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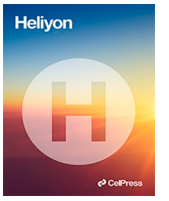

Research article

Using optimized monochromatic energy dispersive X-ray fluorescence to determine the cadmium concentration in cacao and soil samples

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ABSTRACT

Following the implementation of food safety limits on cadmium (Cd) in cacao products, there has been a growing demand for monitoring Cd in cacao tissues and soils. Traditional methods like acid digestion followed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) are time-consuming and costly. X-ray Fluorescence is an alternative technique that offers advantages in terms of speed, cost, ease of use and less environmental impact. However, to date, relatively high detection limits have impeded its application for food safety limits. This study examines a Monochromatic Energy Dispersive X-ray Fluorescence (MEDXRF) method optimized for Cd analysis as an alternative to ICP-MS. Using a measurement time of 200 s, the average limit of quantification (LOQ) was 0.178 mg Cd kg^{-1} for biological samples and 0.205 mg Cd kg^{-1} for soil samples. A strong correlation ($y = 1.013\ x + 0.003$, $R^2 = 0.984$) with ICP-MS results was found for 95 bean, 16 cacao liquor, 75 leaf, and 91 soil samples. The coefficient of variation (CV) among three replicates was below the threshold value of 15 % for most samples with Cd concentrations above the reported LOQ values. Additionally, a significant difference in CV was obtained between soils sieved over 500 μm (median 8.2 %) or 2 mm (median 9.8 %). However, no significant difference in CV was observed between 500 μm unpeeled beans and cocoa liquor with particle size of 20 μm. Based on our data, the proposed procedure is to analyze three replicates for 200 s with a sample size of 500 μm. The optimized MEDXRF technique offers important advantages in terms of cost-effectiveness and efficiency of routine Cd monitoring in the cacao supply chain, large-scale screening, and scientific research, and could be extended to other crops and heavy metals that are subject to food safety regulation at low concentrations.

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1. Introduction

Cadmium (Cd) is a non-essential trace element known for its detrimental effects on human health. Various regulatory bodies have established limits on the allowable Cd levels in a range of foodstuffs, including chocolate products. For example, the European Commission set Cd limits ranging from 0.10 mg kg^{-1} in chocolates with <30 % cacao solids to 0.80 mg kg^{-1} in chocolates with ≥50 % cacao solids [1]. Consequently, monitoring Cd concentrations in cacao beans for export has become an essential practice in the supply chain. Additionally, ongoing research is focused on reducing Cd levels in cacao beans that exceed these limits [2]. This increased need for both monitoring and research has led to a growing demand for Cd analyses of cacao plant tissue and soil samples.

Traditionally, acid sample digestion followed by inductively coupled plasma mass spectrometry (ICP-MS) has been the method of choice for mineralizing solid samples and quantifying their heavy metal concentrations. In Latin America it is also common to find the use of inductively coupled plasma optical emission spectroscopy (ICP-OES) and atomic absorption spectroscopy (AAS). Analyses by ICP-MS are more sensitive and accurate than ICP-OES and AAS, making it the gold standard for heavy metal quantification [3,4]. All these techniques are time-consuming, resource-intensive, and require elaborate sample preparation steps. This makes the process less suitable for commercial actors in the cacao supply chain who require rapid and cost-effective analyses [5]. Additionally, Dekeyrel et al. [6] showed that the inter-laboratory variation in Latin America is high for ICP and AAS Cd analyses in cacao samples. In this context, there is a growing interest in exploring alternative analytical techniques that offer both accuracy and efficiency as well as real-time results.

X-ray fluorescence (XRF) spectrometry has been available for many years as an alternative to acid digestion followed by ICP-MS measurement for elemental analysis in solid samples. This technique does not require extensive sample treatment steps. It is also less expensive, easy to operate and does not require trained personnel or a dedicated laboratory [4]. However, until now, the major disadvantage of the technique was its relatively high detection limit for trace elements such as Cd that occur in the sub mg kg^{-1} concentration range in environmental samples [7–9].

Here, we explored the potential of using monochromatic energy dispersive X-ray fluorescence (MEDXRF) as a rapid and cost-effective method for measuring the total Cd concentration in cacao bean, liquor, leaf, and soil samples. The MEDXRF instruments were optimized for Cd analysis and calibrated at the company without recalibration. First, the calibration curves were used to calculate the limit of detection (LOD) and limit of quantification (LOQ) for both biological and soil samples. Then, a total of 305 samples were selected as a representative subsample (based on concentration) of a previous collection made by this group of authors. These samples originate from Latin America (Colombia, Ecuador, and Peru) where the Cd levels in cacao are higher than in other cacao producing countries due to naturally high levels of Cd in the soil [2]. The samples were analyzed by MEDXRF and compared to the existing ICP-MS results to validate the viability of MEDXRF as an alternative for Cd analysis. Furthermore, we quantified the reproducibility of the method and how it is affected by sample preparation, i.e., different particle sizes.

2. Materials and methods

2.1. Experimental design and preparation of the samples

In total, 102 bean samples, 76 leaf samples, and 108 soil samples were selected from previous field studies conducted in Ecuador and Colombia. The Cd concentrations (ICP-MS data) ranged from 0.048 to 4.9 mg kg^{-1} with medians of 0.81 (beans), 2.12 (leaves) and 0.37 (soil) mg kg^{-1}. This range and these medians are representative for the region with respect to the export of cacao beans and the existing food safety regulations [1,10–12]. All samples were dried at 60 or 65 °C. Cacao and leaf samples were homogenized in a MIXTEC SJ-9669 blender, a KitchenAid Blade BCG111OB Coffee Grinder, or an Oster BLSTBCG-B50-000 blender and sieved through a 500 μm stainless steel sieve. Soil samples were ground with a pestle and mortar and sieved through a 500 μm stainless steel sieve, except for 25 soil samples which were sieved through a 2 mm stainless steel sieve. Additionally, 19 cocoa liquor (100 % cacao mass; particle size 20 μm) samples (ICP-MS median 0.39 mg Cd kg^{-1}) were taken from commercial lots of the Norandino cooperative in Peru. To enhance the robustness of our methods and instrument comparison, we deliberately selected diverse samples, operators, and instruments.

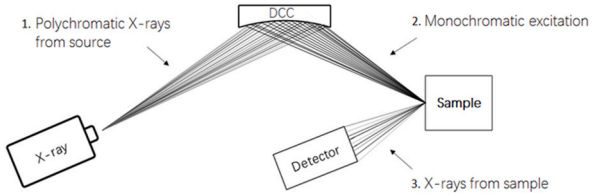

Fig. 1. The monochromatic energy dispersive X-ray fluorescence (MEDXRF) mechanism used in the E-max instrument. The polychromatic X-ray source is focused on a three-dimensional monochromatic beam by a doubly curved crystal (DCC). This setup allows for simultaneous excitation of all elements in the sample. An energy dispersive detector equipped with a multi-channel analyzer then identifies and quantifies the characteristic radiation profile of each element.

2.2. Cadmium analysis with MEDXRF

The MEDXRF instrument used in this study is the E-max Portable Heavy Metal Analyzer for Soil and Food (Z-Spec, East Greenbush, NY, USA), a recently developed desktop instrument that is commercially available. The instrument is calibrated by the manufacturer for both biological and soil samples (using ERM BD151, BD513, BD514, BD513, NIST 1570a and NIST SRM1646a for biological samples and NIST SRM2586, SRM2709a, and SRM2710a for soil samples). Fig. 1 shows a schematic overview of the MEDXRF mechanism behind the E-max [13].

The E-max instrument uses a MEDXRF configuration with a 50 W X-ray source emitting a high energy beam of 30 keV for optimal excitation of atoms in the samples. This beam is focused by doubly-curved crystal (DCC) optics to a beam with a spot size of 0.8 mm in Full Width Half Maximum (FWHM). The incident angle of the beam on the sample surface is approximately 10°, so a 10 mm line-type profile is formed on the sample surface. A fast silicon-drift detector (SDD) collects the fluorescence X-ray signals at a 5 mm distance from the sample. Although MEDXRF can analyze many elements at the same time, these specifications make it optimal for the analysis of heavy metals like Cd. A subsample of approximately 1 g was added to the sample holder cup and compressed manually with a plunger against the cup end covered with a disposable 12 μm polypropylene film. The sample holder was subsequently inserted into the instrument, the correct sample matrix (biological or soil) was selected, and the data collection time was set to 200 s per measurement. Each sample was measured three times. Between measurements, the sample holder was removed from the instrument, rotated approximately 120°, and inserted again to change the optical path of the X-rays incident on the sample. In total, four MEDXRF instruments (same reference and models) were used in this study.

2.3. Cadmium analysis with ICP-MS

Two different laboratories – further distinguished by the numbers 1 and 2 – conducted acid digestion and ICP-MS analysis of the samples. Biological samples (cacao beans, liquors or leaves) were digested by either (1) dissolving a 100 mg subsample in 8 mL Normatom® nitric acid (HNO_3 67–69 % w/w, VWR International, Radnor, PA, USA) in a closed vessel microwave system (MARS6, CEM Corporation, Charlotte, NC, USA) at 180 °C or by (2) dissolving a 200 mg subsample in 3 mL Primar Plus™ HNO_3 (68 % w/w, Thermo Fisher, Waltham, MA, USA), 2 mL Primar™ hydrogen peroxide (H_2O_2 30–32 % w/w, Thermo Fisher) and 3 mL Milli-Q water in a closed vessel microwave system (Multiwave Pro, Anton Paar, Graz, Austria) at 140 °C. For soil samples, either (1) 100 mg was digested with aqua regia, i.e., 2 mL Normatom® HNO_3 and 6 mL Suprapur® hydrochloric acid (HCl 30 % w/w, Merck, Darmstadt, Germany) at 180 °C in a closed vessel MARS6 microwave system or (2) 400 mg was digested with 3 mL HNO_3, 2 mL H_2O_2, and 9 mL Suprapur® HCl (37 % w/w, Thermo Fisher) at 108 °C on a hotplate. Diluted samples were measured by ICP-MS (1) Agilent 7700x (Agilent Technologies, Santa Clara, CA, USA) or (2) Thermo-Fisher Scientific iCAP-Q (Thermo Fisher Scientific, Bremen, Germany) with limits of quantification (LOQs) of 0.014 mg Cd kg^{-1} and 0.007 mg Cd kg^{-1}, respectively (calculated as the median LOQ of all measurements). The following certified reference materials were used (with certified Cd concentration, recovery range, and average recovery in brackets): NIST®2384 baking chocolate (0.073 ± 0.008 mg Cd kg^{-1}, 91–105 %, 96 %) and BCR-142R Light Sandy Soil (0.249 ± 0.010 mg Cd kg^{-1}, 94–104 %, 100 %) for lab 1, and ERM BD512 Dark Chocolate (0.302 ± 0.013 mg Cd kg^{-1}, 95–113 %, 104 %), NIST® SRM® 1573a Tomato Leaves (1.52 ± 0.04 mg Cd kg^{-1}, 93–103 %, 97 %) or BCR-142R Light Sandy Soil (0.249 ± 0.010 mg Cd kg^{-1}, 89–98 %, 94 %) for lab 2. Table 1 provides the number of samples analyzed by each laboratory and instrument for each sample type. Detailed data and equipment specifics are available in the Supplementary Material and at Mendeley Data (https://doi.org/10.17632/xzwzh3kmns.1).

2.4. Statistical analysis

The variability of replicate MEDXRF measurements is reported below as the coefficient of variation (CV, in %; Equation (1)) among the three readings of the *same* sample:

$$CV = \frac{\sigma}{\mu} * 100\% \quad (1)$$

where σ is the standard deviation among the three readings and μ is the average Cd concentration of the measurements, both in mg

Table 1
Number (n) of different samples analyzed in different laboratories: two ICP-MS laboratories (see method section) with certified reference materials in each laboratory and four identical MEDXRF instruments used in different countries (total n = 305).

	MEDXRF 1	MEDXRF 2	MEDXRF 3	MEDXRF 4
ICP-MS lab 1	cacao bean: 51 soil: 52			cacao liquor: 19
ICP-MS lab 2		cacao bean: 23 leaf: 53 soil: 25	cacao bean: 28 leaf: 23 soil: 31	

kg^{-1}. Because these three readings are the replicate readings on a different 10 mm line measured at different angles on a round surface, they may be considered as technical sampling replicates. We considered a CV < 15 % reliable in line with international criteria (modified Horwitz equation) as described in Dekeyrel et al. [6]. All statistical analyses were done using JMP® Pro (version 17.0.0, The SAS Institute, Cary, NC, USA).

3. Results and discussion

3.1. Calibration of MEDXRF instruments and figures of merit

Calibration curves of all four instruments were similar for both biological and soil samples (Fig. 2, Table 2). From these equations, the LOD (in mg kg^{-1}) was calculated as follows:

$$LOD = \frac{3}{b}\sqrt{\frac{BG}{t}} \quad (2)$$

Where b is the slope of the calibration curve (in cps per concentration of the analyte), BG is the background of the measurement (in cps), and t is the irradiation time (in s). The LOQ was estimated by multiplying the LOD by a factor 3. The BG is overall higher for soil samples which resulted in an overall higher LOQ for soil samples (mean 0.205 mg kg^{-1}) than for biological samples (mean 0.176 mg kg^{-1}) for an irradiation time of 200 s (Table 2). In total, the Cd concentration of 28 samples out of the 305 selected samples was below the LOQ. An LOQ of 0.176 mg kg^{-1} is sufficiently low when analyzing Cd concentrations in cacao. For example, the most stringent European limit is the limit of 0.60 mg kg^{-1} in cocoa powder, which translates to 0.30 mg kg^{-1} in the cacao beans when assuming 100 % pure, defatted cocoa powder [1]. The 200 s used in this study is a trade-off between sensitivity and time. Lower LOQs can theoretically be reached when extending the irradiation time. According to equation (2), the LOQ of biological samples would reduce to 0.102 mg kg^{-1} when using a longer irradiation time of 600 s. For soil samples, the LOQ would reduce to 0.126 mg kg^{-1} at $t = 600$ s.

3.2. Comparing MEDXRF and ICP-MS results

A strong correlation between the Cd concentration measured by MEDXRF (y) and ICP-MS (x) was obtained for all samples together (Fig. 3, $y = 1.013\ x + 0.003$, $R^2 = 0.984$) and for each sample matrix separately (Table 3). In case of biological samples, both techniques determine total elemental concentration. In case of soil, the ICP-MS measurement is based on an aqua regia extraction, while MEDXRF measures total concentrations. Nontheless, total and aqua regia digestible soil Cd concentrations are generally comparable. Taraškevičius et al. [14] reported a median extractability (aqua regia versus *real total* content) of 94 %. Similarly, Sastre et al. [15] and Florian et al. [16] found aqua regia extractable Cd results to be comparable to total Cd concentrations, with the latter suggesting that Cd bound to aluminosilicate layers is negligible.

3.3. Variability of MEDXRF measurements

Across all 280 samples with a particle size of 500 μm, the CV increased with decreasing Cd concentration (Fig. 4). The critical CV of 15 % is reached around the calculated LOQs, indicating that the method is sufficiently precise above these concentrations. It should be reiterated that these MEDXRF results are for a measurement time of 200 s and that the variability could decrease further with increasing measurement times. Overall, the CV among replicates was found to be higher for soil samples than for the other three matrices ($p = 0.05$; Tukey-Kramer HSD).

The combination of low LOQs and both accurate and precise results without the need for a pre-concentration step is unprecedented

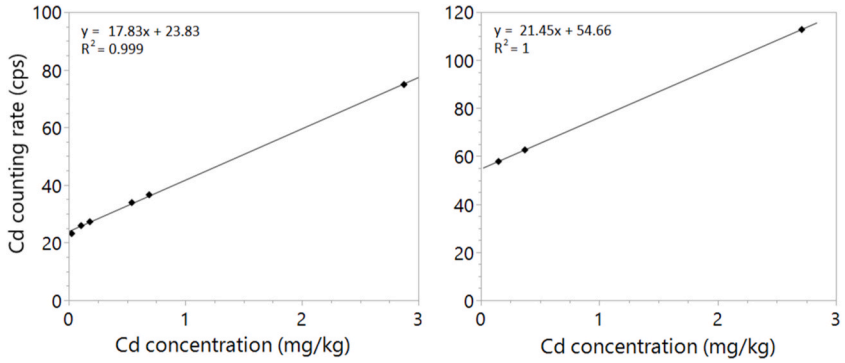

Fig. 2. Calibration curves for MEDXRF equipment 1 for both biological (left) and soil samples (right). Certified samples ERM BD151, BD513, BD514, BD513, NIST 1570a and NIST SRM1646a were used to calibrate the instruments for biological samples and NIST SRM2586, SRM2709a, and SRM2710a were used for soil samples.

Table 2
Calibration curves, R^2 values, LOD, LOQ and RMSE values of all MEDXRF instruments for both biological and soil samples (t = 200 s).

MEDXRF instrument	Matrix	Calibration curve	R^2	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	RMSE
1	Biological	y = 17.83x + 23.83	0.999	0.058	0.174	0.68
	Soil	y = 21.45x + 54.66	1	0.074	0.223	0.01
2	Biological	y = 16.65x + 21.83	0.998	0.060	0.179	1.00
	Soil	y = 21.75x + 46.72	1	0.069	0.208	0.51
3	Biological	y = 15.54x + 20.80	0.996	0.062	0.187	1.33
	Soil	y = 18.79x + 43.38	1	0.060	0.181	0.30
4	Biological	y = 20.08x + 26.91	0.999	0.055	0.164	0.45
	Soil	y = 19.66x + 51.26	1	0.069	0.208	0.52

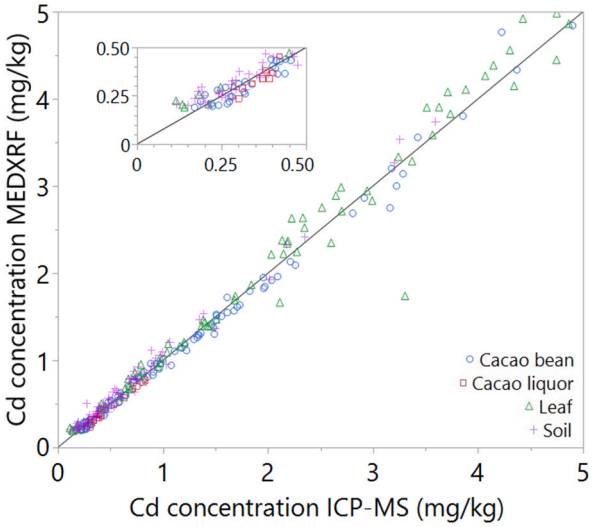

Fig. 3. The Cd concentration measured by MEDXRF in function of the Cd concentration measured by ICP-MS for different sample types (o cacao bean, □ cocoa liquor, Δ cacao leaf, + soil), both in mg kg^{-1}. The diagonal black line represents the 1:1 line.

Table 3
The amount of samples (n), equations, R^2 values, and standard errors (SE) of the slope for the Cd concentration measured by MEDXRF (y) as a function of the Cd concentration measured by ICP-MS (x, both in mg kg^{-1}), for each sample matrix separately.

Sample matrix	n	Equation	R^2	SE slope (mg kg^{-1})
Cacao bean	95	y = 0.990 x – 0.017	0.99	0.009
Cocoa liquor	16	y = 0.963 x – 0.017	0.98	0.036
Cacao leaf	75	y = 1.014 x + 0.036	0.97	0.022
Soil	91	y = 1.027 x + 0.039	0.99	0.010

for XRF measurements of Cd concentration in biological and soil samples. Compared to the LOQs around 0.2 mg kg^{-1} in the current study, Guimarães et al. [8] reported an LOD of 9–11 μg g^{-1} for Cd, which translates to an LOQ of approximately 30 mg kg^{-1}. Almeida et al. [17] did report a similar LOQ value for Cd in ground coffee samples (0.186 mg kg^{-1}). However, Cd concentrations measured by XRF were not similar to Cd concentration measured by ICP (y = 0.7803x + 47.541, R^2 = 0.920).

3.4. Variability of MEDXRF measurements for different sample preparation protocols

For soil samples, we compared the variability of the MEDXRF measurements for two different particle sizes: 2 mm (n = 25), which is the standard preparation for most soil analyses, and 500 μm (n = 83), to obtain a more homogeneous material. The median CV was 9.8 % for the 2 mm samples and 8.2 % for the 500 μm samples. A Wilcoxon two-sample test (Mann-Whitney *U* test) indicated a significant, albeit small, difference in the CVs between these two sample sizes (p = 0.0353). This indicates that the optimal preparation for soil samples, considering the precision of the measurement alone, is to use finely ground material.

A similar comparison was conducted between cocoa liquor samples with a particle size of 20 μm (n = 19, median CV = 3.4 %) and cacao bean samples with a particle size of 500 μm (n = 102, median CV = 4.2 %). The Wilcoxon two-sample test (Mann-Whitney *U* test) showed no significant difference in CV (p = 0.7084), indicating that processing cacao beans to a liquor does not further reduce

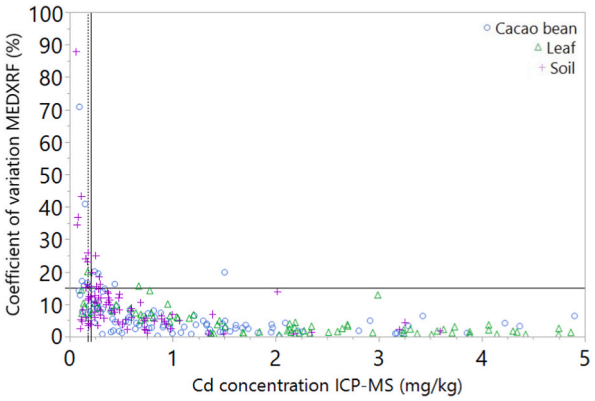

Fig. 4. The CV of the MEDXRF measurements in function of Cd concentrations measured by ICP-MS. Only samples with a particle size of 500 μm were included in this calculation to exclude the effect of particle size on variability among replicates (n = 280). Results below the calculated LOQ values were included, but average LOQs for both biological (—) and soil (—) samples are indicated by vertical lines. The horizontal black line represents the threshold of CV = 15 %.

variability in replicate MEDXRF measurements of the same sample.

4. Conclusion

The average limit of quantification (LOQ) among the different MRFXRF instruments was 0.178 mg Cd kg^{-1} for biological samples and 0.205 mg Cd kg^{-1} for soil samples. Cadmium concentrations measured by the MEDXRF instruments were very close to corresponding values obtained with ICP-MS for 95 bean, 75 leaf, 91 soil, and 16 cacao liquor samples ($y = 1.013\,x + 0.003$, $R^2 = 0.984$) with CV values among replicates mostly below the threshold of 15 %. In terms of sample preparation, using soil samples sieved at 2 mm proved to be slightly more variable than 500 μm soil samples. The precision obtained for 500 μm sieved cacao bean samples was not significantly different from cacao liquor samples. Combining precision and practical considerations, we recommend three 200 s readings of a 500 μm sample as the standard procedure for analyzing Cd concentration in soil, cacao, and leaf samples.

These quantification limits, accuracy and precision are unprecedented for XRF measurements of Cd concentrations in biological and soil samples. Our study confirms MEDXRF as an accurate, rapid, and cost-effective alternative to the conventional combination of acid digestion and ICP-MS technology for the analysis of cacao and soil samples at Cd levels that are relevant for food safety compliance in cacao, serving both commercial and research purposes. In the broader context of food regulation, MEDXRF can also be applied to the analysis of fruits, vegetables, and other foodstuffs. Additionally, while capable of detecting other elements, like lead (Pb) and arsenic (As), its detection limits and accuracy for these elements should be validated before application in food safety analyses.

CRediT authorship contribution statement

Jesse Dekeyrel: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Rachel Atkinson:** Writing – review & editing, Resources, Methodology, Conceptualization. **Eduardo Chavez:** Writing – review & editing, Resources, Methodology, Conceptualization. **Mayesse da Silva:** Writing – review & editing, Resources, Methodology, Conceptualization. **Orlando Idarraga-Castaño:** Writing – review & editing, Resources, Methodology. **Mirjam Pulleman:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Erik Smolders:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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