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Cd and Pb in cocoa beans: Occurrence and effects of chocolate processing

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ABSTRACT

The aim of this study was to quantify Cd and Pb levels in cocoa beans from three cocoa producing regions of the world, along with their derived products (liquor, cocoa powder and cocoa butter). The contaminants were determined by inductively coupled plasma optical emission spectrometry (ICP OES) and two sample preparation procedures were evaluated: microwave assisted acid digestion and dry ashing. The limits of detection and quantification for Cd and Pb were 0.5 and 1.5 μg kg^{-1} and 7.0 and 22 μg kg^{-1}, respectively. The study examined 90 samples of cocoa beans and the concentration range found for Cd was <0.0015–1.598 mg kg^{-1} and for Pb was <0.022–2.528 mg kg^{-1}. In 8% (Cd) and 66% (Pb) of the samples, levels detected were higher than the maximum allowed limits. Dry ashing decomposition method was shown to be adequate to the determination of Cd and Pb levels in cocoa beans and their derivatives, with satisfactory results for accuracy and precision. High levels of Pb and Cd were found in beans from Brazil and Ecuador, respectively. The derived products obtained in the process showed Cd levels between <0.0015 and 0.118 mg kg^{-1} and Pb between <0.022 and 0.136 mg kg^{-1}. A tendency of the inorganic contaminant to remain in the non lipidic fractions of the beans (cocoa powder) was observed. The ingestion of chocolate produced from contaminated beans can contribute to consumer exposure to inorganic contaminants, exceeding Cd PTMI for children. The use of cocoa beans from different regions (blends) in the manufacture of cocoa products can be an alternative in reducing the levels of these contaminants in the final product intended for consumption.

1. Introduction

Cocoa tree is historically cultivated on West Africa, Central and South America and Southeast Asia. Annually, 4651 thousand tons of cocoa beans are produced around the globe (ICCO, 2019). Cocoa beans composition as well as the flavor attributes of their derivatives are directly influenced by genetic variability, geographic origin, harvest and processing. Hence, chemical and biochemical characteristics and their relation to the environment are key features for quality control and technological aspects (Cohen et al., 2003; Loureiro et al., 2017). Cocoa based products consumption is very common in the whole world, and the consumers have been more concerned about products quality (such as origin certificate), food safety, sustainable practices and flavor refinement (ICCO, 2007, pp. 11–14).

The intake of chocolate and its derivatives may provide health benefits due to the presence of essential minerals such as Ca, Cu, Fe, K, Mg, Mn and Zn (Bertoldi et al., 2016) and antioxidant components with high flavonoids concentration. These compounds have different sorts of biological activity, as on the prevention of heart diseases or on the modulation of immunological and/or anti-inflammatory responses (Martín & Ramos, 2016). However, recent works have reported the presence of inorganic contaminants (Al, As, Cd, Ni, Cr and Pb) in cocoa products (Lo Dico et al., 2018; Villa et al., 2014). Long term exposition to toxic elements such as Cd and Pb can cause adverse effects to the kidney, bones and neurological system, with food being one of the main sources of exposure to these contaminants (Lippi, 2013).

The soil is among the main contamination sources of inorganic contaminants in cocoa beans. The relation between contaminants level in cocoa beans and the soils from cultivation regions are being studied (Bertoldi et al., 2016; Mohamed et al., 2020; Romero-Estévez et al., 2019). Studies also show a relation between the increase in the proportion of cocoa solids in cocoa product (dark, milk and white

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chocolates) and the inorganic contaminants levels (Abt et al., 2018; Villa et al., 2014; Yanus et al., 2014). Nevertheless, there is little information regarding the distribution of inorganic contaminants along the different processing stages of cocoa beans.

Due to the increasing concern regarding safe levels of Cd and Pb in cocoa beans and their derivatives, several regulations have been established. In 2019, the European Union laid down maximum levels for Cd in different cocoa products categories from a legislation formulated in 2014: 0.10 mg kg^{-1} for milk chocolate with cocoa content <30%; 0.30 mg kg^{-1} for chocolate with cocoa content <50% and milk chocolate with cocoa content ≥30%; 0.80 mg kg^{-1} for milk chocolate with cocoa content ≥50% and 0.60 mg kg^{-1} for cocoa powder (EU, 2014). Maximum levels are also established by Mercosul, China and Codex Alimentarius for both Cd and Pb (Brazil, 2013, pp. 33–35; CAC 2019; MERCOSUL, 2011; USDA, 2014).

Brazil is the sixth world's cocoa producer, having harvested 255 thousand tons in 2018. However, there is a lack of information about Cd and Pb levels in cocoa beans from Brazil's different production regions (IBGE, 2019).

Therefore, the main objectives were to: (i) evaluate the occurrence of Cd and Pb in cocoa beans from Brazil, Africa and Ecuador; (ii) study the behaviour and occurrence of Cd and Pb in cocoa beans derivatives obtained after beans processing (liquor, cocoa powder and cocoa butter) and (iii) estimate the exposure to Cd and Pb through the consumption of chocolate by adults and children.

2. Material and methods

2.1. Samples

Samples of fermented and dried cocoa beans (n = 90) were acquired from different locations, representing the main productive regions of Brazil (Bahia (BA) (n = 33), Pará (PA) (n = 29), Espírito Santo (ES) (n = 4) and Rondônia (RO) (n = 08)); Africa (Côte d'Ivoire (CI) (n = 12)) and Ecuador (n = 4). Samples with shells were homogenized and stored in a freezer until the moment of analysis. A sample is a batch of beans from the same farm.

A fermented and dried cocoa bean sample from Bahia was used for processing in a pilot scale in order to study the behaviour of Cd and Pb during cocoa beans processing and obtaining of derivatives (liquor, cocoa powder and cocoa butter). A 2 kg portion of the sample was roasted in a rotating electric oven (JAF Inox, Tambaú, SP) with forced air circulation for 60 min at 120 °C. Afterwards, the cocoa beans were crushed in an inox knife mill (ICMA, Campinas, SP) and the particles separated in different sizes (mesh sizes 5.66 mm and 2.83 mm). Shells and nibs were separated by density in a winnowing (JAF, Inox, Tambaú, SP) and the nibs grinded in a three horizontal cylinders Melanger mill (Spectra 10, India) for liquor obtaining. The maximum particles diameter was controlled with a digital micrometer (Mitutoyo, Suzano, Brazil) being in the range of 20–25 μm. Cocoa butter and cake were obtained through the pressing of 500 g of liquor in a hydraulic press (Ercitec, Bauru, SP), and the cocoa powder by cake grinding.

To understand the elements behaviour during cocoa beans processing steps (roasting, grinding and pressing) a mass balance was calculated taking into account the products weight and Cd levels.

2.2. Reagents

All reagents were analytical grade or superior. Hydrogen peroxide 30% (w/v) and chloridric acid 37% (w/v) were from Merck (Darmstadt, Germany) and nitric acid was obtained by sub-boiling destilation (Berghof, Eningen, Germany). Water (18,2 MΩ cm) was purified in a reverse osmose system (Gehaka, São Paulo, Brazil).

2.3. Cd and Pb determination

Two different sample preparation procedures were evaluated: microwave assisted acid digestion (closed system) and dry ashing decomposition. All glassware used was kept immersed in HNO_3 20% (v/v) for at least 2 h, followed by rinsing with deionized water. Quantification of inorganic contaminants was performed by ICP OES, using 5100 VDV (Agilent Technology, Tokyo, Japan) with a radiofrequency (RF) of 27 MHz and a simultaneous multielemental detector of solid state CCD (Charge Coupled Device).

ICP OES experimental conditions were optimized as follows: radiofrequency power of 1200 W, nebulization rate of 0.5 L min^{-1}, plasma gas flow rate (Ar) of 12 L min^{-1}; auxiliary gas flow rate (Ar) of 1.0 L min^{-1}, 2 points background correction, integration and reading time of 10 s for the 3 replicates, axial vision, double pass nebulization chamber and seaspray nebulizer. Liquid Argon 99,996% (Air Liquid, SP, Brazil). Analytical line wavelengths were: Cd (214.439 nm) and Pb (220.353 nm). Analytical curves were prepared from a 100 mg L^{-1} standard solution of Cd e Pb (Specsol – Quimlab, Jacareí, Brazil) in HCl 5% (v/v) in concentrations range of 0.0001–1.00 mg L^{-1} (Cd) and 0.002–1.00 mg L^{-1} (Pb). The determination of inorganic contaminants was carried out in triplicate.

2.3.1. Microwave assisted acid digestion

Cocoa beans digestion was done in a closed microwave system (Start D, Milestone, Sorisole, Italy), adapted from Butaciu et al. (2016): 0.5 g of the sample were placed into a PTFE digestion vessel and followed by the addition of 8 mL of concentrated HNO_3, standing overnight. After, 2 mL of H_2O_2 30% (m/v) were added. The vessels were sealed and transferred to the microwave digester using 2 heating ramps applying 1000 W of power: (a) room temperature to 170 °C in 15 min; (b) maintain a constant temperature of 170 °C for 25 min. The digested resulting solutions were transferred to 20 mL volumetric tubes with deionized water.

2.3.2. Dry ashing decomposition

This method was adapted from Latimer and G (2012): 2.5 g of the sample were weighted in porcelain capsules, pre-carbonized in a hot plate and incinerated in a muffle furnace (F3-DM/T, Fornitec, São Paulo) with a heating ramp of 1 °C/min until 450 °C for 15 h. The ashes were solubilized using deionized water and 1 mL of HCl 37% (w/v). The final solution was transferred quantitatively to 20 mL volumetric tubes with deionized water and filtered in quantitative filter papers (Nalgon, Itupeva, Brazil).

The evaluation of the sample preparation methods (microwave and dry ashing) and the method validation were performed according to INMETRO (2018) guidelines using as figures of merit linearity, sensibility, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. To these purposes, the sample preparation methods were evaluated using certificate reference material (CRM) Tea Leaves (INCT-TL-1, Instytut Chemii I Techniki Jądrowej, Warszawa, Poland) and a sample of cocoa beans dried from Brazil (in the region of Bahia). In the validation of the dry ashing method, another CRM was used: Peach Leaves (NIST 1547, NIST, Maryland, EUA).

2.4. Statistical analysis

The results were expressed as mean and concentration range. Data were processed using one-way analysis of variance (ANOVA) with mean comparison Tukey (*t*-test) with a significance level of 95% ($p < 0.05$) using software XLSTAT 2012.6.03 (Addinsoft, France).

2.5. Dietary exposure assessment

An intake of Cd and Pb through the consumption of chocolate was estimated considering the worst case scenario: the highest levels of Cd e Pb detected, a consumption of 100% cocoa chocolate and a daily consumption of 15.75 g (5.75 kg year per person) (MDCI, 2018). Body weight was considered as 60 kg for adults and 15 kg for children (FAO/WHO, 2013). Estimated intakes of Cd and Pb were calculated according to Equation (1). Cd intakes were compared to the recommended Provisional Tolerable Monthly Intake (PTMI) of 25 μg kg^{-1} $month^{-1}$ (FAO/WHO, 2013), and Pb levels were compared to Benchmark Dose Lower Confidence Limit ($BMDL_{01}$) of 12 μg kg^{-1} day^{-1} (EFSA, 2013).

$$Ingestion = \frac{Concentration\ x\ Consumption}{Body\ weight}\ \frac{mg}{kg}\ b.w. \quad (1)$$

where: concentration = highest contaminant level detected in the cocoa beans samples (mg kg^{-1}); consumption = consumption of chocolate in a particular period (kg) (15.75 g per day).

Target Hazard Quotient (THQ), a parameter that evaluates the potential risk to health associated with a long term exposition to a certain substance, was also calculated. THQ was proposed by the Environmental Protection Agency (US EPA), being expressed by Equation (2). THQ values lower than or equal to 1 do not suggest adverse effects for the exposed population, while values higher than 1 are considered of concern.

$$THQ = \frac{E_{FR}\ x\ Ed\ x\ F_{IR}\ x\ C}{RfD\ x\ BWa\ X\ ATn} \times 10^{-3} \quad (2)$$

where: E_{fr} is the frequency of exposition to the element (365 days), E_d is the duration of exposition (70 years); F_{ir} is the daily intake rate in g/day; C is the element concentration in food (mg/kg), R_{fD} is the element reference dose in mg/kg/day (Khan et al., 2008; US-EPA 2016), B_{wa} is the reference body weight of 60 kg, AT_n is the average exposition time (365 days × 70 year) and 10^{-3} an unit correction factor.

3. Results and discussion

3.1. Evaluation of sample preparation methods and validation of Cd and Pb analytical method

Both sample preparation methods were evaluated using CRM of Tea Leaves (INCT-TL-1) and a sample of cocoa beans from Brazil (BA). CRM results are depicted in Table 1 and indicate that both methods presented recovery values in the range recommended by AOAC (2016) (80%–110%).

When applying T test to the cocoa beans sample from Brazil, satisfactory results were achieved. Average results (n = 3) for Cd were 0.056 ± 0.001 mg kg^{-1} and 0.055 ± 0.005 mg kg^{-1} for the dry decomposition and microwave methods, respectively. A p-value of 0.760 (t-critical: 2.776) was obtained, indicating no statistical differences between both methods ($p > 0.05$). In the case of Pb, average values (n = 3) were 2.43 ± 0.12 mg kg^{-1} e 2.35 ± 0.34 mg kg^{-1}, with a p-value of 0.711 (t-critical: 2.776), also corresponding to no significant statistical differences. Thus, both methods may be considered adequate for Cd and Pb determination in cocoa beans. For the present study, the dry ashing method was selected due to the lesser need of chemicals, which allows the analyses of a greater number of samples simultaneously and presents lower cost.

Results obtained in the validation of the dry ashing method are shown in Table 1 (B). The limits of detection and quantification were calculated from the standard deviation values (s) of 10 analytical blanks, with LOD = 3s and LOQ = 10s; precision was evaluated by the coefficient of variation of 6 analytical replicates of the same sample of cocoa beans, expressed as a coefficient of variation (CV). The obtained values were satisfactory, with maximum CV of 6%. Accuracy was evaluated using the Tea Leaves CRM, varying from 90% to 104%. Both results are in agreement with values established by AOAC (2016), maximum CV = 11%, acceptable range 80–110%.

Table 1
A- Accuracy of wet and dry methods (n = 3) for the determination of Cd and Pb using Tea Leaves CRM. **B-** Validation parameters of the dry method. Correlation coefficient (r), limits of detection (LOD) and quantification (LOQ), accuracy (CRM of Peach Leaves, n = 3) and precision (coefficient of variation - CV, n = 6).

	Method	Element	Certified value[a] (mg kg^{-1})	Value found (mg kg^{-1})		Recovery (%)
A	Microwave assisted acid digestion	Cd	0.030 ± 0.004	0.032 ± 0.002		107 ± 5
		Pb	1.78 ± 0.24	1.53 ± 0.10		86 ± 6
	Dry ashing decomposition	Cd	0.030 ± 0.004	0.031 ± 0.004		103 ± 14
		Pb	1.78 ± 0.24	1.53 ± 0.16		86 ± 9
B	Dry ashing decomposition		Certified value[b] (mg kg^{-1})	Value found (mg kg^{-1})		Recovery (%)
		Cd	0.0261 ± 0.0022	0.0271 ± 0.0024		104 ± 9
		Pb	0.869 ± 0.018	0.785 ± 0.043		90 ± 5
			r	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	CV[c] (%)
		Cd	0.9998	0.0005	0.0015	3
		Pb	0.9999	0.007	0.022	6

[a] Tea Leaves INCT-TL-1.
[b] Peach Leaves Nist 1547.
[c] CV = Coefficient of variation.

3.2. Occurrence of Cd and Pb in cocoa beans

Cd and Pb levels in cocoa beans from different Brazilian regions (BA, PA, ES and RO), Africa (CI) and Ecuador (EQ) are placed in Table 2 and Fig. S1. It is possible to observe that most samples presented quantifiable levels for Cd (89%) and Pb (94%).

Cd was detected in all samples analysed, with concentrations ranging from <0.015 to 1.59 mg kg^{-1}. The highest levels of Cd were found in the samples from the Brazilian state of Pará (PA), with a mean value of 0.129 ± 0.149 mg kg^{-1}, and Ecuador (EQ), with mean value of 1.15 ± 0.36 mg kg^{-1}. Samples from these regions, being three from PA (10%) and four from EQ (100%), presented levels above the maximum limit established by Brazil and Mercosul (0.3 mg kg^{-1}). When considering the maximum limit set by European Union, all samples from EQ are in discordance (0.6 mg kg^{-1}).

Results obtained for samples from EQ are similar to those reported in previous studies using coca beans samples from Ecuador and Peru (Arévalo-Gardini et al., 2017; Romero-Estévez et al., 2019). As for CI and the other Brazilian regions, lower Cd levels were detected. The detection of high Cd levels in EQ samples was expected, since South America's Northeast region presents soils rich in Cd (Chavez et al., 2015), which could be related to the oil drilling activity on the Ecuadorian Amazon. Barraza et al. (2017) reported Cd levels above regulation limits in soil samples from this region as well, with the highest concentration in superficial layers (2.23 ± 0.07 mg kg^{-1}).

Table 2 also brings the results for Pb in cocoa samples from Africa, Ecuador and different Brazilian regions, which showed a concentration range from <0.022 a 2.528 mg kg^{-1}. The highest levels of Pb were detected in samples from Brazil, with a maximum of 2.528 mg kg^{-1} in PA. PA also has the highest mean level (0.985 ± 0.656 mg kg^{-1}) followed by BA (0.727 ± 0.583 mg kg^{-1}), ES (0.533 ± 0.178 mg kg^{-1}) and RO (0.397 ± 0.216 mg kg^{-1}). The lowest mean values were detected in samples from Ecuador (EQ).

When comparing data obtained with Brazil and Mercosul regulations one can observe that the values detected are above the maximum limits

Table 2
Mean (n = 3), concentration range and percentage of samples above the maximum tolerable limit (MTL) for Cd and Pb (mg kg^{-1}) in cocoa beans from Brazil, Africa and Ecuador.

Country/Region	N	Cd (mg kg^{-1})			Pb (mg kg^{-1})		
		Mean ± SD (range)	Samples >MTL (%)		Mean ± SD (range)	Samples >MTL (%)	
			Brazil/Mercosul[1]	UE[2]		Brazil/Mercosul[1]	UE[2]
Brazil/BA	33	0.060 ± 0.052[a] (<0.015–0.213)	0	0	0.727 ± 0.583[a] (<0.022–2.17)	58	–
Africa/CI	12	0.038 ± 0.028[a] (<0.015–0.064)	0	0	0.629 ± 0.307[a] (0.037–1.04)	75	–
Brazil/ES	04	0.142 ± 0.017[a] (0.124–0.158)	0	0	0.533 ± 0.178[a] (0.365–0.756)	75	–
Brazil/PA	29	0.129 ± 0.149[a] (0.038–0.819)	10	0	0.985 ± 0.656[ab] (0.291–2.53)	79	–
Brazil/RO	08	0.063 ± 0.008[a] (0.052–0.076)	0	0	0.397 ± 0.216[ab] (<0.022–0.624)	63	–
Ecuador/EQ	04	1.153 ± 0.364[b] (0.721–1.59)	100	100	0.019 ± 0.024[b] <0.022–0.050)	0	–

[ab] means values in the columns with the same letter do not differ at the 95% confidence level.
MTL = Maximum tolerable limits for cocoa liquor: Cd = 0.3 mg kg^{-1}; Pb = 0.5 mg kg^{-1} (Brazil, 2013, pp. 33–35; MERCOSUL, 2011)[1] and cocoa powder Cd = 0.6 mg kg^{-1} (EU, 2014)[2].
Regions: CI: Côte d'Ivoire; BA: Bahia; ES: Espírito Santo; PA: Pará; RO: Rondônia e EQ: Equador.

for Pb (0.5 mg kg^{-1}) in nine samples from CI (75%), 23 samples from PA (79%), 19 from BA (58%), five from RO (63%) and three from ES (75%), while EQ samples are in agreement with the same.

A summary of Cd and Pb levels in cocoa beans reported in the last five years is shown in Table 3. It is possible to observe a great variation in the levels of both elements among the different production regions, with the highest levels being reported in samples from Ecuador and Peru. The same high variation was observed in the present study.

3.3. Occurrence of Cd and Pb in roasted cocoa beans and derivate products

Cocoa beans were processed in a pilot scale using the "bulk" method in order to study the presence of Cd and Pb during different stages of the process (ICCO, 2007, pp. 11–14).

Table 3
Means concentrations of Cd and Pb (mg kg^{-1}) reported in recent studies for cocoa beans and derivatives (cocoa powder), from the main producing regions in the world.

Cocoa	Origen		n	Analyte (mg kg^{-1})		Reference
				Cd	Pb	
Beans	Malaysia		86	0.25	0.50	Mohamed et al (2020)
Powder			97	0.33	0.27	
Beans	Ecuador	Esmeraldas	9	1.22	1.83	Romero-Estévez et al (2019)
		Santo Domingo	9	0.420	1.97	
		Guayas	9	1.73	1.75	
		Napo	9	0.280	0.502	
Beans	Peru	Tumbes	10	1.78	2.75	Arévalo-Gardini et al (2017)
		Piura	10	1.55	3.78	
		Cajamarca	10	0.770	1.00	
		Amazonas	10	0.970	2.15	
		San Martín	10	0.790	1.67	
		Huánuco	10	0.640	1.50	
		Junin	10	0.410	2.67	
		Cuzco	10	0.170	1.00	
Nibs	Latin America		7	0.620	0.003	Abt et al (2018)
Beans	Dominican Republic		6	0.128	0.162	Kruszewski et al. (2018)
Powder	Italy		35	0.159	0.417	Lo Dico (2018)
Beans	Western Africa		21	0.093	0.108	Bertoldi et al (2016)
	East Africa		8	0.508	0.101	
	Asia		8	0.328	0.097	
	South America		14	1.39	0.068	
	Central America		10	0.544	0.053	
Beans	Cameroon		10	0.050	0.370	Vītola and Ciproviča (2016)
	Nigeria		10	0.020	0.520	
	Ghana		10	0.017	0.320	

*Samples obtained from Italian markets.

The fermented and dry cocoa beans sample presented 0.113 ± 0.003 mg kg^{-1} of Cd. After processing, this contaminant was also observed in cocoa derived products: shells (0.124 ± 0.004 mg kg^{-1}), cocoa powder (0.111 ± 0.010 mg kg^{-1}), liquor (0.060 ± 0.006 mg kg^{-1}) and cocoa butter (<0.0015 mg kg^{-1}). Yanus et al. (2014) evaluated the contaminants during processing of cocoa beans and, oppositely to the present study, the lower levels of Cd were found in the shells and the higher were in the cocoa powder.

The mass balance of cocoa processing and Cd distribution indicates that, during grinding stage, most of the Cd was retained in the residue in the mill (59%), while 35% was transferred to the liquor and 6% remained in the shell. During pressing, all Cd present in the liquor was transferred to cocoa powder, hence the butter and pressing residue were not contaminated. Therefore, the obtained data show that Cd occurrence is mainly in the non lipidic fractions of cocoa beans. As dark chocolates contain higher levels of solid cocoa, they should be most contaminated by Cd in comparison to milk and white chocolates, which present higher amounts of cocoa butter and powder milk (Villa et al., 2014).

As for Pb, values were below LOQ (<0.022 mg kg^{-1}) in all derivatives, except for the shells, 0.142 ± 0.006 mg kg^{-1}. Therefore, it was not possible to study its distribution during processing.

3.4. Dietary exposure assessment

An intake of Cd and Pb through the consumption of chocolate was estimated considering the worst case scenario: the highest levels of Cd and Pb detected were transferred in its totality to the final product (chocolate), a daily intake of 15.75 g of chocolate in Brazil (MDCI, 2018) and a body weight of 60 kg for adults and 15 kg for children. These results are summarized in Table 4.

According to Table 4, monthly intake of Cd varied from 0.51 to 12.80 μg/kg b.w., for adults, and from 2.04 to 51.10 μg/kg b.w., for children. The highest value was from EQ, where the intake reached 51% of PTMI for adults and 204% for children. In the case of PA samples, intake of Cd reached 26% of PTMI for adults and 105% for children. In both scenarios the consumption of chocolate alone would be enough to reach the monthly tolerable intake values of Cd.

Given the same considerations for Pb, daily intake resulted in values ranging from 0.01 to 0.66 μg/kg b.w. for adults and from 0.05 to 2.65 μg/kg b.w. for children, reaching a maximum of 6% of $BMDL_{01}$ for adults and 22% for children in PA samples.

The biggest consumer of chocolate in the world is Germany, with a mean year consumption of 11.1 kg/year (30.4 g/day) (Chocosuisse, 2018). Keeping the same considerations made so far, monthly intake of Cd would be of 99% of PTMI for adults and 398% for children. Pb daily intake would be of 11% of $BMDL_{01}$ for adults e 43% for children.

The risk assessment based on THQ calculation, using the same

Table 4
Cd and Pb dietary exposure. Considerations: maximum levels (mg kg^{-1}) by region studied and consumption of 479.16 g (monthly) and 15.75 g (daily) of 100% cocoa chocolate for adults (60 kg) and children (15 kg).

Element	Cocoa beans origin	Maximum concentration found (mg kg^{-1})	Adults (60 kg)		Children (15 kg)	
			Estimated monthly intake (μg/kg bw)	% PTMI	Estimated monthly intake (μg/kg bw)	% PTMI
Cd	BA	0.213	1.70	7	6.80	27
	CI	0.064	0.51	2	2.04	8
	ES	0.158	1.26	5	5.05	20
	PA	0.819	6.54	26	26.16	105
	RO	0.076	0.61	2	2.43	10
	EQ	1.60	12.80	51	51.10	204
			Estimated daily intake (μg/kg bw)	% $BMDL_{01}$	Estimated daily intake (μg/kg bw)	% $BMDL_{01}$
Pb	BA	2.17	0.57	5	2.28	19
	CI	1.04	0.27	2	1.09	9
	ES	0.756	0.20	2	0.79	7
	PA	2.52	0.66	6	2.65	22
	RO	0.624	0.16	1	0.66	5
	EQ	0.050	0.01	0	0.05	0

PTMI (Provisional Tolerable Monthly Intake) for Cd = 25 μg kg^{-1} body weight; **$BMDL_{01}$** (Benchmark dose of 1% extra risk for developmental neurotoxicity) for Pb = 12 μg kg^{-1} body weight.
CI: Côte d'Ivoire; BA: Bahia; ES: Espírito Santo; PA: Pará, RO: Rondônia e EQ: Equador.

considerations applied for the assessment of PTMI for Cd and $BMDL_{01}$ for Pb, showed results below 1. For THQ values to exceed the unit (THQ>1), with the possibility of risk, it is necessary to consume 42 g/day for Cd and 80 g/day for Pb – values that can be reached by frequent chocolate consumers. The data obtained in the present study show that the consumption of chocolate produced from cocoa beans containing Cd and Pb can contribute to the ingestion of toxic elements.

4. Conclusions

The dry ashing decomposition method followed by quantification using ICP OES was shown to be adequate to the determination of Cd and Pb levels in cocoa beans and their derivatives, with satisfactory results for accuracy and precision.

Cd and Pb levels were found to be above the maximum limits established by Brazil and Mercosul in 8% and 66% of the samples, respectively. EQ samples presented the highest mean levels for Cd and the lowest for Pb, while the highest levels of Pb were from Brazil samples originated from PA and BA states, which could be correlated to the origin of harvest and production. The use of beans from different regions (blends) in the production of cocoa products may be a viable alternative for reducing inorganic contaminants levels in the final product.

The obtained results show that a consumption of chocolate higher than 42 g per day may represent a health risk for adults and that children are most susceptible to the risk due to the appeal for this public. Finally, the results point the presence of Pb in cocoa beans as an issue that must be addressed in future studies.

Declaration of competing interest

The authors declare that there is no potential competing interest with respect to the research, authorship, and/or publication of this paper.

CRediT authorship contribution statement

Ana Paula Ferreira de Oliveira: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Raquel Fernanda Milani:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Priscilla Efraim:** Conceptualization, Methodology, Validation, Investigation, Resources, Supervision. **Marcelo Antonio Morgano:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Silvia Amelia Verdiani Tfouni:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2020.107455.

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