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Trace elements in bean-to-bar chocolates from Brazil and Ecuador

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ABSTRACT

Background: The high quality and unique flavor and aroma of bean-to-bar chocolates have resulted in an increase in the consumption of these products. Nevertheless, cocoa beans may present inorganic contaminants from environmental and anthropogenic sources which can contribute to contamination of the chocolates, despite the fewer processing steps and few ingredients used in bean-to-bar manufacturing process compared to the industrial one. Therefore, this study aimed to evaluate the content of trace elements (As, Cd, Co, Cu, Hg, Pb, Se) in bean-to-bar chocolates and traceable cocoa beans from Brazil and Ecuador.

Methods: Bean-to-bar chocolate samples were acquired in Brazil (n=65) and Ecuador (n=10), considering the main products available: white, milk, semisweet and dark chocolate. Cocoa samples from dedicated farms (n=23) were analyzed for trace elements and inorganic contaminants regulated by Brazil and European agencies. Samples were mineralized using acid digestion (nitric acid and hydrogen peroxide) in a closed microwave-assisted system. Quantification of trace elements was performed using Inductively coupled plasma mass spectroscopy (ICP-MS) and Inductively coupled plasma optical emission spectroscopy (ICP OES) in optimized conditions. The analytical control was performed with certified reference materials (ERM BD512 - Dark Chocolate, Tort-2 and Tort-3 - Lobster Hepatopancreas and SRM 1547 - Peach leaves) and recoveries ranged between 84% and 105% for all elements.

Results: The trace element levels in the bean-to-bar chocolates were (mg/kg): As (<0.022–0.023), Cd (<0.002–0.74), Cu (0.11–21.2), Co (<0.003–1.88), Hg (<0.010-<0.010), Pb (<0.007–0.22), and Se (<0.029–0.35). The exposure assessment from inorganic contaminants in chocolates revealed up to 93% of provisional tolerable monthly intake (PTMI) for Cd and 123% of tolerable upper intake level (UL) for Co for children. Inorganic contaminants were also analyzed in cocoa beans from dedicated farms and Cd and Pb levels were found above the thresholds established by Brazil health agency.

Conclusion: The results observed for both bean-to-bar chocolates and raw materials (cocoa beans from dedicated farms) indicated a need for monitoring these trace elements.

1. Introduction

The interest in artisanal chocolates has been growing, with an emphasis on bean-to-bar chocolates, due to few manufacturing processes, the preservation of cocoa butter, and a few ingredients used in the formulation (organic sugar, milk powder, dried fruits, or oilseeds) compared to the industrial scale production [1,2]. In general, cocoa beans are ground under high temperatures in an electric stone grinder, known as melanger [1,2]. In addition, bean-to-bar chocolates are produced with traceable cocoa beans, which provides a high-quality standard and valorization for small-scale producers. Brazil is one of the world's largest producers of cocoa, accounting for approximately 5% of its global production [3].

Although several compounds with health benefits have been reported for cocoa beans (e.g., antioxidants, flavonoids, minerals), recent studies have shown the presence of inorganic contaminants in this product and its raw material (cocoa beans) [4–7]. Several studies have focused on Cd and Pb levels in cocoa beans and chocolates due to its association to adverse health effects, including cancer [8] and being consumed by children [9]. The mechanism for Cd and Pb accumulation in the cocoa fruit is currently limited. Blommaert *et al.* (2022) [10] recently studied the Cd translocation in a high Cd accumulating cultivar of *Theobroma cacao L.* and identified three major groups of ligands that bind Cd in cacao: O(C)-ligands (Cd-hydrated, Cd-cellulose, Cd-cell wall,

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Cd-Ca oxalate and Cd-organic acids), O(P)-ligands (R-PO_4H_2 ligands represented by Cd-phosphate and Cd-phytate) and S-ligands (Cd-phytochelatin, Cd-cysteine, Cd-glutathione and Cd-metallothionein). The authors also reported and 10–28-fold increase on Cd concentration in plant versus the topsoil and the following pattern of Cd levels in cocoa: placenta < nib < testa < pod husk < root < leaf < branch.

De Oliveira *et al.* [6] studied cocoa bean samples from Brazil, Ecuador, and West Africa reported levels higher than the threshold established by the Brazilian regulatory agency, ranging from <1.5–1590 µg/kg and <22–2530 µg/kg for Cd and Pb, respectively. Yannus *et al.* [7] also examined Cd and Pb in cocoa powder and cocoa bean samples of chocolate importers from Israel and reported values ranging from 125 and 103 ng/g and 72 and 40 µg/kg), respectively. Inorganic contaminants in conventional chocolate samples from Europe, the USA, and Israel were also studied by Yannus *et al.* [7], revealing values between 65 and 141 and 86–230 µg/kg for Cd and Pb, respectively. Villa *et al.* [5] investigated Cd and Pb in chocolates made with cocoa contents between 34% and 85% and reported similar concentrations, ranging from <1.7–0.108 and <21–0.138 µg/kg, respectively.

Even though bean-to-bar chocolates represent an expanding market, and it is largely consumed by children and adults, to the best of our knowledge, there is still a lack of studies concerning trace elements in this product. Thus, the main objective of this study was to evaluate the occurrence of trace elements arsenic, cadmium, cobalt, copper, mercury, lead, and selenium in samples from Brazil and Ecuador. Sampling considered the products available at the market (local and e-commerce) and bean-to-bar chocolates with different percentages of cocoa solids (white, milk, semisweet and dark chocolate). In addition, traceable cocoa beans from farms in the main producing regions of Brazil (Amazonas, Bahia, Pará, Espirito Santo) were also acquired to verify their trace element contents.

2. Material and methods

2.1. Sampling

The bean-to-bar were acquired from local markets and e-commerce, consisting of 75 samples made with cocoa beans from Brazil (Amapa, AP; Amazonas, AM; Espirito Santo, ES; Bahia, BA; Rondonia, RO; Para, PA; Atlantic Forest and Amazon Forest) and Ecuador (Cayambe, Esmeraldas, Los Rios, Manabi and Quito). The samples were classified according to their labels (Table S1), considering the percentage of cocoa mass in the chocolate and if milk was an ingredient: 0% (white), 40–56% (milk chocolate), 40–68% (semisweet), and 70–100% (dark).

Traceable cocoa bean samples (n=23) were acquired from farms located in the main producing regions of Brazil: BA (Arataca, Ibirapitanga, Itabuna, Itajuipe, and Porto Seguro), PA (Novo Repartimento and Mocajuba), AM (Borba, Manicore, and Nova Olinda), and ES (Linhares).

Samples were homogenized in analytical mill (IKA, Staufen, Germany) (cocoa beans) or grater (bean-to-bar chocolates) and were kept in their original packaging or sterile plastic bags under refrigeration until analysis.

2.2. Reagents and solutions

The reagents used in the experiment were of analytical grade, as follows: nitric acid purified by sub-boiling distillation (Distillacid, Berghof, Eningen, Germany), hydrogen peroxide 30% (Merck, Darmstadt, Germany), reverse osmosis-purified water (Gehaka, São Paulo, Brazil), and certified standard solutions (Specsol/Quimlab, Jacarei, Brazil) of 100 mg/L for As, Cd, Cu, Co, Cd, and Pb and 1000 mg/L for Se and Hg.

2.3. Determination of trace elements in bean-to-bar chocolates

The samples were mineralized using a microwave system (Start D, Milestone, Sorisole, Italy). Briefly, 0.5 g of samples were weighed into a PTFE digestion flask and 6 mL of nitric acid was added and kept overnight. Then, 2 mL of hydrogen peroxide was added, the vessels were closed, and heated to 170 °C for 37 minutes. At the end of the digestion, the resulting solution was transferred to graduate tubes with reverse osmosis-purified water (25 mL). The assays were performed in analytical triplicate, in the presence of blank experiments. As, Cd, Co, Cu, Hg, Pb, and Se quantification was conducted by Inductively Coupled Plasma Mass Spectroscopy, ICP-MS (iCAP RQ, Thermo Fisher Scientific, Bremen, Germany) under optimized conditions [11]: frequency power 1.55 kW, Ar flow rate 14.0 L/min, auxiliary Ar flow rate of 0.80 L/min, helium (He) flow rate of 5.00 mL/min, MicroMist nebulizer at a flow rate of 0.98 L/min, cooled double-pass nebulization chamber (2.8 ºC), KED mode (Kinetic Energy Discrimination), isotopes (*m/z*) ^{75}As, ^{59}Co, ^{63}Cu, ^{78}Se, ^{80}Se, ^{111}Cd, ^{202}Hg, and ^{208}Pb, and internal standards (50 µg L^{-1}) ^{72}Ge, ^{103}Rh, ^{45}Sc, ^{209}Bi, and ^{195}Pt. Analytical curves for all analytes ranged from 0.0001 to 0.1 mg/L.

2.4. Determination of trace elements in traceable cocoa beans

Traceable cocoa beans were mineralized using a microwave digester, as previously described in item 2.3. Due to the total dissolved solid content in the resulting solution, As, Cd, Cu and Pb quantification was conducted by Inductively Coupled Plasma Optical Emission Spectroscopy, ICP OES (5100 VDV, Agilent Technology, Tokyo, Japan). Optimized conditions were [6]: frequency power of 1.20 kW, seaspray nebulizer with a flow rate of 0.50 L/min, auxiliary argon (Ar) flow rate of 1.0 L/min, Ar flow rate of 12.0 L/min, axial view, double-pass nebulizer chamber, and the wavelengths (nm) for As (193.696), Cd (214.439), Cu (324.754) and Pb (220.353). Analytical curves ranged from 0.005 to 1.00 mg/L (As), 0.001–1.00 mg/L (Cd, Cu), and 0.0025–1.00 mg/L (Pb).

2.5. Analytical validation and statistical analysis

The proposed methods were evaluated considering the figures of merit [12,13]: limit of detection (LOD) and quantification (LOQ), linearity, accuracy, and precision. The LOD and LOQ were estimated using blank experiments (n=7), being the analyte concentration corresponding to the blank value plus 3 and 5 times the standard deviation, respectively. Linearity was evaluated using residuals plot and the correlation coefficient and method accuracy was evaluated using certified reference materials available for trace elements (n=3): Dark Chocolate (ERM BD512, Joint Research Center, Geel, Belgium), Lobster Hepatopancreas (Tort-2 and Tort-3, National Research Council, Ottawa, Canada) and Peach leaves (SRM 1547, National Institute of Standards and Technology, Gaithersburg, USA). The precision (coefficient of variation CV, n=7) was estimated using seven independent analytical replicates for each matrix (chocolate and cocoa beans).

Statistical analysis was performed using one-way ANOVA (Analysis of Variance) and Tukey test, at 95% confidence (Statistica - StatSoft, Tulsa, USA).

2.6. Exposure assessment

To estimate the exposure to trace elements from chocolate consumption, we considered the daily intake (DI) of 15.75 g of chocolate (472.5 g/month or 5.75 kg/year) [14]; the standard body weight for adults (60 kg) and children (15 kg); the maximum levels found for As, Cd, Co, Cu, Hg, Pb, Se, using Eq. (1) [15].

$$Exposure(mg / kg \quad bw) = \frac{Maximum \quad level \quad x \quad Daily \quad intake}{Body \quad weight} \tag{1}$$

To characterize the risk associated with exposure to trace elements, the results were compared to the available health-based guidance values

Table 1
Analytical parameters for the determination of the trace elements in bean-to-bar chocolates and traceable cocoa beans.

Trace element	Technique	Parameters						
		Certified value (mg/kg)	Experimental value (mg/kg, n=3)	Recovery (%)	Correlation coefficient (r)	Limit of detection (mg/kg)	Limit of quantification (mg/kg)	CV (%, n=7)
As	ICP-MS	59.5 ± 3.8	60.8 ± 0.7	102 ± 1	0.9996	0.015	0.022	11
Cd	ICP-MS	0.302 ± 0.013	0.271 ± 0.008	90 ± 3	1.0000	0.001	0.002	3
Co	ICP-MS	0.07	0.066 ± 0.001	94 ± 1	0.9999	0.002	0.003	2
Cu	ICP-MS	14.3 ± 0.7	14.0 ± 1.1	98 ± 8	0.9996	0.007	0.01	2
Hg	ICP-MS	0.292 ± 0.022	0.245 ± 0.028	84 ± 10	0.9999	0.007	0.01	9
Pb	ICP-MS	0.869 ± 0.018	0.881 ± 0.011	101 ± 1	0.9999	0.005	0.007	14
Se	ICP-MS	0.120 ± 0.017	0.126 ± 0.016	105 ± 14	0.9999	0.019	0.029	12
As	ICP OES	21.6 ± 1.8	19.0 ± 0.4	88 ± 2	0.9996	0.17	0.27	13
Cd	ICP OES	26.7 ± 0.6	25.1 ± 0.6	94 ± 2	0.9999	0.02	0.03	5
Cu	ICP OES	106 ± 10	105 ± 2	99 ± 2	0.9999	0.04	0.07	4
Pb	ICP OES	0.35 ± 0.13	0.34 ± 0.05	98 ± 14	0.9998	0.06	0.09	7

Certified reference materials: Cd and Cu (ERM BD512, Dark Chocolate); Pb, Se and Co (SRM 1547, Peach leaves); As and Hg (Tort-2 and Tort-3, Lobster Hepatopancreas). Experimental values expressed as mean ± uncertainty (type A); CV= coefficient of variation.

Table 2
Mean (range) of trace element levels in cocoa beans (n=23) from cocoa dedicated farms in Brazil.

Region	Trace elements (mg/kg)						
	As (ICP OES)	Cd (ICP OES)	Co (ICP-MS)	Cu (ICP OES)	Hg (ICP-MS)	Pb (ICP OES)	Se (ICP-MS)
Amazonas (AM, n=8)	<0.17	0.68[c] (0.52–0.89)	0.80[a] (0.70–0.91)	18.2[a] (15.2–24.8)	<0.010	<0.06 (<0.06–0.094)	<0.029 (<0.029–0.030)
Bahia (BA, n=11)	<0.17	0.056[a] (0.027–0.15)	0.62[a] (0.59–0.64)	19.1[a] (10.3–26.9)	<0.010	<0.06 (<0.06–0.31)	0.22[a] (<0.029–0.44)
Para (PA, n=2)	<0.17	0.064[a] (0.042–0.087)	0.75[a] (<0.003–1.96)	22.4[a] (16.6–28.1)	<0.010	<0.06	<0.029
Espirito Santo (ES, n=2)	<0.17	0.30[b] (0.16–0.44)	1.19[a] (0.87–1.60)	19.5[a] (16.0–23.0)	<0.010	<0.06	0.13[a] (0.10–0.14)

[a,b]Different letters for the same element (column) indicates significant difference, according to Tukey's test ($p < 0.05$).

Table 3
Mean (range) of trace elements (ICP-MS) in bean-to-bar chocolates (n=75) produced with cocoa beans from Brazil and Ecuador.

Cocoa bean origin		Trace elements (mg/kg)						
		As	Cd	Co	Cu	Hg	Pb	Se
Brazil	Amazonas (AM, n=7)	<0.015	0.14[a] (<0.001–0.74)	0.17[a] (<0.002–0.54)	7.34[a] (0.20–12.8)	<0.007	0.014[ab] (<0.005–0.027)	0.075[a] (<0.029–0.28)
	Amapa (AP, n=2)	<0.015	0.054[a] (0.007–0.10)	0.15[a] (0.054–0.25)	7.17[ab] (5.67–8.66)	<0.007	0.019[ab] (0.016–0.021)	0.044[a] (0.034–0.053)
	Bahia (BA, n=36)	<0.015	0.046[a] (0.009–0.18)	0.39[a] (0.027–0.92)	8.51[a] (1.63–15.7)	<0.007	0.012[a] (<0.005–0.054)	0.083[a] (<0.029–0.28)
	Espirito Santo (ES, n=8)	<0.015	0.046[a] (0.008–0.094)	0.49[a] (<0.002–0.94)	7.16[a] (1.69–11.4)	<0.007	0.016[ab] (<0.005–0.037)	0.058[a] (<0.029–0.23)
	Amazon Forest (n=2)	<0.015	0.081[a] (0.04–0.12)	0.77[a] (0.46–1.07)	12.6[ab] (7.36–17.9)	<0.007	<0.005[ab] (<0.005–0.008)	<0.029[a] (<0.029–0.017)
	Atlantic Forest (n=4)	<0.015	0.036[a] (0.012–0.061)	0.29[a] (0.15–0.43)	7.55[ab] (3.60–8.42)	<0.007	<0.005[ab] (<0.005–0.008)	<0.029[a] (<0.029–0.063)
	Para (PA, n=4)	<0.015	0.021[a] (<0.001–0.067)	0.62[a] (0.012–1.88)	8.74[ab] (0.11–19.3)	<0.007	0.010[ab] (<0.005–0.039)	0.064[a] (<0.029–0.18)
	Rondonia (RO, n=2)	<0.015	0.024[a] (0.016–0.031)	0.15[a] (0.15–0.15)	4.46[ab] (2.96–5.96)	<0.007	0.015[ab] (0.012–0.017)	0.11[a] (0.084–0.14)
Ecuador (n=10)		<0.015[a] (<0.015–0.023)	0.43[b] (0.24–0.63)	0.32[a] (0.19–0.55)	14.0[b] (9.77–21.2)	<0.007	0.052[b] (<0.005–0.22)	0.17[a] (0.091–0.35)

[a,b]Different letters for the same element (column) indicates significant difference, according to Tukey's test ($p < 0.05$).

established in the literature: Provisional Tolerable Monthly Intake (PTMI) for Cd [8], Provisional Tolerable Weekly Intake (PTWI) for Hg [8] and Provisional Maximum Tolerable Daily Intake (PMTDI) for Cu [8], Tolerable upper intake level (UL) for Co [16,17] and for Se [18], and Benchmark Dose Lower Limit (BMDL) for Pb [19] and As [8].

3. Results and discussion

3.1. Trace elements in traceable cocoa beans for bean-to-bar chocolate production

The method for As, Cd, Cu and Pb in traceable cocoa beans (ICP OES) was validated and the results were presented in Table 1.

LOD and LOQ ranged from 0.02 to 0.17 mg/kg and 0.03–0.27 mg/kg, respectively, for all analytes and were considered adequate

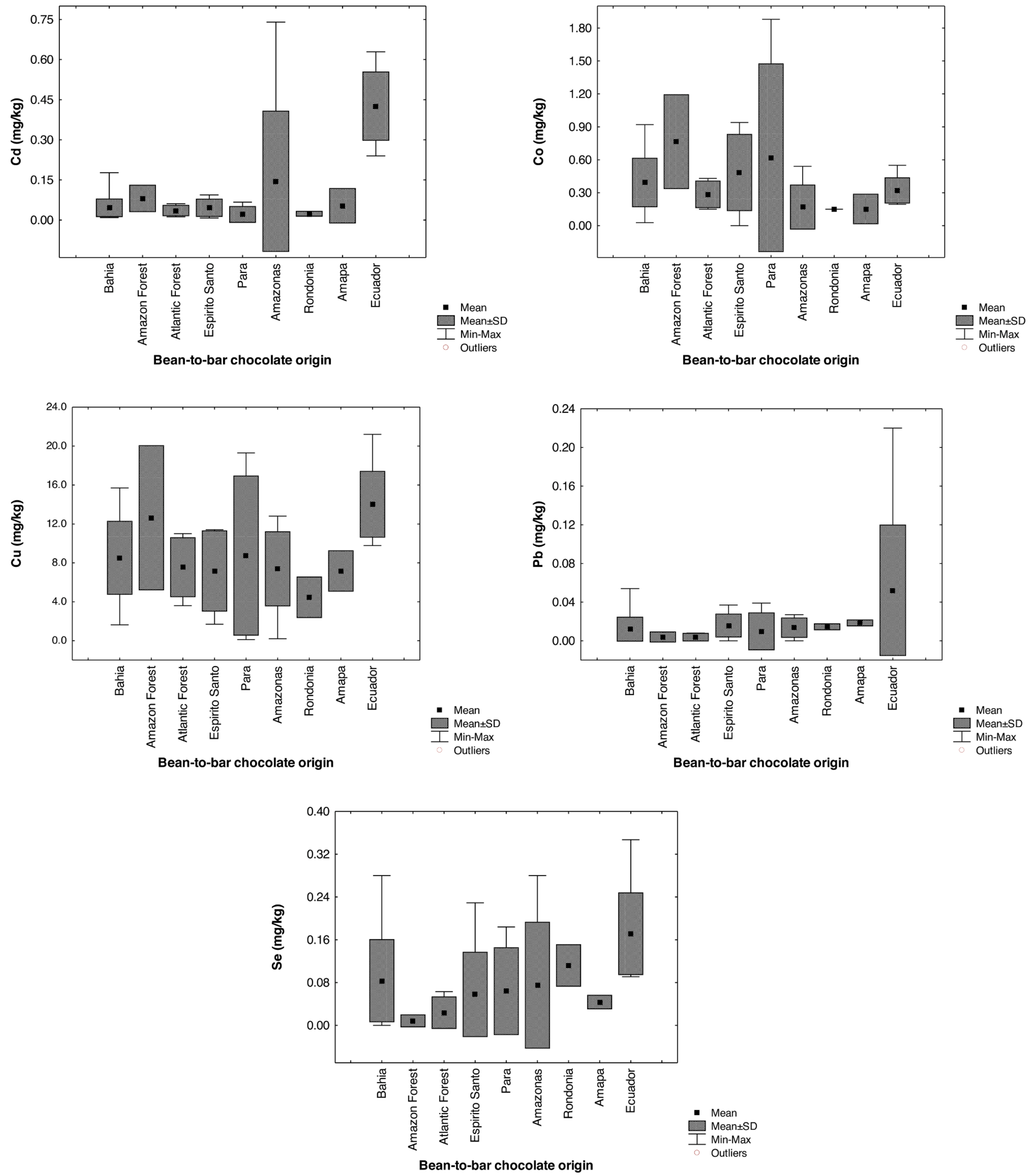

Fig. 1. Boxplot diagrams for Cd, Co, Cu, Se and Pb (ICP-MS) in bean-to-bar chocolates (n=75) produced with cocoa beans from Brazil and Ecuador.

according to the thresholds established for cocoa mass or cocoa beans (As = 0.50 mg/kg; Cd = 0.3 mg/kg; Pb = 0.50 mg/kg; Cu = 40 mg/kg) [20] and for cocoa powder (Cd = 0.60 mg/kg) [21] by Brazilian and European regulatory agencies, respectively. Analytical curves were considered linear (r>0.999, null residual plot) for all elements and the coefficient of variation (CV) for 7 replicates was between 4% and 13%. The recovery for all certified reference materials were within the AOAC [13] and INMETRO [12] recommendations, ranging from 88% to 99%.

Table 2 presents the results for trace elements in traceable cocoa beans intended for bean-to-bar chocolate production.

In general, the results indicated a tendency in the trace element levels in cocoa beans: [Cu] > [Co] > [Cd] > [Pb] > [Se] > [As] and [Hg], being As and Hg not detected in any sample from Brazil. Co and Se maximum levels were found in samples from PA and BA, respectively. In addition, samples were found within the maximum tolerable limits established by the Brazilian and European regulations [20,21], except for Cd in samples from the AM region, which presented mean values of 0.68 mg/kg.

For Cd, the results range within two orders of magnitude, with higher and lower values of the samples from AM and PA regions (0.68 and

Table 4
Mean (range) of trace elements (ICP-MS) in bean-to-bar chocolates (n=75), according to their composition.

Trace element (mg/kg)	Chocolate composition			
	White (n=2)	Milk (n=15)	Semisweet (n=18)	Dark (n=40)
As	<0.015	<0.015	<0.015[a] (<0.015–0.023)	<0.015
Cd	<0.001	0.030[a] (0.009–0.094)	0.11[a] (0.004–0.63)	0.13[a] (0.008–0.74)
Co	0.006[a] (<0.002–0.012)	0.29[a] (0.06–0.94)	0.31[a] (0.027–0.59)	0.46[a] (<0.002–1.88)
Cu	0.15[b] (0.11–0.20)	5.87[ab] (1.63–12.2)	8.03[a] (2.51–16.0)	10.9[c] (2.4–21.2)
Hg	<0.007	<0.007	<0.007	<0.007
Pb	0.020[a] (<0.005–0.039)	0.012[a] (<0.005–0.038)	0.019[a] (<0.005–0.12)	0.019[a] (<0.005–0.22)
Se	<0.029	0.059[a] (<0.029–0.25)	0.086[a] (<0.029–0.35)	0.099[a] (<0.029–0.28)

[a,b]Different letters for the same element (lines) indicates significant difference, according to Tukey's test ($p < 0.05$).

0.064 mg/kg, respectively). For Pb, few samples showed detectable levels, with the highest value observed in the BA sample (0.31 mg/kg). For Cu, values found ranged from 10.3 to 28.1 mg/kg, agreeing with the study reported by Medauar *et al.* [22] for not-traceable cocoa beans, who found levels from 18.8 to 30.4 mg/kg, with the highest level found in samples from PA region (22.4 mg/kg).

In general, the Cd and Pb levels reported in literature for not-traceable cocoa beans were lower than those found in our study, except for samples originates in Ecuador and Peru. Kruszewski *et al.* [23] reported mean Cd and Pb levels of 0.128 and 0.162 mg/kg, respectively, in samples from the Dominican Republic, which were lower than those found by Bertoldi *et al.* [24] in 61 cocoa samples from Africa, Asia, South America, and Central America, ranging from 0.093 to 1.39 and 0.053–0.108 mg/kg for Cd and Pb, respectively. Vītola *et al.* [25] studied samples from Africa (South Africa, Cameroon, Nigeria, and Ghana) and found lower Cd and Pb levels than our study, ranging from 0.017 and 0.050 and 0.320 and 0.520 mg/kg, respectively.

Cocoa beans intended for bean-to-bar chocolate are mainly produced on small farms, comprehending the harvesting and post-harvest process (such as fermentation, drying and storage) [26]. Higher values were reported by Romero-Estévez *et al.* [27], who evaluated 36 samples from Ecuador and observed contents ranging from 0.280 to 1.73 mg/kg and 0.502–1.97 mg/kg, respectively. De Oliveira *et al.* [6] reported Cd and Pb levels from 0.060 to 0.142 mg/kg and 0.019–0.985 mg/kg, respectively, in cocoa bean samples from Brazil, Ecuador, and Ivory Coast. Arévalo-Gardini *et al.* [28] found high contents in samples from Peru, ranging from 0.170 to 1.78 mg/kg for Cd and 1.00–3.78 mg/kg for Pb.

3.2. Occurrence of trace elements in bean-to-bar chocolate

The results of quality control for trace elements in bean-to-bar chocolate are presented in Table 1. LOD and LOQ ranged from 0.001 to 0.015 mg/kg and 0.002–0.022 mg/kg, respectively, and were considered adequate for all trace elements. Accuracy was verified using certified reference materials and recovery values ranged between 84% and 102% for all elements in accordance with AOAC [13] and INMETRO [12] recommendations. Analytical curves were considered linear (r>0.999, null residual plot) and the coefficient of variation (CV) for 7 replicates were between 2% and 14%.

Table 3 and Fig. 1 show the results of trace elements in bean-to-bar chocolates classified according to the origin of the cocoa beans used in the manufacturing processes.

As can be seen in Table 3 and Fig. 1, the highest Cd, Pb and Se contents were observed for the samples from Ecuador, with values of 0.63, 0.22 and 0.35 mg/kg, respectively. Mean values of these trace elements were also higher than chocolate samples produced with cocoa beans from Brazil, indicating a possible influence of environmental conditions in producing farms, such as soil [10]. The element Cu was detected in all samples, with an average content of 8.93 ± 4.51 mg/kg (n=75), and the highest concentrations were observed in samples from PA and Ecuador (19.3 and 21.2 mg/kg, respectively). For Co, the highest levels were found in samples from PA and Amazon Forest regions (1.88 and 1.07 mg/kg), respectively.

Conventional chocolate samples were studied by several authors, mainly focusing on the inorganic contaminants As, Cd and Pb. In this type of chocolate, the information concerning the origin of cocoa beans is not available and, generally, the industries use raw material from different cocoa producers (pool or cocoa bulk) [1]. The contents of trace elements reported in the literature are similar to those found in our study. Salama [29] studied 19 chocolate samples commercialized in the United Arab Emirates and reported As, Co, Cu, Hg, and Pb contents of 0.025, 2.09, 0.039, 0.048, and 0.078 mg/kg, respectively. Peixoto *et al.* [4] investigated trace elements in white chocolate, milk chocolate, and dark chocolate and found As, Co, Cu, and Se levels of 0.001, 0.005 (LOQ), 0.17, and 0.02 mg/kg; 0.01, 0.087, 3.30, and 0.04 mg/kg; and 0.01, 0.36, 12.18, and 0.07 mg/kg, respectively. Lower Cd and Pb contents were reported by Kruszewski *et al.* [23] in conventional chocolates, ranging from 0.107 to 0.429, and 0.0013 (LOD) to 0.03 mg/kg, respectively.

Bravo *et al.* [30] reported a decrease of up to 40% in initial Cd contents in cocoa beans when compared to chocolate, which ranged from 4.17 to 1.60 mg/kg, respectively. Abt *et al.* [31] also reported higher Cd and Pb contents in cocoa powder and nibs, with values of 0.70 and 0.11, and 0.62 and 0.003 mg/kg, respectively, when compared to dark chocolate and milk chocolate (0.27–0.03, and 0.06–0.01 mg/kg, respectively). To further investigate statistical differences between the bean-to-bar chocolates, Table 4 and Fig. 2 present the results of the trace elements as a function of the concentration of cocoa solids (white, semisweet, and dark chocolate) and presence of milk (vegetal, bovine or goat) (milk chocolate).

Trace element contents in bean-to-bar chocolates indicated a tendency to [Cu] > [Co] > [Cd] > [Se] > [Pb] > [As]. Levels below the detection limit were observed for Hg for all samples studied. The elements Cu, Co, and Cd were detected in more than 97% of the samples, with values ranging from 0.11 to 21.2 mg/kg, <0.003–1.88 mg/kg, and <0.001 and 0.74 mg/kg, respectively. For Pb and Se, about 70% of the bean-to-bar chocolates presented detectable levels, ranging from <0.005–0.35 mg/kg and from <0.029–0.22 mg/kg, respectively.

Concerning the maximum limits established by Brazilian and European regulations (Table S2) [20,21], only Cd were found higher than the Brazilian thresholds: four samples (22%) of semisweet chocolate from Ecuador (0.24, 0.27, 0.43, and 0.63 mg/kg) and seven samples (18%) of dark chocolate from Ecuador and AM region (0.31, 0.35, 0.46, 0.49, 0.53, 0.55, and 0.74 mg/kg).

The highest trace element concentrations were observed in bean-to-bar chocolates containing higher cocoa contents (dark or semisweet),

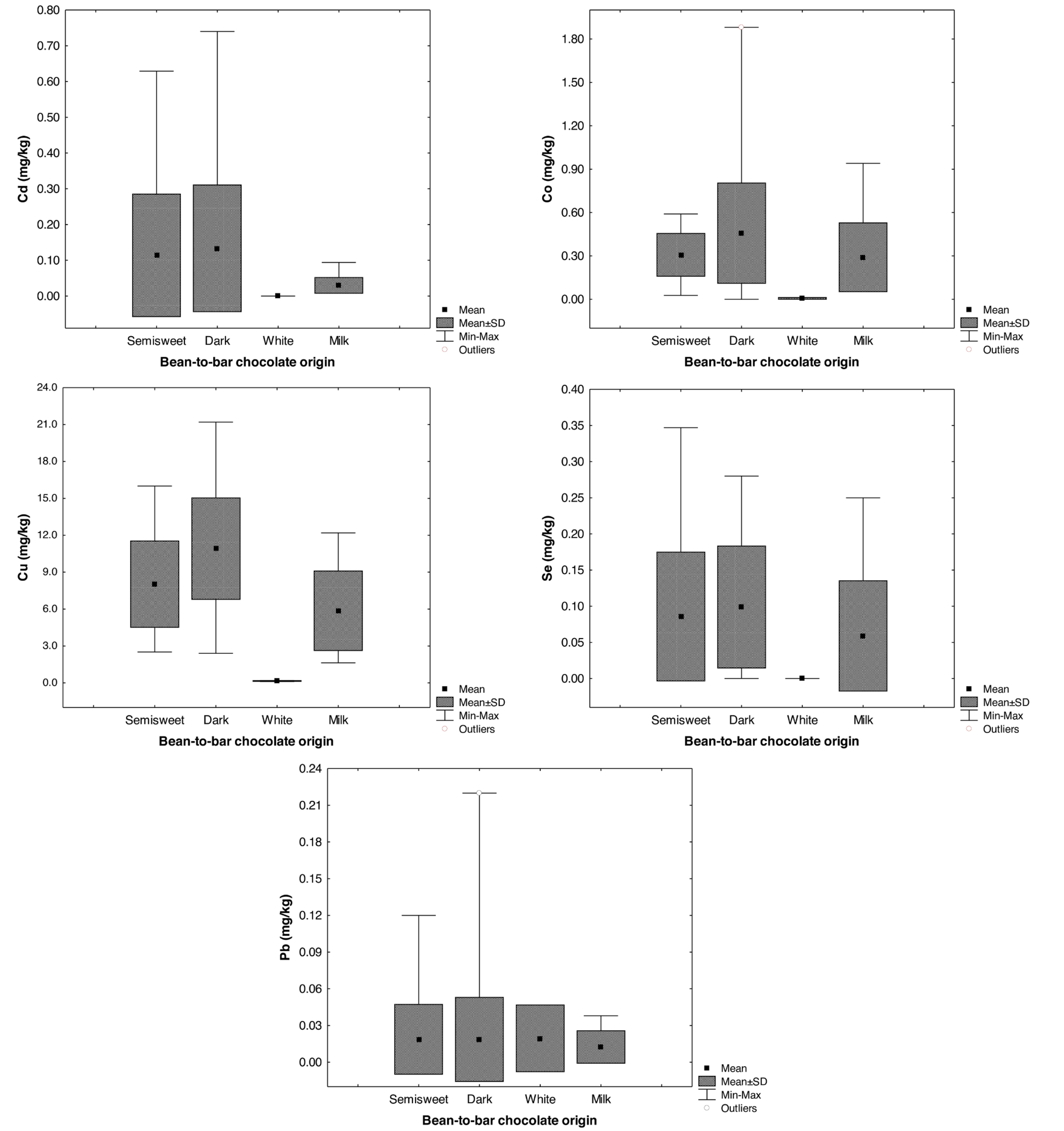

Fig. 2. Boxplot diagrams for Cd, Co, Cu, Se and Pb (ICP-MS) in bean-to-bar chocolates (n=75), according to their composition.

indicating a direct correlation between cocoa solids and the presence of trace elements. This tendency corroborates the results reported by Villa *et al.* [5], who analyzed 30 chocolate samples from Brazil (55–85%) and reported a linear correlation between cocoa contents and the levels of the contaminants Cd and Pb in chocolates.

3.3. Exposure assessment

To estimate the exposure of trace elements through bean-to-bar chocolate consumption, the highest concentrations observed were considered: As = 0.015 mg/kg (LOQ), Cd = 0.74 mg/kg,Co = 1.88 mg/kg, Cu = 21.2 mg/kg, Hg = 0.007 mg/kg (LOQ), Pb = 0.22 mg/kg, and Se = 0.35 mg/kg. Standard body weight for adults (bw = 60 kg) and children (bw = 15 kg) and an annual consumption of 5.75 kg of chocolate were considered [14], corresponding to a daily consumption of 15.75 g and a month consumption of 472.5 g. The estimated values were compared with established exposure values of PTMI for Cd (25 μg/kg bw); PTWI for Hg (4 μg/kg bw); the UL for Co (0.0016 mg/kg bw) and Se (255 and 130 μg daily for adults and children aged 7–10 years, respectively); BMDL for Pb (12 μg/kg bw) and for As (3 μg/kg bw); and PMTDI for Cu (0.5 mg/kg bw) [8,16–19].

The daily consumption of one serving of bean-to-bar chocolate revealed low As, Cu, Hg and Pb exposure for adults and children, corresponding to 0.13% and 0.53% of BMDL, 1.1% and 4.4% of PMTDI, 0.32% and 1.3% of PTWI, and 0.48 and 1.9% of BDML, respectively. For Se, the daily consumption was also found to be safe, reaching 2.2 and 4.2% of the UL for adults and children, respectively.

In contrast, values above 20% were estimate of Co and Cd exposure through chocolate consumption. For Co, the concentration found in a dark chocolate sample corresponded to 31 and 123% of UL for adults and children, respectively. For Cd, PTMI contributions of 23 and 93% for adults and children, respectively, were calculated. If a high consumption is considered, such as 11.1 kg (30.4 g per day) [32], which corresponds to the annual per capita consumption in Germany; the concentration found in some dark chocolate samples corresponded to 180% of PTMI for Cd and 238% of UL for Co for children.

It is important to note that these levels were found in semisweet and black chocolates and these foods do not represent the only possible source of these trace elements in the diet [33]. In addition, it is well known that food undergoes biotransformation in the gastrointestinal system and the trace element total content may not represent their bioaccessible content [33,34]. Nevertheless, aspects such as the bioaccessibility of trace elements in chocolate are still limited in the literature. Recent studies reported bioaccessibility ranging from 7% to 32% in chocolates [35] and from 12% to 60% in different products with cocoa [36]. Vanderschueren *et al.* [36] estimated that the daily Cd exposure in the Belgian population from chocolate and chocolate products was 0.015 ± 0.035 μg/kg bw, which corresponded to 7% of the total Cd exposure. The authors also suggested that the Cd intake from cocoa consumption has been underestimated because of hidden cacao in non-chocolate food categories. Thus, these results emphasize the need for monitoring of these elements in these products.

4. Conclusion

Occurrence of trace elements As, Cd, Co, Cu, Hg, Pb and Se in bean-to-bar chocolates from Brazil and Ecuador were evaluated, considering the available brands and composition (white, milk, semisweet and dark chocolate). In general, the concentration of trace elements followed the tendency of [Cu] > [Co] > [Cd] > [Se] > [Pb] > [As]. Cu, Co, and Cd were detected in more than 97% of the samples studied, being positively correlated with the higher concentration of cocoa solids. Samples from AM and Ecuador regions were found above the maximum limits allowed by Brazilian regulation for Cd: 22% and 18% samples of semisweet and dark chocolate, respectively. In addition, cocoa beans intended for bean-to-bar chocolate production were also evaluated for inorganic contaminants (As, Cd, Cu, Pb) conformity within Brazilian and European regulations. Traceable cocoa bean samples were found within those thresholds, except Cd in samples from the AM region.

The exposure assessment revealed that values 93% of PTMI for Cd and 123% of UL for Co may be reached when considered the consumption of a daily portion (15.75 g) of chocolate for children. Since few studies have addressed this new class of chocolates, monitoring of these trace elements in these products is recommended.

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Author statement

I confirm that this manuscript is original, has not been published, and is not under consideration by any other journal, in both printed and electronic formats. All the authors actively contributed to the planning, execution, or analysis of this study and have reviewed approved the final version of the manuscript.

CRediT authorship contribution statement

Raquel Fernanda Milani: Writing – review & editing, Visualization, Resources, Methodology, Investigation. **Manuela Luísa Nunes Silva:** Validation, Investigation, Formal analysis, Data curation. **Vitor Hugo Burgon:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Marcelo Antonio Morgano:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, 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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtemb.2024.127431.

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