analyse the results of the experiments designed to assess method robustness, using the influence of sample pretreatment and measurement time, analyses of variance (ANOVA) were performed on the results of the designs. The assumptions of the ANOVA were checked using the Shapiro-Wilk and Levene tests. The comparison between the MXRF and ICP techniques was assessed using a Pearson test. All statistical tests were performed at a 95 % confidence level using the RStudio software version 2024.04.2. A total of 363 samples previously digested and quantified by ICP (average of three replicate readings) from the cacao system (100 soil, 100 soil litter, 12 root, 10 stem, 10 leaf, 20 pod, 10 shell, and 101 nibs samples) were used to construct correlograms with the MXRF technique (average of two replicate readings). The slope of the correlation plots and the % R were used as criteria to evaluate the similarity of the two analytical techniques, using the concentration measured by ICP as a reference. The traceability of the ICP quantifications was ensured through previously characterized internal reference materials (IPE 907: plant material, and IRM 172: soil). Similarly, the traceability of the MXRF measurements was established through the quantification of CRM in solid samples and control points in liquid samples. Furthermore, all the statistical plots were performed using QtiPlot version 2025 5.12.8. The sketches were edited using the vector graphics editor and the design program package of Adobe Illustrator (Ai) version 29.3.1, 2025.

3. Results and discussion

3.1. Validation of the analytical method

As first result, the LOQs for Cd in this study were lower than the real Cd concentration values reported in Colombian soils in a previous study, with mean values between 0.40 and 2.83 mg kg^{-1} Cd [5]. The assessing of the performance of each parameter of validation is detailed below.

3.2. Robustness

For the soil and cacao nib samples, the full lab processing pretreatment (drying, grinding, and sieving) showed a higher homogeneity (average CV of 5.3 %) than the other pretreatments (average CV of 7.6 %). The summarised results of the experimental design are shown in Table 1. The homogeneity of the samples related to the pretreatment process of the solid samples could be ordered as follows: for soils: full lab processing > field > in situ, for nib full lab processing > partial processing ≈ field. However, the CV obtained for the three replicates of each treatment were lower than the CV values required by the validation guidelines. According to these guidelines, CV between 9.7 and 13.5 % are considered acceptable for analyses performed under repeatability conditions (with at least seven replicates) at the concentration levels studied. This means that, although the treatments involving full lab processing pretreatment showed lower variability, any of the treatments could potentially meet the required repeatability criteria. This is based on the common observation that CV tend to decrease as the number of replicates increases [28]. This implies that, although the data generated by the in full lab processing pretreatment are more precise, any pretreatment can be used to quantify Cd by MXRF.

Similarly, when analysing the experimental design results related to the measurement time in the soil and nib samples, a higher variability of the results was found when the time of 60 s was used (average CV of 8.9 %). The 200 and 600 s times showed greater precision (average CV of 5.6 and 6.0 %, respectively). Nevertheless, the variability found in each measurement time was lower than required by the validation guidelines [28]. Thus, any of the three measurement times can be used for Cd quantification by MXRF.

The ANOVA of the experimental design results applied to soil and cacao nib samples indicated that using any of the three pretreatments evaluated had no significant effect ($p > 0.05$) on Cd quantification by MXRF. Similarly, varying the measurement time between 60 and 600 s on the solid samples also showed no significant effect (p > 0.05).

The behaviour registered in the solid samples was not observed for the LB medium when the measurement time was altered. The summarised results of the experimental design are shown in Table 1. In this case, the findings were diverse, and in some cases, the results with the 60 s measurement time were more precise than those with 200 and 600 s. The CVs obtained in this sample type were, on average, lower (1.9 %)

Table 1
Cd content found (mean ± standard deviation of three replicates) in the treatments of the experimental design used to evaluate the robustness of the method for Cd quantification by MXRF.

Treatment	Pretreatment*	Measure time	Cd in soil [mg kg^{-1}]			Cd in nibs [mg kg^{-1}]				Cd in liquid medium LB [mg L^{-1}]		
			So1	So2	So3	Cn1	Cn2	Cn3	Cn4	M1	M2	M3
1	Full lab processing	60 s	0.222 ± 0.024	0.984 ± 0.074	1.09 ± 0.05	0.335 ± 0.021	0.444 ± 0.022	0.499 ± 0.009	1.74 ± 0.05	0.809 ± 0.025	29.89 ± 0.36	102.5 ± 0.7
2		200 s	0.207 ± 0.003	1.03 ± 0.06	1.08 ± 0.03	0.330 ± 0.005	0.459 ± 0.022	0.486 ± 0.021	1.78 ± 0.07	0.800 ± 0.040	29.59 ± 0.53	102.5 ± 0.1
3		600 s	0.214 ± 0.019	0.989 ± 0.053	1.14 ± 0.02	0.346 ± 0.049	0.449 ± 0.045	0.482 ± 0.021	1.72 ± 0.06	0.825 ± 0.025	30.40 ± 0.25	102.9 ± 1.2
4	In situ (soils) /partial processing (nibs)	60 s	0.209 ± 0.062	0.953 ± 0.058	1.07 ± 0.09	0.346 ± 0.041	0.419 ± 0.015	0.489 ± 0.072	1.71 ± 0.06			
5		200 s	0.204 ± 0.038	1.00 ± 0.08	1.04 ± 0.07	0.335 ± 0.011	0.417 ± 0.021	0.495 ± 0.029	1.84 ± 0.09			
6		600 s	0.221 ± 0.022	1.03 ± 0.03	1.10 ± 0.01	0.321 ± 0.010	0.449 ± 0.006	0.490 ± 0.017	1.71 ± 0.01			
7	Field	60 s	0.202 ± 0.044	1.01 ± 0.09	1.11 ± 0.04	0.356 ± 0.041	0.461 ± 0.034	0.489 ± 0.057	1.86 ± 0.11	ND		
8		200 s	0.203 ± 0.029	0.961 ± 0.047	1.07 ± 0.04	0.335 ± 0.030	0.472 ± 0.030	0.484 ± 0.003	1.81 ± 0.02			
9		600 s	0.214 ± 0.030	0.972 ± 0.094	1.09 ± 0.04	0.311 ± 0.014	0.442 ± 0.051	0.489 ± 0.060	1.80 ± 0.01			

Abbreviations: So = Soil samples; Cn = Cacao nibs samples; M = Luria - Bertani liquid medium samples. ND = No Data.
* The pretreatment information is not applicable to the liquid medium LB samples.

than in the solid samples. However, as with the solid samples, no significant effect of measurement time ($p > 0.05$) was observed in the Cd quantified by MXRF for the microbial culture medium samples.

The higher homogeneity found in the full lab processing pretreatment in soil and nib samples was attributed to the sieving step performed only for this pretreatment. The results agree with those reported by other authors using XRF techniques [30,31]. In these studies, a reduction in the variability of the results of different elements was observed by increasing the homogeneity of the sample. The importance of producing homogeneous samples and flat surfaces has also been highlighted to obtain representative results when using XRF [32]. A particle size <0.5 mm, as used in the full lab processing pretreatment, is preferable in XRF measurement, especially when analysing light elements such as Na, Mg, and S [32].

The decrease in precision observed when using the 60 s measurement time in soil and nib samples is consistent with the findings of the study by [33]. These authors reported that increasing the measurement time significantly increased the count rates. This may be because the X-ray emission from the source does not fully excite the analyte in the sample in a short time, or the detector does not collect all the fluorescent radiation emitted by the sample [34]. This behaviour was not observed in the analysis of the LB medium, which is explained by a greater homogeneity in the liquids, even though its chemical composition is not complex enough to compare with selective media, such as the Schlegel and Mergeay media [35,36], used to isolate oxalotrophic and cadmium-tolerant bacteria, respectively. This is relevant, as classical spectrometric techniques require an additional acid digestion step for accurate quantification due to microbial activity. Therefore, the novel MXRF technology is presented in the current study as a faster technique for analysing Cd immobilisation activity in LB liquid medium for CdtB-tolerant bacteria.

The importance of the results of the robustness study lies in the fact that the equipment can be taken out of the laboratory, i.e., it is portable, in contrast to the equipment necessary for a thorough pretreatment of the sample that may not be available, and yet reliable results can be obtained using a simple pretreatment such as maceration with a mortar and pestle. Hence, the analytical method was validated with a measurement time of 200 s and a full lab processing pretreatment for solid samples to ensure the highest analytical precision. However, there is an exception in the LOD, LOQ, repeatability, and intermediate precision sections, where a measurement time of 60 s was good enough for comparative purposes.

3.3. Limits of detection (LOD), limit of quantification (LOQ), repeatability and intermediate precision

The LOD and LOQ found for the soil, nibs, and LB medium were similar (Table 2). The highest value of the verified LOQ (0.148 mg kg^{-1}) is still considered the reported limit to treat a single value from the sample type indiscriminately (Table 2). The verification test showed an adequate CV and % R for the LOQ, as required by international regulations for these characteristics [28]. For both soils, nibs and liquid medium, the CVs were below 10 %. The lowest variability was present in nibs where the CV was 7.35 %, however, this same sample type presented the lowest % R with a value of 89.6 %.

Decreasing the measurement time from 200 to 60 s showed a significant increase in the LOQ (from 0.148 mg kg^{-1} to 0.285 mg kg^{-1}), which explains the variability obtained in the robustness studies for soil sample So1 (Table 1: Cd ~ 0.211 mg kg^{-1}). Thus, it is emphasised that reliable results are generated with a measurement time of only 60 s if concentrations higher than 0.285 mg kg^{-1} Cd are assessed.

Lower CVs than expected were found in the analysis of soil, cacao,

Table 2
Estimated limits of detection (LOD) and quantification (LOQ) for Cd measurement by MXRF in each sample type, along with their respective coefficient of variation and recovery percentage obtained during LOQ verification.

Cd in sample types***	Estimation		ICP-MS value	Verification		
	LOD [3 × SD]	LOQ [10 × SD]		MXRF [± SD] - Measured value	% R	CV [%]
Cd in soil [mg kg^{-1}]	0.037	0.124	0.124	0.136 ± 0.013	109.9	9.37
Cd in nibs [mg kg^{-1}]**	0.041	0.138	0.148	0.133 ± 0.010	89.6	7.35
Cd in liquid LB medium [mg L^{-1}]	0.040	0.133	0.135	0.138 ± 0.013	101.9	9.28

Abbreviations: CV = coefficient of variation. % R = recovery percentage. SD = Standard deviation.
* Cd concentration in cacao and liquid LB medium is expressed on a fresh weight basis, while in soil, it is expressed on a dry weight basis at 105 °C.
** The nib and soil samples used were subjected to a full lab processing pretreatment.

and microbial culture medium samples under repeatability and intermediate precision conditions [28] (Table 3). Data with enough precision was obtained, even when the measurement was performed using 60 s as the time of exposure (at time of exposure above 60 s, LOQ > 0.285 mg kg^{-1}) and even when the environmental analysis conditions were variable.

The LOQ obtained was similar to that reported by other authors [37–39], using the same technology (MXRF) for Cd quantification in various sample types (LOQ between 0.102 and 0.233 mg kg^{-1}). Other techniques associated with XRF have reported significantly higher LOQ [40–42] than those reported in this study, with values up to 32 mg kg^{-1} [42]. Thus, this work confirms the innovation of the MXRF technique to quantify Cd concentrations at trace levels. There are no previous studies in the literature where the Cd LOQ has been reported for microbial culture media or liquid solutions using the MXRF technique. Compared to ICP-OES and ICP-MS with an LOQ of 0.043 mg kg^{-1} and 0.004 mg kg^{-1}, respectively [26], the LOQ found was higher using MXRF, as shown in this study. Nonetheless, due to international regulations, the LOQ recorded by MXRF is also appropriate for studying Cd in the cacao system and liquid cultures.

Likewise, the repeatability and intermediate precision results were similar to those found by other authors using the same technology to measure Cd in other sample types [17,38]. This is the first time repeatability and intermediate precision results are presented for Cd quantification in cacao system samples by MXRF, including the LB microbial liquid culture medium.

Together with the robustness results, the intermediate precision results contribute to developing an analytically reliable measurement tool that can be used outside the laboratory. Some of the beneficiaries of this tool could be cacao bean concentration and storage centres (central collection centres) in high-producing departments of Colombia, such as Arauca [43]. This tool would allow producers in the cacao sector to monitor Cd levels prior to commercialisation. Therefore, they can make decisions to alleviate the presence of Cd. One strategy relies in blending cacao mass using low and medium Cd contents in cacao coming from different subregions of Colombia, to decrease Cd content into the final commercial products [44]. Besides, the bioremediation strategy is one of the most cost-effective techniques used for other crops, such as rice [45] and lettuce [46], in which the role of cadmium-tolerant bacteria (CdtB) has been emphasised [2,47]. However, in cacao, only a few studies have addressed the issue of cadmium (Cd) presence, either by performing bioprospection of CdtB [36] or by applying viable and culturable CdtB populations in field experiments in Colombia [48,49]. Further recent work on this subject in the Arauca region of Colombia is under review [48].

3.4. Reproducibility

In the proficiency test (PT) carried out for the soil samples (ISE 2024.2), concentrations of 0.290 and 0.858 mg kg^{-1} were reported for the evaluated samples labelled as 849 and 856, respectively. The value assigned by the PT supplier was 0.308 ± 0.039 mg kg^{-1} for sample labelled as 846 and 0.823 ± 0.087 mg kg^{-1} for sample number 856, resulting in a satisfactory performance with a Z'-score of −0.5 and 0.4 for these, respectively. Ten participants of the PT were presented in the round of this interlab test for the total Cd parameter, using quantification techniques such as XRF, ICP-MS, flame atomic absorption (AA), and graphite furnace AA, as well as acid digestion methods including mixtures such as $HClO_4$/ HNO_3/ HF and HNO_3/ HF (Fig. S1.A).

In the PT carried out on the cocoa powder (PT-CT-718), a concentration of 0.439 mg kg^{-1} was reported. The value assigned by the PT supplier was 0.414 ± 0.040 mg kg^{-1}, showing a satisfactory performance with a Z'-score of 0.6. Other PT participants obtained satisfactory results with different quantification techniques, such as AA, ICP-OES, and ICP-MS (Fig. S1·B).

The PT results reported for soil and cacao by MXRF demonstrate that the method is reproducible and comparable to conventional Cd analysis techniques. To date, no properly accredited PT following ISO 17043:2010 standards have been reported for cacao samples using the MXRF technique; therefore, these results are novel as they show via an in depth study for the validation of MXRF as an analytical technique in the cocoa system, not only soils or cocoa beans, but specifically in plant tissue material such as roots, stems, pods, husks and, as a novelty, liquid LB media for growing of soil cadmium-tolerant bacteria associated with the cacao-cadmium system.

3.5. Trueness and uncertainty

The MXRF method showed adequate trueness [28] for solid (Table S2) and liquid samples (Table S3 and Table S4), depending on the % R obtained, not only in the cacao system samples but also in other crops (e.g., tomato leaves, spinach, and cabbage). The % R obtained varied between 96.7 and 104.6 % in soil, 94.2 and 103.3 % in plant samples, and 90.2 and 108.0 % in liquid samples. The type of CRM used allowed a wide range of certified concentrations from 0.371 to 12.30 mg kg^{-1} for soil, 0.073 to 2.876 mg kg^{-1} for plant samples, and 0.200 to 1000 mg L^{-1} for liquid samples.

The expanded uncertainty estimated for cadmium (Cd) measurements by MXRF was 22.1 % for soil samples and 19.5 % for plant samples. Three main features were considered in the estimation: the use of certified CRM, moisture content, and method precision. The feature

Table 3
Coefficient of variation obtained from the analysis by MXRF of samples with different Cd concentrations in soil, cacao, and LB liquid media under repeatability conditions (10 replicates) and intermediate precision (30 replicates).

Sample type	Conditions	Repeatability						Intermediate precision	
	Day & analyst	Day 1, Analyst 1		Day 2, Analyst 2		Day 3, Analyst 2		Three days, two analysts	
	Temperature	19.7–23.0 °C		27.5–28.5 °C		19.2–20.8 °C		19.2–28.5 °C	
	Relative moisture	64.6–66.1 %		49.9–53.8 %		57.4–64.8 %		49.9–66.1 %	
	Measure time	200 s		60 s		60 s		60–200 s	
	Sample	Cd [mg kg^{-1}]	CV (%)	Cd [mg kg^{-1}]	CV (%)	Cd [mg kg^{-1}]	CV (%)	Cd [mg kg^{-1}]	CV [%]
Cacao	Cn5	1.124 ± 0.088	7.83	1.304 ± 0.067	5.14	1.296 ± 0.056	4.32	1.241 ± 0.109	8.78
	Cn6	3.854 ± 0.289	7.5	3.992 ± 0.157	3.93	4.091 ± 0.108	2.64	3.979 ± 0.217	5.45
	Cn7	19.13 ± 0.23	1.18	19.35 ± 0.22	1.16	19.33 ± 0.38	1.97	19.27 ± 0.29	1.5
Soil	So4	0.339 ± 0.027	7.96	0.346 ± 0.028	8.09	0.339 ± 0.045	13.27	0.341 ± 0.034	9.97
	So5	1.205 ± 0.072	5.98	1.202 ± 0.052	4.33	1.162 ± 0.068	5.85	1.190 ± 0.066	5.55
	So6	21.73 ± 0.56	2.58	22.07 ± 0.66	2.99	21.52 ± 0.37	1.72	21.77 ± 0.57	2.62
Microbial culture medium	M4	1.047 ± 0.012	1.15	1.119 ± 0.044	3.93	1.161 ± 0.059	5.08	1.109 ± 0.064	5.77
	M5	30.48 ± 0.20	0.66	29.84 ± 0.56	1.88	29.94 ± 0.35	1.17	30.09 ± 0.48	1.6
	M6	102.3 ± 0.5	0.49	101.5 ± 1.3	1.28	100.6 ± 1.8	1.79	101.4 ± 1.5	1.48

Abbreviations: CV = coefficient of variation; So = Soil samples; Cn = Cacao nibs samples; M = Luria - Bertani liquid medium samples. n = number of replicates.

with the greatest contribution was the CRM, classified as a 'type A' uncertainty source. It accounted for 82.5 % of the total uncertainty in soil samples and 70.77 % in plant samples. The estimated expanded uncertainty in the LB medium was the lowest among all matrices (2.3 %). In this study, the largest individual contributor to the total uncertainty was the precision component of the method, accounting for 85.1 % of the total.

Trueness results were similar to those found by other authors [16] using MXRF for Cd quantification in CRM (89–113 % R) and also similar to those found in previous work and studies by other authors (89.7–108.8 % R) using conventional (ICP) Cd analytical techniques [24,26,50]. No previous information has been presented regarding the trueness of MXRF for Cd quantification in microbial culture media. The expanded uncertainty of MXRF was higher than that reported (< 8 %) for Cd by ICP [28].

3.6. Cd quantification

The 363 samples quantified by the validated MXRF method and the reference ICP method (Table S1) covered a wide range of concentrations ranging from 0.08 to 310.00 mg kg^{-1} (Table 4). Most of the samples used, i.e., 91.2 %, were from Arauca, 7.4 % from Santander, 0.6 % from Huila, 0.6 % from Guaviare, and 0.3 % from Norte de Santander (Table 4). As a function of median and mean, Cd concentrations in the different sample-types increased in the following order: pod < leaf < soil < nibs < stem < shell < soil litter < root (see Table 4). The highest median concentrations were tested in the root samples, with a maximum value of 310 mg kg^{-1}. The LOQ for the ICP-MS method was 0.004 mg kg^{-1} in plant samples. For soil samples, the LOQ obtained were 0.005 mg kg^{-1} and 0.043 mg kg^{-1} for ICP-MS and ICP-OES, respectively. The analytical controls used during ICP quantification showed recovery results ranging from 80 to 110 % for the control points, and with concentrations falling within the established range for the internal reference material (mean ± 2 × standard deviation, $n = 30$). Similarly, the solid CRM analysed as controls in MXRF yielded recoveries ranging from 94.2 to 104.6 %. Finally, the liquid control points analysed by MXRF showed recoveries ranging from 90 to 110 %.

Arauca was the primary department of origin of the samples used in this study. This was due to the diversity of Cd concentrations in cacao and soil reported throughout the department. This characteristic could be reflected in the current study, where concentrations varied from 35-fold in nibs to 76-fold in soil, between the minimum and maximum values recorded (Table 4). Due to this variability, compliance with international regulations on maximum permissible Cd levels is a target that can be achieved in the department of Arauca through blending cacao beans [45]. To achieve this, it is critical to assess the Cd content in the production lots. This could be done with fast and lower-cost measurements, using the MXRF technology, playing a crucial role in the starting point of any mitigation strategy.

3.7. MXRF and ICP correlations

The results for Cd in soil measured by MXRF showed a high correlation (Fig. 1A and B) with those measured by ICP-MS (Pearson correlation coefficient of 0.9939 and determination coefficient of 0.988) and lower correlation with those measured by ICP-OES (Pearson correlation coefficient of 0.9349 and determination coefficient of 0.874). However, the % R (74.8 to 129.9) was greater than expected [28] (Table S1). Only 20 % of the results showed % R below 100 %, demonstrating the tendency of the MXRF technique to quantify Cd values above those obtained by acid digestion/ICP.

The results for Cd in plant samples measured by MXRF showed a higher correlation level (Pearson correlation coefficient between 0.9962 and 0.9990 and determination coefficient ranging from 0.992 and 0.997) with the ICP-MS technique used as a reference (Figs. 1C, 2 and 3). The slope defining the straight line characterising this correlation was within 8 % of the value of 1.00, which would define a correlation where the two variables are exactly equal. The following are the % R found for soil litter 86.6 to 109.6 %), root (91.1 to 108.1 %), stem (97 to 114.1 %), pod (90.3 to 109.2 %), and leaf (101.4 to 109.2 %). Besides, as shown in Fig. 3, the correlation was also high for nibs (90.0 to 109.3 %) and shell (93.0 to 126.1 %). All correlations were within the expected range in 97 % of the samples (Table S1). The highest % R value in the plant samples was found in shells (126.1 %), but this value corresponded to a sample concentration (0.080 mg kg^{-1}) below the LOQ. Except for these data, the highest % R in shells was 108.2 %, and in all plant samples, which is an expected value [28].

The high % R found in Cd quantification in soil was associated with

Table 4
Origin and descriptive statistics of Cd content quantified in the different sample types that make up the cacao system.

Sample type	Department	Municipality	n	Cd [mg kg^{-1}]			
				Minimum – Maximum	Median	Mean	SD
Soil	Arauca	Arauca	4	0.09–1.78	1.30	1.29	0.35
		Arauquita	56	0.18–2.57	1.21	1.24	0.50
		Fortul	7	0.17–1.88	0.53	0.71	0.57
		Saravena	23	0.42–2.31	1.08	1.09	0.46
		Tame	10	0.17–3.12	0.46	0.68	0.79
Soil litter		Arauquita	37	5.98–31.09	14.90	15.97	6.45
		Fortul	9	0.65–8.84	2.36	3.78	2.84
		Saravena	33	1.81–36	16.81	17.50	8.59
		Tame	21	1.94–15.56	6.10	6.24	3.16
Root	Santander	Rionegro - C.I. La Suiza Research Centre	12	0.13–310.00	28.23	77.18	103.79
Stem	Huila	Rivera	1	0.16–0.17	0.16	0.16	0.00
		Campoalegre	1	0.53–0.58	0.55	0.55	0.03
Leaf	Santander	Rionegro - C.I. La Suiza Research Centre	8	1.08–24.73	8.87	11.24	8.12
Pod	Arauca	Tame	10	0.25–1.24	0.47	0.56	0.28
		Saravena	20	0.09–1.82	0.37	0.50	0.44
Shell	Guaviare	San José del Guaviare	1	0.08–0.10	0.09	0.09	0.01
		El Retorno	1	0.14–0.14	0.14	0.14	0.00
	Norte de Santander	Convención	1	5.15–5.53	5.34	5.24	0.27
	Santander	Rionegro - C.I. La Suiza Research Centre	7	1.63–28.33	14.19	13.28	9.11
Nibs	Arauca	Arauquita	22	0.59–6.86	3.47	3.72	1.57
		Fortul	6	0.09–4.61	0.88	1.52	1.62
		Saravena	42	0.28–6.85	2.14	2.41	1.68
		Tame	31	0.22–5.51	1.11	1.34	1.03

Abbreviation: SD = Standard deviation; n: number of samples.

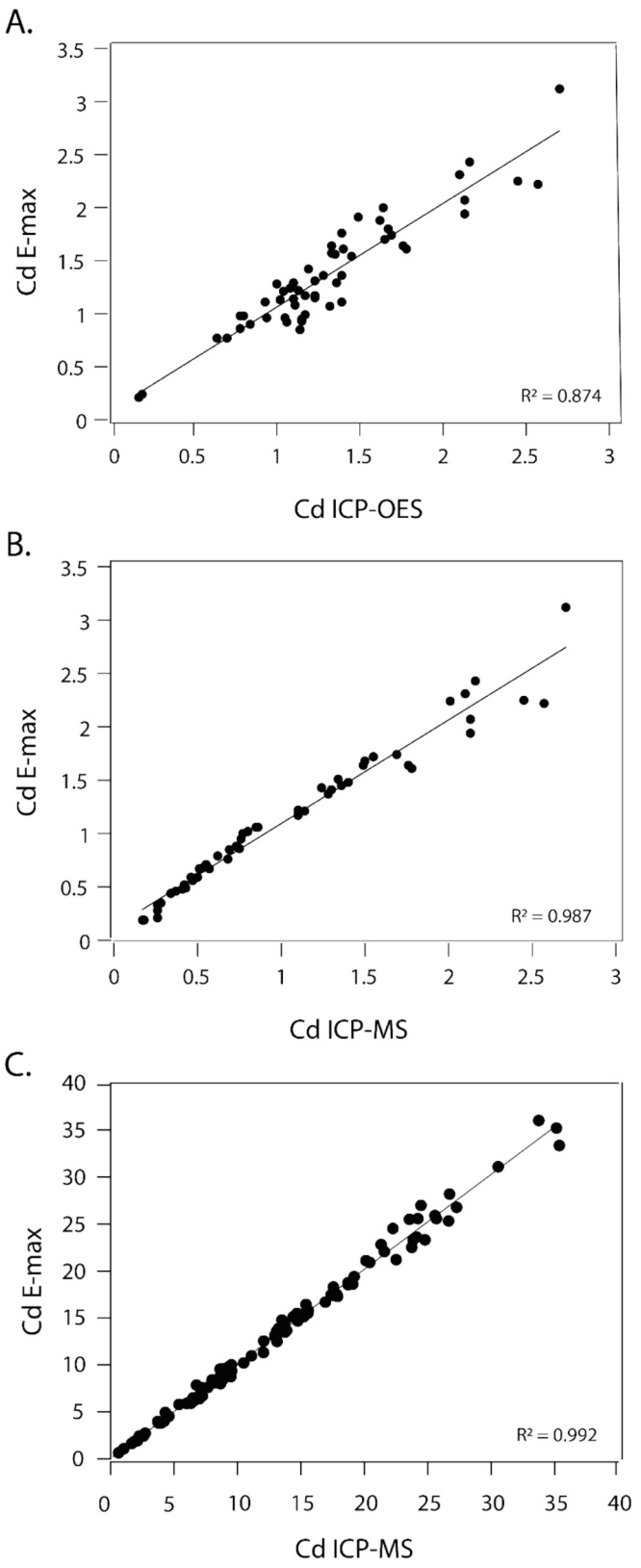

Fig. 1. Correlation between MXRF and ICP concerning Cd quantification in A. soil (n = 111), using ICP-OES; B. soil (n = 90), using ICP-MS; and C. soil litter (n = 100) of cacao crop using ICP-MS. Cd concentration is expressed in mg kg^{-1}. R^2 represents the coefficient of determination.

the acid digestion used in this research, which did not use HF to solubilise Cd bound to soil silicates [50], so the Cd values reported by ICP correspond to a pseudo-total content in the soil samples. The correlation observed in this study for soil samples was consistent with previous findings (R^2 = 0.997) [14] and comparable to the correlation reported for plant samples in another study, which showed an R^2 higher than 0.970 [37]. In contrast to the findings of the present study, previous research on soil samples [17] did not report significant deviations in Cd quantification by MXRF compared to values obtained through acid digestion followed by ICP-MS. However, it is important to highlight that the referenced study [17] used a digestion method with aqua regia, whereas the present study used reverse aqua regia. Other authors [38,51] have also reported a strong correlation (R^2 = 0.920–0.999) between Cd results obtained by MXRF and ICP for other sample types.

3.8. Cd quantifications using MXRF

The validation of the method for quantifying Cd in cacao system samples using MXRF provided objective evidence that the method is fit for this purpose [27]. This was demonstrated over a wide concentration range: 0.100–310 mg kg^{-1} for plant samples, 0.110–22.07 mg kg^{-1} for soils, and 0.110–1000 mg L^{-1} for a microbial culture medium. The measure of Cd in solid samples and in LB medium, using ICP techniques, require an acid digestion step to eliminate organic interferences originating from both the solids and the culture medium. In the case of MXRF, the organic matter it is not find as interference, therefore the quantification is not requiring the digestion process [52]. Furthermore, while CdtB were not included in this study, ensuring accuracy in determining Cd in the LB medium at different concentrations is noteworthy. Future studies with MXRF should incorporate the microbial biomass of CdtB genera isolated from cacao soils, such as *Burkholderia, Ensifer, Enterobacter, Rhizobium,* and *Streptomyces* [36].

However, to ensure routinary implementation of this technique, it is recommended that random measurements be performed using conventional analytical techniques for quality control purposes and CRM analysis during each analytical session performed at MXRF. Here it is worth to mentioning that MXRF users may not have always access to parallel ICP MS measurements. Although work has been reported on the use of MXRF in samples including chocolate [53], cigarette filters [17], scallops [38], tea [34], processed foods [18], cacao samples and soils [17], and even with analytes such as arsenic and lead [20], research on MXRF is becoming popular.

3.9. MXRF and final repercussion in food science research

Monochromatic X-ray fluorescence (MXRF) spectrometry, specifically the *E*-max technology, has emerged as a robust and cost-effective method for cadmium (Cd) quantification in cacao systems, as validated in this study. This technique offers high sensitivity (LOQ 0.124–0.148 mg kg^{-1}), portability, and non-destructive analysis of samples, making it ideal for real-time Cd monitoring in cacao soils, plant tissues, and microbial cultures, with strong correlations to ICP methods (Pearson coefficient 0.964–0.999). Recent literature [14,37,53] highlights the growing application of MXRF in Colombian agriculture, extending beyond cacao to crops like coffee and rice, where Cd and other heavy metals pose food safety risks [24,54].

The E-max is not the only MXRF instrument on the market. Others include desktop versions of EDXRF and WDXRF systems that, in terms of costs, it can start around $100,000 up to $500,000 USD [12]. These systems have been routinely used in food analysis but have largely been replaced by smaller, more powerful ED-XRF devices. Here are some examples of other ED-XRF technologies available on the market: i. HORIBA provides high-performance ED-XRF analysers, notably the XGT series (LOD ~100 mg kg^{-1} Cd, [55]), which are well-suited for agriculture and food safety applications. ii. Shimadzu's ED-XRF spectrometers, such as the EDX-7000/8000/8100 series (LOD 0.07–1 mg kg^{-1} Cd,

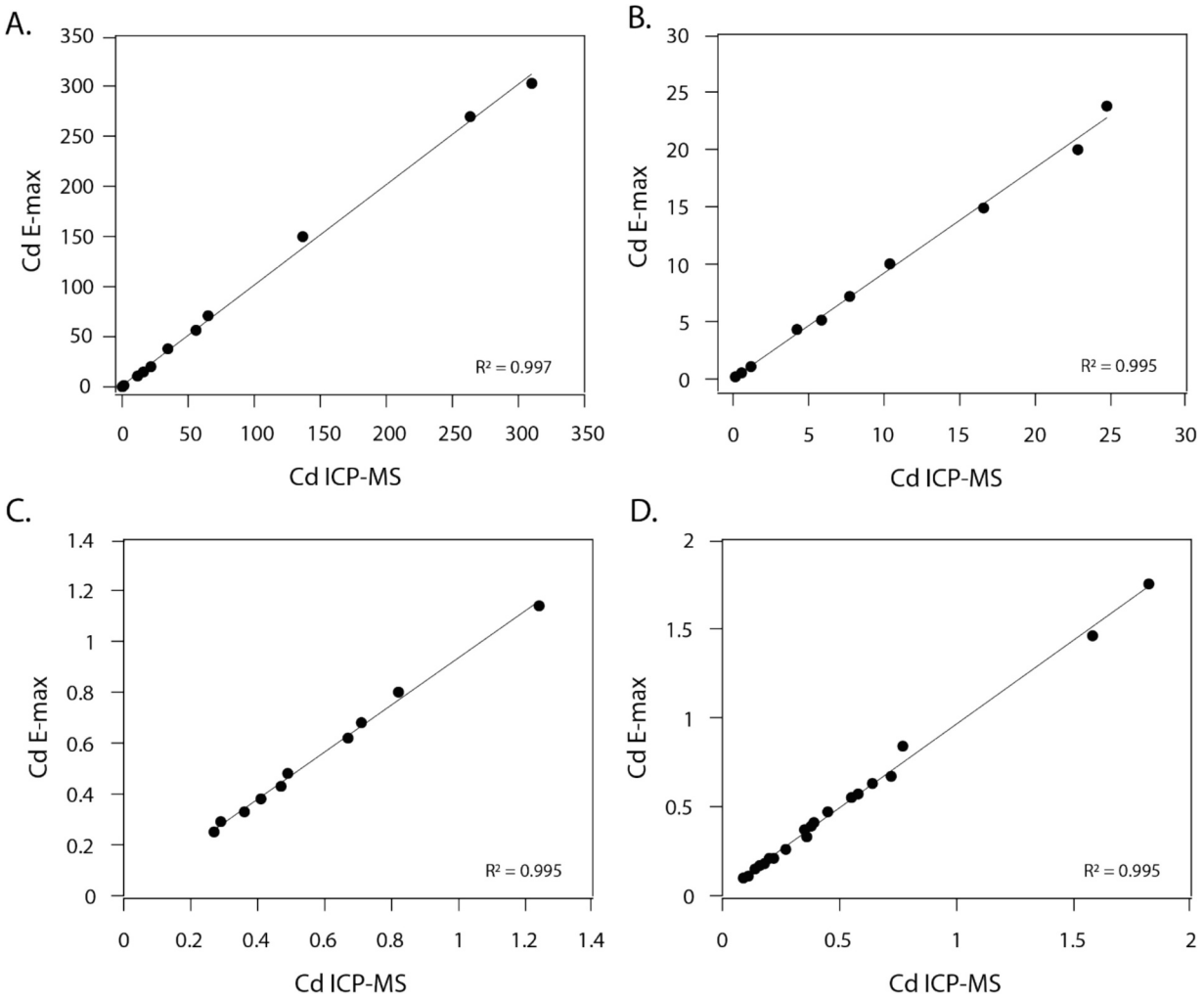

Fig. 2. Correlation between MXRF and ICP concerning Cd quantification in: A. Cacao root ($n = 12$), B. Cacao stem ($n = 10$), C. Cacao leaf (n = 10), and *D. cacao* pod ($n = 20$). Cd concentration is expressed in mg kg^{-1}. R^2 represents the coefficient of determination.

[56, 57]), are designed for non-destructive elemental analysis in solid, powder, and liquid samples. Iii. Bruker offers a range of ED-XRF spectrometers, including the S2 PUMA and S1 TITAN series (LOD 4 mg kg^{-1} Cd, [58]), which are effective for agriculture and food safety. iv. Malvern Panalytical provides ED-XRF solutions like the Epsilon 1 and Epsilon 4 (LOD 0.004–0.01 mg kg^{-1} Cd, [12, 59]), which are tailored for agriculture and food safety. The Epsilon 4 prototype is particularly noted for its ability to analyse heavy metals in food products and soils, supporting compliance with regulations like RoHS; and v. Thermo Fisher Scientific's with the equipment Niton XL5 Plus handheld ED-XRF analyser and ARL PERFORM'X benchtop systems (LOD 5 mg kg^{-1} Cd, [18]), are designed for rapid, non-destructive elemental analysis. Despite being able to analyse Cd, the aforementioned ED-XRF are not monochromatic. Besides, none of the above-mentioned equipment have been described to test Cd in cacao systems. A non-commercial monochromatic XRF has been reported [55] for use with tipping paper and cigarette filters. Another monochromatic device called the HD Mobile®, is also available from the X-ray Optical Systems - XOS supplier [18]. However, this device is not useful for cadmium measurements in food science, since XOS focuses on low-energy excitations for petroleum applications. Interestingly, in terms of low-cost (below 100,000 USD), efficiency, portability and high accuracy, needed for cacao stakeholders (and their cooperatives) in Colombia, the *E*-max make great difference in comparison to the above-mentioned brands.

Studies also emphasise its potential in environmental monitoring, such as assessing polluting metals content in mining-impacted agricultural soils [56]. This technique could be extrapolated to other crops, such as rice and avocado, which are important crops in Colombia. The use of this method would greatly benefit the monitoring processes carried out by AGROSAVIA and other cacao-related institutions, as well as in other crops. The assessment of As and Pb content can also be performed using MXRF, although similar validation to that required for Cd will be necessary. Using the new technique might help to have other criteria to ensure exports of these crops to Europe and other markets, complying with the maximum limits for Cd, such as 0.05 mg kg^{-1} for avocado regulation 2023/915 [4].

Future trends point towards integrating MXRF with machine learning technology for enhanced data precision and its adoption in portable field devices for on-site analysis. This would address the need for sustainable and accessible tools in Colombia to ensure food safety and compliance with international Cd regulations. The next step will be integrating *E*-max technology with robotics to asset a multi-approach technique to automate the samples received and deliver analytics and suggestions for specific crops using the metadata and the IA of the generated databases.

4. Conclusions

The validation of monochromatic X-ray fluorescence (MXRF) using the E-max system for cadmium (Cd) quantification in the cacao system

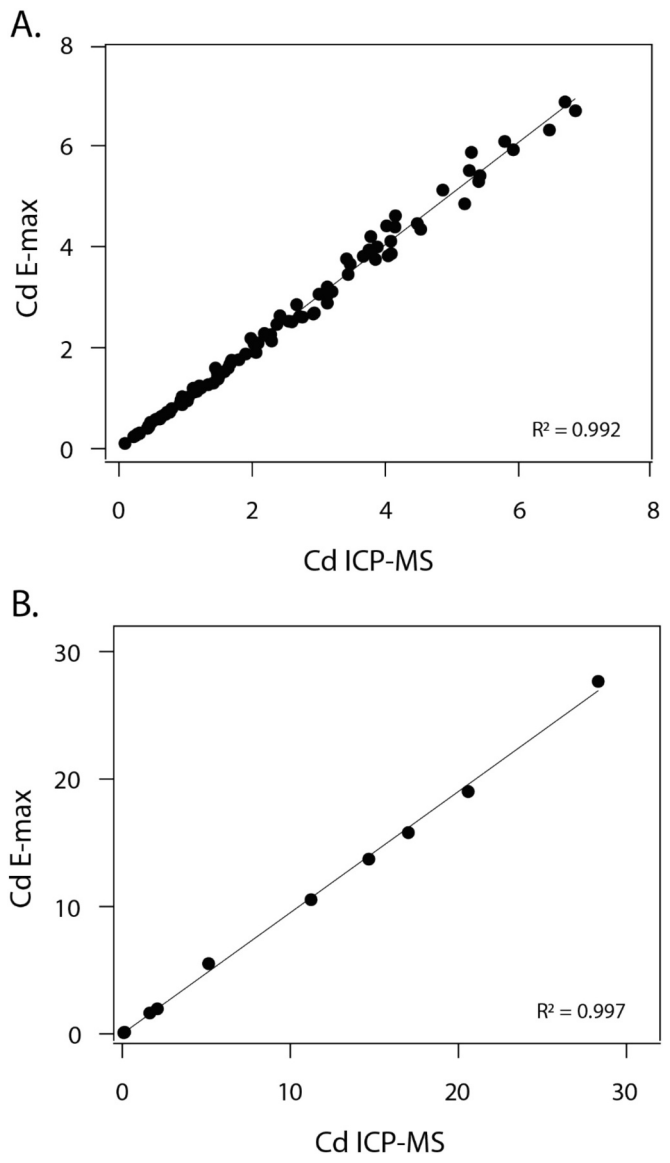

Fig. 3. Correlation between MXRF and ICP concerning Cd quantification in A. Cacao nibs ($n = 101$) and B. Cacao shell (n = 10). Cd concentration is expressed in mg kg^{-1}. R^2 represents the coefficient of determination.

demonstrated adequate analytical performance across diverse sample types, including soil, soil litter, root, stem, leaf, pod, shell, nibs, and the microbial LB liquid culture medium. The method achieved a limit of quantification (LOQ) of 0.148 mg kg^{-1}, well within international regulatory thresholds (0.1–0.8 mg kg^{-1}), with recovery percentages ranging from 90.7 % to 109.0 % and Z'-scores for reproducibility between −0.50 and 0.62. Robustness was confirmed through minimal variability across full lab processing, field, and in situ pretreatments, with 60 and 200 s measurement times proving highly sensitive. A strong correlation with inductively coupled plasma (ICP) techniques was evidenced by Pearson's coefficients from 0.964 to 0.999 and % R between 74.8 and 129.9 %, for MXRF in comparison to ICP. These results, supported by satisfactory proficiency test outcomes and trueness values of 90.2 to 108.0 %, verified that MXRF is a reliable, cost-effective, and portable alternative to conventional methods, enabling precise Cd monitoring in cacao production and facilitating compliance with food safety standards in Colombia.

Furthermore, MXRF was found to correlate highly with conventional Cd analysis techniques (ICP) for all plant material samples of the cacao system evaluated. In the soil samples, MXRF evidenced that Cd concentrations measured by MXRF were higher compared to ICP, but this was associated with the absence of HF in the digestion process accompanying the ICP technique. This is the first time that a validation using MXRF as an analytical technique on cacao system samples has been reported in the literature, including all the performance parameters shown in this study: robustness, LOD, LOQ, repeatability, intermediate precision, reproducibility (through proficiency testing) and trueness. This study is also the first to include soil samples alongside all possible parts of the cacao plant (soil-leaf litter, roots, stems, leaves, pods, shells, and nibs), as well as the microbial LB liquid culture medium to assess the MXRF technique against conventional Cd analysis techniques (ICP).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sab.2025.107352.

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CRediT authorship contribution statement

Cifuentes MEA: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Y. Rodríguez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **L.M. Avellaneda-Torres:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **R. Quiroga-Mateus:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **D. Bravo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing financial or personal interests that might influence this study.

Data availability

Data will be made available on request.

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