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Voltammetric methods to quantify hazardous elements (Ni, Pb and Cd) in chocolate and cacao products

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ABSTRACT

This study focuses on optimizing voltammetric methods to quantify three elements of toxicological interest (lead, cadmium and nickel) in 52 chocolate and cocoa-based products. Using microwave-assisted mineralization followed by anodic re-dissolution and differential pulse voltammetry, the methods demonstrated high sensitivity, precision, and low cost-efficiency for detecting trace metals in complex food matrices. Ni was the most abundant element followed by Pb and Cd. Results revealed Ni exceeds legal limits in over 50 % of samples. While concentrations of Cd were generally compliant with regulations, Pb levels surpassed legal limits in certain dark chocolate samples. The Ni concentration varies between the limit of quantification (23 $\mu g\,kg^{-1}$) and 12100 $\mu g\,kg^{-1}$ with an average of 2064 $\mu g\,kg^{-1}$. It is present in higher concentrations (8500–12100 $\mu g\,kg^{-1}$) in three samples of white chocolate. A dark chocolate cream produced by a famous industry, shows the highest concentration. Cadmium in the analysed samples is the element having the lowest concentrations. It is present in the range between the limit of quantification (37 $\mu g\,kg^{-1}$) and 610 $\mu g\,kg^{-1}$ with an average of 63 $\mu g\,kg^{-1}$. The highest concentration was quantified in a gianduia chocolate.

1. Introduction

Chocolate is consumed daily all over the world due to its organoleptic characteristics and in the last century become an accessible luxury to more and more people who often use it as personal gratification or as a dessert. The largest consumers are the Swiss with a per capita intake of around 9 $kg\,y^{-1}$ while the Chinese people consume the least (100 $g\,y^{-1}$ per capita) (Del Prete and Samoggia, 2020). Cocoa is obtained through various stages of processing from the fruit of a tree (*Theobroma cacao L.*). Each plant, in a year, produces from 20 to 50 fruits, called cabosse. To obtain a kilogram of cocoa, approximately, are sufficient 10 carbosses (Kew). Inside the whitish and gelatinous pulp of the carboxa, from 25 to 40 pale red seeds, called cocoa beans, are allied in 5–8 longitudinal series. The beans, freed from the white mucilage that surrounds them, subjected first to fermentation, then roasting and grinding, are the raw material to obtain chocolate and their products (Barišić et al., 2019). In some countries, refreshing juices, smoothies, jellies and creams can be obtained from the pulp. The seeds contain sugars, cocoa butter fats, albuminoids, alkaloids, minerals and colourings which make them a highly energetic food. Also contains B (B1, B2, B3, B5, B6), K and J vitamins, amino and substances providing beneficial properties such as caffeine, serotonin, phenylethylamine and tyramine (Villa et al., 2015). Among the alkaloids, the most important are theobromine (diuretic and cardiotonic) and caffeine: the first is a euphoric while the second is a stimulant. Large amounts of cocoa products can, in fact, induce a physiological dependence. Due to its content of mineral salts such as iron, potassium, magnesium, calcium, selenium, zinc and flavonoids, cocoa products are important not only for its antioxidant action, but also for the strengthening of cardiac muscle tissue and the walls of the arterial system and venous which make it, for this reason, a food substance useful for reducing cardiovascular, hepatic and autoimmune diseases (Villa et al., 2015). Since cocoa products induce the production of serotonin they are also natural antidepressants.

Depending on the ingredients, several types of chocolate can be obtained: dark, milk, white, etc., however, on the market, there are chocolates containing dried fruit (hazelnuts, almonds, peanuts, etc.), pepper, salt etc. (Villa et al., 2015). Dark chocolate contains the highest percentage of cocoa, while the other ingredients are sugar and cocoa butter. Milk chocolate has similar ingredients to dark one but with the addition of milk, while white chocolate contains cocoa butter, milk and

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sugar (Mrmošanin et al., 2018; Rovasi Adolfo et al., 2024). Other ingredients can be added: hydrogenated vegetable oil, salts, buffering agents and emulsifiers (Rehman and Husnain, 2012). Gianduia hazelnut chocolate is produced following an Italian (Piedmontese) recipe created for the first time in 1865, using cocoa mass mixed with hazelnut powder and sugar. Ruby chocolate has a natural pink colour obtained without the addition of artificial colourings. The pink colour is given by the pigments of cocoa beans, which are a very rare variety called Ruby cocoa bean, or Brazil Lavados, grown exclusively in Ecuador, Brazil and the Ivory Coast.

In addition to the main essential elements for the normal functioning of the human body, as in other food, cocoa-based sweets may contain undesirable chemical substances (heavy metals, PAHs, pesticides, etc.) (Amorello and Orecchio, 2013; Barreca et al., 2023; Amorello et al., 2023; Orecchio et al., 2014). Although the intake levels of hazardous substances for several foods are documented (Orecchio et al., 2014; [12] 2006. Inorganic and organic lead compounds. IARC Monogr. Eval. Carcinog. risks Hum. 87, 1–471.Inorganic and organic lead compounds, IARC monographs on the evaluation of carcinogenic risks to humans, 87 (2006) 1-471.; Arıca et al., 2018; Yüksel et al., 2023), limited information are still available for consumers of chocolate and cocoa products sold in Italy (Villa et al., 2015; Mrmošanin et al., 2018; Rovasi Adolfo et al., 2024; Rehman and Husnain, 2012).

Several elements have an importance in human biology, for example, traces of some metals, (manganese, copper, zinc, etc.), are essential micro nutrients and have more biochemical functions in all living organisms, while, others may constitute a potential health risk if consumed above the tolerable upper in-take levels over an extended period (Barreca et al., 2023). Examples of these are the three elements (Ni, Pb and Cd) investigated in this research paper.

International Agency for Research on Cancer (IARC) ([12]2006. Inorganic and organic lead compounds. IARC Monogr. Eval. Carcinog. risks Hum. 87, 1–471.Inorganic and organic lead compounds, IARC monographs on the evaluation of carcinogenic risks to humans, 87 (2006) 1-471.) in 2006 classified Pb compounds and inorganic lead in the Group 2 A probably carcinogenic to humans. Depending on exposure and duration, these compounds have toxic effects on people producing inhibition of several enzymes and consequently pathological conditions or death (Arıca et al., 2018). In adults, high exposure to lead compounds can cause peripheral neuropathy and hypertension, damage to the immune, skeletal, nervous, endocrine, renal and respiratory systems (Arıca et al., 2018). Even low exposures can have negative effects on heme synthesis and other biochemical mechanisms and can impair neurobehavioral and psychological activities (Arıca et al., 2018; Yüksel et al., 2023). Some researchers (Yüksel et al., 2021) claim that the relationship between the occurrence of tumours depends on industrial exposure to nickel, in fact it is associated with malignant tumours of the kidney, stomach, breast and neck/head and nose. Furthermore, exposure to high concentrations of Ni can cause contact dermatitis, epigenetic changes, alteration of gene regulation, induction of apoptosis, negative effects on development and reproduction (birth defects, abortion, fertility or subfertility) (Yüksel et al., 2021). These evidences highlight that exposure to Ni is a major problem for human health and for the quality of environmental matrices. No tumours were observed in researches carried out on experimental animals following oral administration of soluble nickel compounds (Goodman et al., 2009). Cadmium is generally known to be a carcinogen and nephrotoxic element (Shannon et al., 2007), and as such is monitored and/or regulated in many food types.

Analysis of potentially toxic elements in cocoa foods is important as it can be useful to assess the estimated daily intake (EDI) and the corresponding health risk, necessary to monitor food safety (Yüksel et al., 2023).

Despite the numerous (often fake) news published on social media, there are very little recent scientific data regarding the concentrations of hazardous elements in chocolates from the European market (Yanus et al., 2014; Godebo et al., 2024; Altunay et al., 2019; Mohamed et al., 2020; Babaahmadifooladi et al., 2020), in particular, the Italian one. Considering that several adults and in particular, children, are large consumers of chocolate, the optimization of an efficient, fast and low-cost analytical procedure for the control of concentrations of metals of toxicological interest is important and, all together, an analytical challenge due to the complexity of the matrix.

In literature (Villa et al., 2015; Mrmošanin et al., 2018; Rovasi Adolfo et al., 2024; Rehman and Husnain, 2012; Yanus et al., 2014; Godebo et al., 2024; Altunay et al., 2019; Mohamed et al., 2020; Dereń et al., 2021), several procedures for the treatment and analysis of chocolate samples are described, but are mainly based on sample calcination, wet acid digestion by microwaves and subsequent analysis by atomic absorption spectrometry (FAAS, GFAAS), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical spectrometry (ICP-OES) etc. Some of the above methods require the use of expensive equipment both for acquisition and for routine management, requiring large volumes of argon and others gases (Gaudio et al., 2023).

The main aim of this investigation was to optimize voltammetric methods to quantify three metals of environmental and toxicological interest (lead, cadmium and nickel), selected as major heavy metal contaminants in chocolate and cocoa-based food samples in which concentrations should be, theoretically, at ppb levels. Voltammetric methods are based on the measure of the current flowing through an electrode immersed in a solution containing electroactive compounds, while a potential scanning is imposed upon it (Amorello et al., 2023). Voltammetric methods are very advantageous to quantify trace elements in food due to their high sensitivity derived from the electrochemical pre-concentration of the analyte at the electrode surface, the ability to discriminate between different elements and low costs for purchasing and managing the equipment which result accessible also to laboratories of food producers, in our case, to the confectionery industries to carry out quality controls both on raw materials and on products intended for final consumers.

2. Experimental section

2.1. Reagents and solutions

Analytical grade chemicals (HCl, HNO_3, H_2O_2, $NaNO_2$ and dimethylglyoxime tartrate) were acquired from Merck and Fluka, stock standard solutions 1000 mg L^{-1} from Merck (Darmstadt, Germany) and ultrapure water (R > 20 MΩ cm^{-1}) was obtained by Milli Q system.

2.2. Laboratory equipment

All glassware and sample containers were thoroughly washed with hot HNO_3 3 % solution followed by rinsing with Milli Q water. To avoid contaminations during the entire procedure, different glassware and pipettes were used for standards and solutions obtained from samples.

2.3. Quality control and quality assurance

The procedural blanks were obtained by subjecting seven different aliquots of a solution containing 2 mL of HNO_3 65 % and 2 mL of H_2O_2 30 %, to the complete mineralization procedure. The detection limits (LOD) and quantification limits (LOQ) (Table 1), referred to samples ready to be consumed, as described in previous papers (Amorello and Orecchio, 2013; Barreca et al., 2023; Amorello et al., 2023), were calculated as the blank signal (current) plus the three- and ten-fold standard deviation of signal found in 7 procedural blanks, respectively, which were prepared in the same way as the chocolate samples. The initial weight of a sample, the final volume of the solution obtained from microwave mineralization and the slope of calibration curves were used to calculate LOD and LOQ values in μg kg^{-1}. Every fifth sample the procedural blank was analysed to ensure good quality assurance. For

Table 1
Detection and quantification limits of some instrumental analytical techniques.

		Seker (Gaudio et al., 2023)	Godebo (Yanus et al., 2014)	Erdogan (Godebo et al., 2024)	Altunay (Godebo et al., 2024)	Antoine (Erdoğan et al., 2024)	Mrmošanin (Mrmošanin et al., 2018)	Peixoto (Antoine et al., 2017)	Oliveira (Peixoto et al., 2012)	Rovasi (Rovasi Adolfo et al., 2024)	Kruszewski (Oliveira et al., 2021)	Villa (Villa et al., 2015)	This paper
		ICP-MS	ICP-MS	ICP-MS	FAAS	FAAS	ICP-OES	ICP-OES	MIP-OES	GFAAS	GFAAS	GF AAS	DPV/a
		µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg L^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}
Ni	**LOD**	**0.13**	**0.05**	**0.1**	**0.3**		**8.3**	**240**	**100**	**49**	**5**		**14**
	LOQ	**1.42**			**1**		**28.3**	**790**	**300**	**163**	**16**		**23**
Pb	**LOD**	**0.04**	**0.009**	**1**			**31.7**	**630**			**0.4**	**6.3**	**15**
	LOQ	**0.52**				**3–65**	**107**	**2090**			**1.3**	**21**	**40**
Cd	**LOD**	**0.01**	**0.009**	**0.01**			**3.3**	**20**			**0.7**	**0.5**	**13**
	LOQ	**0.07**				**1–20**	**10.1**	**80**			**2**	**1.7**	**37**

comparison between the voltammetric method used by us, Table 1 shows the quantification limits of some other methods and Table 2 the characteristics of several instrumental analytical techniques in relation to the elements we took into consideration in this paper.

Not having a specific certified material for cocoa products (ERM®-BD513, ERM®-BD514, and ERM®-BD515) in the quantification of some trace elements in others matrices (cereal and food samples), we evaluated the reliability of the voltammetric methods for the determination of Cd, Ni and Pb using a certified sample (ERM-CE278K, Joint Research Centre) carrying out the same method for the preparation of the samples (microwave mineralization). The recovery percentages for the three elements were between 94 % and 102 % which we consider satisfactory for the purpose of this research.

Also, the employed methods were tested for accuracy (recovery) by analysing several samples prepared by the authors: in the Teflon vessels of the mineralizer, together with the mixture of acids and hydrogen peroxide, volumes (5–20 µL) of a solution with known concentration (20 mg L^{-1}) of the three elements were added to 5 g of sample, in which, according to preliminary tests, the elements were lower than the detection limits. Meanly, the recoveries of enriched samples were 97, 87 and 85 for Ni, Pb and Cd respectively.

The repeatability of the whole method, was calculated as the mean relative standard deviation (RSD %) for five analyses of three identical samples of every category (dark, milk, etc.). RSD % ranged from 1.4 % to 7.8 % that are satisfactory for the purpose of this research. From each sample, three aliquots were mineralized and each sample solution was analysed in duplicate.

2.4. Calibration and measures

Before each calibration, working standard solutions were prepared by sequential dilutions of concentrated stock using Milli-Q water. Because the solutions obtained from cocoa matrix could contribute to the analytical signal, the quantification of the three elements in cocoa-based products was carried out by means of 5–7 standard additions solution to the sample, but in the case of analysis on numerous samples, the additions can be reduced to 2–3 with time savings.

Table 2 -
Characteristics of some instrumental analytical techniques.

	ICP-MS	ICP-OES	FAAS	GF-AAS	DPV/a
Combustible gases	no	no	yes	no	no
Operating cost	high	high	low	medium	Very low
Capital cost	very high	high	low	medium/high	Low

ICP-MS = Inductively Coupled Plasma – Mass Spectrometry
ICP-OES = Inductively Coupled Plasma – Optical Emission Spectroscopy
FAAS = Flame Atomic Absorption Spectroscopy
GF-AAS = Graphite Furnace Absorption Spectroscopy
DPV/a = Differential Pulse Voltammetry

The matrix effect, thus making it impossible to associate directly the analytical signal (in this case current) between sample and standard using the traditional calibration curve approach. Using the intercept (negative) of the straight, the employed volumes of solutions and the quantity of samples, the concentrations of Ni, Cd and Pb were calculated in the different cocoa products. Calibration graphs were built using data from measurements and evaluated by the least squares linear regression method. Considering the obtained proportion of the variance in the dependent variable (R^2 = 0.991–0.999 for nickel 0.994–0.999 for cadmium and 0.999–1 for lead), under the optimum conditions, very good linear correlations were obtained between the monitored voltammetric current peak current and metal concentrations. The uncertainties regarding the slopes did not exceed 1 %, while those regarding the intercepts were always less than 1.5 %. When the aforementioned conditions were not satisfied, the analyses were repeated. The test of homoscedasticity was performed by applying the F test as follows where F (exp) is the experimental F value expressed as a ratio of variance obtained at the lowest concentration level (s12) and at the highest concentration level (s22) of the working range. The test of homoscedasticity is accepted for F (exp) < F (tab). The F (exp) was result of 0.0204 which is < F(tab) obtained from the F-table at a confidence level of 99 %.

The calibration curves, one of which is shown, as an examples, in Fig. 1, indicate that the methods are linear starting from concentrations very close to the limits of quantification.

2.5. Samples

The 52 investigated cocoa products samples are of Italian origins and from different brands. The samples were categorized respect to their composition as dark, milk, white, etc. Specifically, 5 samples were cocoa powder (identified by CO), 23 samples dark chocolate (identified by DK), 11 milk chocolate (identified by MK), 6 white chocolates (identified by WH), 7 gianduia (identified by GI). Commercially available chocolate samples were acquired at local markets.

2.6. Mineralization procedure

Microwave oven (Milestone model MLS-1200 Mega, Milestone Laboratory Systems, Italy), 1200 watt rated magnetron with 1000 watt delivered power, equipped with a Controller (MLS Mega 240), an Exhaust module (EM 45), a rotor with 6 high pressure (up to 100 bar) Teflon digestion vessels (HPR 1000/6), was used for chocolate products sample mineralization.

About 5 g of previously homogenized samples were weighted, transferred inside Teflon vessels and 2 mL of HNO_3 (65 %) (Fluka, Milano) and 1 mL of H_2O_2 (30 %) (Fluka, Milano) were added. The instrumental conditions used for the mineralization were: 1 min at 250 W, 1 min at 0 W, 5 min at 250 W, 5 min at 450 W, 3 min at 600 W and 5 min at 300 W. At the end of the cycle, a clear colourless solution

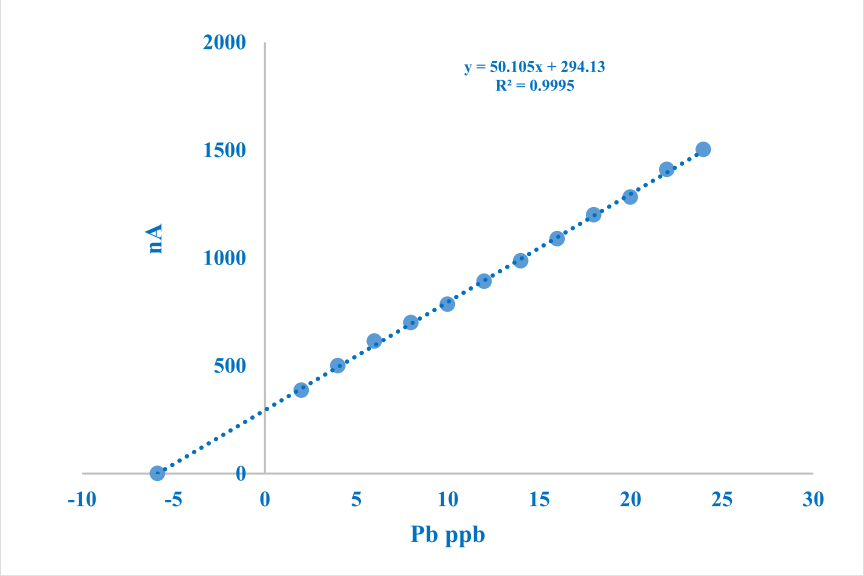


Fig. 1. Calibration curve for Pb quantification in a chocolate sample.

free of solid residues was obtain, confirming the success of the complete decomposition of the sample. The solution was transferred into a volumetric flask and brought to volume with Milli-Q water.

2.7. Analytical method

For the quantitative determination of Pb and Cd we used an AMEL model 433 computerized polarograph equipped with a pendant mercury drop electrode, an Ag/AgCl reference electrode in saturated KCl and a platinum counter electrode. For the determination of each element, a known volume of the solution obtained from mineralization (generally 2 mL) was added to an HCl solution, brought to a volume of 20 mL with Milli Q water and transferred to the voltammetric cell. Before signal acquisition, the dissolved oxygen was removed by a nitrogen flow, keeping the system under stirring. All quantifications were carried out using the standard addition method. The standard working solutions were prepared by dilution of the concentrated stock solutions (1 mg mL^{-1}), at the time of analysis. After each standard addition (generally 2 ppb), an increase in the intensity of the instrumental signal was observed as a function of the concentration (Fig. 2). This voltammetric method allowed to quantify Pb and Cd simultaneously. Table 2 shows the operating parameters for the quantification of Pb and Cd. Fig. 2 shows the voltammograms obtained for the two metals.

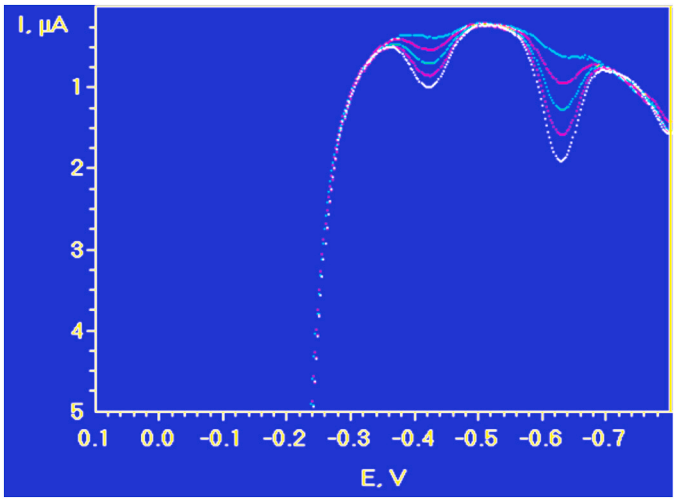


Fig. 2. Lead and cadmium voltammograms.

Since nickel not form mercury amalgam, it was quantified separately from Cd and Pb, using differential pulse voltammetry (DPV/a). Also in this case, the quantifications were carried out using the standard addition method. To a known volume (100 µL) of the solution obtained from mineralization, 1 mL of the supporting electrolyte (1 M tartrate buffer at pH= 9), 500 µL of 5 M $NaNO_2$ and 100 µL of 1 % dimethylglyoxime solution were added and the whole was brought to the final volume of 20 mL with distilled water. Table 3 shows the operating conditions for the nickel quantification.

Figs. 2 and 3 show the voltammograms of the three investigated elements. Each curve represents the addition of a small amount (2 ppb) of standard to the initial solution.

2.8. Statistical and chemometric analysis

Statistical and chemometric analyses were performed in order to obtain information on the contaminant profile of the analysed samples. These analyses were performed by using PAST 4.0, a free software for scientific data analysis. Prior to the chemometric investigation, the experimental data were scaled by subtracting the mean and dividing by the standard deviation.

Table 3
Instrumental parameters for the quantification of Pb, Cd and Ni.

Pb and Cd		Ni	
Deposition Time (s)	120	Start Potential (mV)	−700
Deposition Potential (mV)	−800	End Potential	−1300
Number of Cycles	1	Current Range (µA)	±20.48
Delay Before Sweep (s)	5	Scan Speed (mV/s)	50
Purge and Stir Time (s)	300	Number of Cycles	3
Stirring Speed (r.p.m)	300	Delay Before Sweep (s)	5
Drop Size (a.u)	60	Purge and Stir Time (s)	300
Electrode Type	Hg	Stirring Speed (r.p.m)	300
Initial Mercury Drops	3	Drop Size (a.u)	60
Potential hi graphic limit (mV)	Auto	Electrode Type	Hg
Potential low graphic limit (mV)	0	Initial Mercury Drops	3
Current hi graphic limit	0	Potential hi graphic limit (mV)	Auto
Current low graphic limit	−20	Potential low graphic limit	0

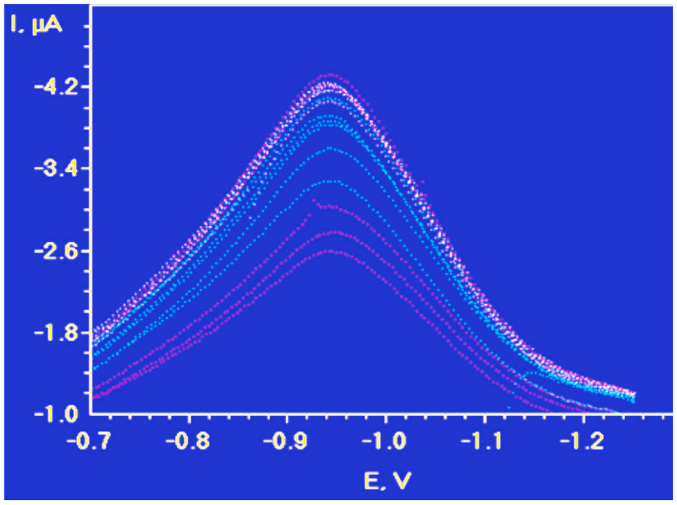


Fig. 3. Nickel voltammograms.

3. Results and discussion

Considering that cocoa based products are very complex matrices and contain high percentages of organic compounds, the choice of specific mineralization method that limits the losses of the analyte and doesn't adds contaminant is very important. In this study, the wet digestion method by microwave was used, followed by voltammetric analysis. Meanly, the recoveries of enriched samples were 97, 87 and 85 for Ni, Pb and Cd respectively. From the preliminary tests to evaluate the features of the voltammetric analytical methods employed to quantify the three elements in the cocoa-based products, the LOD values (Table 1) for Ni, Pb and Cd ranging from 13 to 15 µg kg^{-1} and LOQ from 23 to 40 µg kg^{-1} are of the same order of magnitude as those obtained by several authors (Villa et al., 2015; Mrmošanin et al., 2018; Rovasi Adolfo et al., 2024; Altunay et al., 2019; Şeker, 2023; Godebo et al., 2024; Erdoğan et al., 2024; Antoine et al., 2017; Peixoto et al., 2012; Oliveira et al., 2021; Kruszewski et al., 2018). The good linearity of the standard addition method is confirmed by the values of R^2 ranging from 0.991 to 1. Considering all the analyzed samples, the obtained precision ranged from ± 1.4 to ± 7.8 %. For all the elements investigated the values of these parameters are quite satisfactory to assess any risks due to the consumption of the above sweets. Table 4 shows the concentrations (average of at least three determinations) of the different elements, expressed in µg kg^{-1} of sample.

The results show that the most abundant element is Ni, followed by Pb and Cd. The Ni concentration range is a very wide and varies between the limit of quantification (23 µg kg^{-1}) and 12100 µg kg^{-1} with an average of 2064 µg kg^{-1}. Ni is present in higher concentrations (8500–12100 µg kg^{-1}) in three samples of white chocolate: WH 1, WH 2, WH 3. In the 50 % of all the samples Ni concentration is higher than the legal limit (1000 µg kg^{-1})(JECFA, 2003; [33]1995. FAO/WHO, Gen. Stand. Contam. Toxins Food Feed,.FAO/WHO, General Standard for Contaminants and Toxins in food and Feed, in, 1995.). The highest concentrations in white chocolates are justified by the fact that cocoa butter, which is the main ingredient of this type of chocolate, can contain high concentrations of the metal (Selvapathy and Saraladevi, 1995; Jellesen et al., 2006; Leblanc et al., 2005; Noël et al., 2012) since it is used as a catalyst in the hydrogenation of unsaturated fats. Another source of Ni could be due to the processing and packaging phases of the products since stainless steel equipment and containers are used. In cocoa products sold in Italy, the nickel content is higher than that determined by other researchers (Altunay et al., 2019; JECFA, 2003; Leblanc et al., 2005; Noël et al., 2012; Dahiya et al., 2005) and of the same order of magnitude as that determined by others (Sager, 2012; Iwegbue, 2011; C.K.a.L. Lee, 1985; Magazine).

Table 4
Ni, Pb and Cd concentrations (µg kg^{-1}) and MPI values of the investigated samples.

Samples	Ni	Pb	Cd	MPI
µg kg^{-1}				
CO 1	7660	267	65	510
CO 2	280	40	37	75
CO 3	23	40	37	32
CO 4	23	40	37	32
CO 5	23	40	37	32
Mean CO	1601	85	43	136
DK 1	1781	40	37	138
DK 2	2341	40	37	151
DK 3	5290	40	37	198
DK 4	1487	6320	37	703
DK 5	2141	290	240	530
DK 6	1638	5210	37	681
DK 7	1860	210	37	243
DK 8	96	4660	37	255
DK 9	1168	8710	37	722
DK 10	23	40	110	47
DK 11	23	40	37	32
DK 12	2124	40	37	146
DK 13 70 %	1890	40	37	141
DK 14 90 %	1309	40	52	140
DK 15	7384	40	37	222
DK 16	2725	40	110	229
DK 17 50 %	3533	520	440	931
DK 18	2153	40	37	147
DK 19	23	300	37	63
DK 20	595	95	37	128
DK 21	6865	40	37	216
DK 22	6000	120	37	298
DK 23	23	120	37	47
Mean	2281	1276	74	279
MK 1	2530	150	37	241
MK 2	23	360	37	67
MK 3	1716	40	37	136
MK 4	374	40	37	82
MK 5	23	40	37	32
MK 6	376	40	37	82
MK 7	585	40	37	95
MK 8	23	40	37	32
MK 9	350	290	37	155
MK 10	481	40	37	89
MK 11	23	40	37	32
Mean MK	591	108	37	131
WH 1	11700	40	37	259
WH 2	12100	40	37	261
WH 3	8500	40	37	232
WH 4	23	120	37	47
WH 5	215	220	37	120
WH 6	2819	360	37	335
Mean WH	5893	137	37	209
GI 1	23	2750	37	133
GI 2	352	2680	37	327
GI 3	23	68	37	39
GI 4	468	120	37	128
GI 5	2902	120	37	234
GI 6	1200	120	610	444
GI 0	23	120	37	47
Mean GI	828	538	133	193

Lead concentrations in all the analysed samples ranged from quantification limit (40 µg kg^{-1}) to 8710 µg kg^{-1} (Table 4). Sample DK 9, (a dark chocolate cream) produced by an Italian industry, shows the highest concentration. In particular, were found high concentrations in four dark chocolate bars produced by different famous Italian makers: DK 4 (6320 µg kg^{-1}), DK 6 (5210 µg kg^{-1}), DK 8 (4660 µg kg^{-1}) and DK 9 (8710 µg kg^{-1}) respectively and in two gianduia chocolate samples GI 1 (2750 µg kg^{-1}) and GI 2 (2680 µg kg^{-1}).

Considering the most permissive limit regarding nickel (7 mg kg^{-1}) content for the various types of chocolate established by European legislation (O.J.o.t.E.U, 2024), in 5 samples the aforementioned value is

exceeded. Our concentrations are in the same range (240–8710 μg kg^{-1}) as those obtained by Dahyia (Dahiya et al., 2005) in chocolates and candies from suburban areas of Mumbai, India, but higher than those reported by other authors (Mrmošanin et al., 2018) with a mean of Pb (632 μg kg^{-1}) found in Napolitains Dark (Venezuela), followed by Blanxart chocolate (Perù) that contained Pb of 552 μg kg^{-1}. Lead concentrations in the milk and white chocolate samples ranged from quantification limit to 360 μg kg^{-1}. The highest lead concentrations were quantified in samples bars (MK 2 and MK 9) produced by different famous Italian producers. In the gianduia chocolate samples, Pb concentrations were in the range from 68 to 2750 μg kg^{-1}. Again, considering the most permissive limit regarding lead (0.8 mg kg^{-1}) (Europea, 2006), in 6 samples the aforementioned value is exceeded.

Cadmium in the analysed samples is the element having the lowest concentrations. It is present in the range between the limit of quantification (37 μg kg^{-1}) and 610 μg kg^{-1} with an average of 63 μg kg^{-1}. The highest concentration was quantified in the sample (GI 6), a gianduia chocolate. The data given in Table 4 show that Cd concentrations in the samples, with the exception of two (DK 17, GI 6), are lower than the maximum permissible limit in foods (300 μg kg^{-1}) (Mrmošanin et al., 2018; Commission; C.R.E, 2021). The cadmium concentrations determined in this work are of the same order of magnitude as those obtained by Lee and Low for Malaysian samples, while they are on average higher than those for Turkish samples (C.K.a.L. Lee, 1985).

Researchers quantified several elements by ICP-MS in 18 different chocolate samples of different varieties and brands. Regarding the elements analysed by us, the contents were in the range: Ni= 0.21–4.96; Cd= 0.141–0.209; Pb= 0.096–0.136 μg g^{-1} (Erdoğan et al., 2024).

3.1. Statistical and chemometric considerations

Ni, Pb and Cd were found in 100 % of the samples, with concentrations ranging from LOQs level and 12100,8710 and 610 μg kg^{-1} respectively (Table 5). Moreover, Ni, Pb and Cd concentrations show significant variability and non-normal distributions, which is confirmed by the Shapiro-Wilk test ($p < 0.05$). The median concentrations are much lower than the mean arithmetic ones, especially for Pb and Cd, indicating a strong right skewness. Coefficients of variation (CV), especially for Cd (348 %) and Pb (267 %), indicate considerable relative variability. Positive skewness values (2.0 for Ni, 3.2 for Pb and 4.3 for Cd) indicate long right tails, while high kurtosis, especially for Pb (10.5) and Cd (19.2), indicate heavy-tailed distributions with outliers. These evidences suggest that the sources of contamination for the investigated metals may be diverse and sporadic.

The boxplot with jittered data points (Fig. 4) provides an overview of the distribution and variability of Ni, Pb, and Cd concentrations.

Among the three metals, Ni shows the widest range, indicating a higher degree of variability in contamination levels compared to Pb and Cd. For Ni, extreme outliers are observed far beyond the whiskers of the boxplot, which may point to localized or highly concentrated sources of contamination. While Pb, Cd also display some outliers, their values are less extreme than those of Ni. The interquartile range (IQR) of Ni is much larger, reflecting greater variability within the middle 50 % of the data. In contrast, Pb and Cd exhibit narrower IQRs, which suggests that their concentrations are more consistent across most of the samples. Despite their generally uniform distributions, Pb and Cd also show occasional spikes in concentration, possibly related to specific localized inputs or contamination events. The broader spread and frequent outliers observed for Ni suggest that this metal may originate from several sources, such as industrial activities or natural processes, leading to higher contamination variability. On the other hand, the more stable patterns for Pb and Cd might reflect more uniform contamination sources, although the occasional outliers still indicate instances of higher pollution.

Table 5
Statistical parameters.

Parameter	Ni	Pb	Cd
N	52	52	52
Min	23	40	37
Max	12100	8710	610
Sum	106988	34300	1627
Mean	2139	670	63
STD Error	410	244	15
Variance	8744963	3095586	11834
Stand dev	2957	1759	109
Median	881	0	0
25 percentil	0	0	0
75 percentil	2483	255	0
Skewness	2.0	3.3	4.3
Kurtosis	3.6	10.5	19.2
Coeff. var	144	267	348

The biplot reported in Fig. 5 displays that the first two principal components, capture the majority of the variance in the dataset (Component 1 and Component 2 explain a cumulate variance of 71.2). Both Cd and Ni significantly contribute both to the variance along Component 1 and Component 2. In contrast, Pb is negatively correlated with Component 1 and not shows significantly contribute to the variance along Component 2. Most samples are tightly clustered near the origin, indicating similar levels of Ni, Pb, and Cd. However, few samples, such as GI 6, DK 5, and DK 17 (50 %), deviate from the cluster, highlighting samples with elevated contamination. GI 6, DK 5 and DK 17 (50 %) strongly aligns with Cd, suggesting elevated amount of this metal. DK 5 is positioned along Component 2, indicating a distinct pattern, possibly influenced by Pb. DK 17 (50 %) is also located away from the main cluster, pointing to unique contamination profiles. The elliptical confidence region represents the typical sample distribution, with points outside the ellipse considered outliers, likely exhibiting distinct characteristics. In this context, the 90 % of analysed samples can be clustered taking into account a typical metal distribution in analysed samples.

From a contamination perspective, Cd and Pb appear to be the primary metals driving variability in the data, suggesting heterogeneous contamination sources. Ni, on the other hand, has less influence on sample differentiation, implying either lower contamination levels or a more uniform distribution across samples. The tightly clustered samples in the centre reflect similar contamination levels, while the outliers may indicate specific cases with localized or high-intensity contamination, warranting further investigation. This analysis emphasizes the complex variability among samples, particularly driven by Cd and Pb, with Ni playing a less significant role. Furthermore, in order to limit the effect of the higher data, a PCA analysis was carried out by pre-treating the results with the Box-Cox method, the results of which are shown in Fig. 5b. In this last PCA analysis it is possible to highlight 3 different types of clusters, confirming the high variability of the data.

3.2. Metal pollution index

To quickly compare the content of the three elements in the different samples, we calculated the metal pollution index (MPI) (Fig. 6), obtained by the following relation: (Sedeño-Díaz et al., 2020)

MPI (μg kg^{-1}) = $(C_1.C_2 \bullet C_3 \bullet \ldots \bullet C_n)^{1/n}$

where:

C_n = concentration of each single metal in the sample (μg kg^{-1});

n = number of metals in the sample.

Considering the investigated chocolate samples, five dark chocolates (DK 4, DK 5, DK 6, DK 9 and DK 17), among the most consumed and appreciated in Italy, show the highest MPI values (530–931 μg kg^{-1}), while, the lowest ones are relative to a several samples (CO 3, CO 4, CO 5, DK 11, MK 5, MK 8, MK 11). On average, dark chocolate is the one that presents highest MPI values (279 μg kg^{-1}) while the smallest is related to milk chocolate samples (95 μg kg^{-1}). Fig. 7

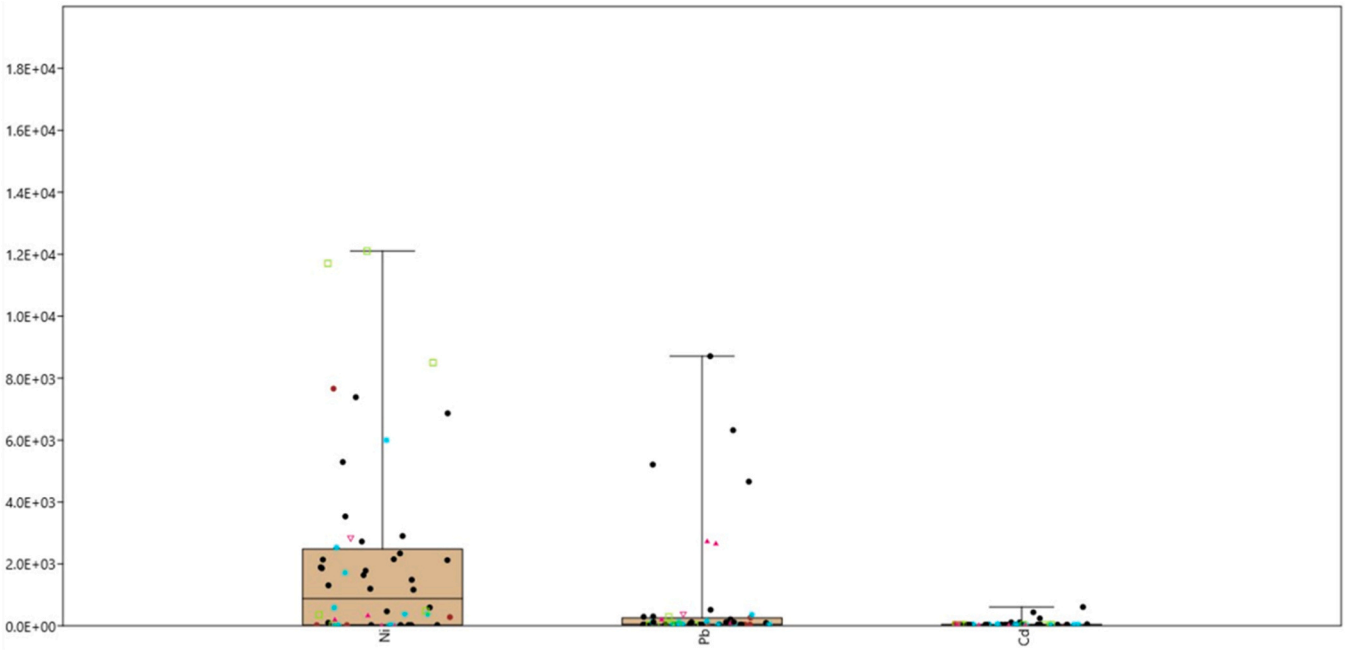


Fig. 4. Boxplot distribution (expressed as μg kg^{-1}) for Ni, Pb, and Cd in analysed samples.

3.3. Enrichment factor

Element enrichment factor (EF), evaluated relative to the background values ([48]Wikipedia, Abundance of Elements in Earth's crust, in..), was used to establish which elements were relatively enriched in the different chocolate samples. EF values close to 1 indicate natural origin while those > 10 are considered to have a non-crustal source. To determine it, we used the following relationship:

$EF = C_{metal\ sample} / C_{metal\ earth's\ crust}$

On the basis of the enrichment factor (Orecchio and Amorello, 2019), five categories of contamination are recognized (Table 5).

Most of the analyzed samples regard to Ni, Pb and Cd are characterized by enrichment factor classified minimal or zero (EF < 2), while two samples (DK 17 and GI 6) show 2 <EF> 4, indicating a modest contamination. Fig. 8

4. Health risk assessment

Numerous studies have investigated the ways through which most living beings, by ingesting contaminated foods, are exposed to hazardous substances. This work evaluates the health risk, relating the presence of Ni, Pb and Cd by the consumption of several chocolate types. Several important parameters such as estimated daily intake (EDI), Target Hazard quotient (THQ), Hazard index (HI) and Target Cancer Risk (TCR) were calculated. The oral reference doses used in this research were taken from the (USEPA-IRIS, 2002; USEPA 2014). Table 6

4.1. Estimated daily intake (EDI)

The Tolerable Daily Intake correspond to the amount of a substance contained in a matrix with which people comes into contact (food, water, air, etc.) that can be consumed over a lifetime without causing important health risks, as specified by the European Food Safety Authority (EC, 2020).

To calculate the EDI and THQ an oral reference dose is necessary. As defined in the US EPA's, the RfD values for Cd (0.003 mg kg^{-1} day^{-1}), Pb (0.0035 mg kg^{-1} day^{-1}) and Ni (0.020 mg kg^{-1} day^{-1}) are considered (USEPA-IRIS, 2002; USEPA 2014).

The Estimated Daily Intake (EDI) in mg kg^{-1} day^{-1} relating investigated elements in chocolates consumed in Italy was calculated as follow:

$$EDI = \frac{(C \times F_{ir})}{BW_a}$$

C represents the element concentration in chocolate sample (mg kg^{-1}), F_{ir} corresponds to the daily average consumption of chocolate (25 g day^{-1}) and BW_a is the average body weight for adults (70 kg) (US-FDA, 2006) and for children (15 kg) (Anyanwu and Adetunji, 2018). For adults and children, the mean values of EDI, THQ and Σ THQ for the different type of investigated chocolate samples are shown in Table 7.

As can be seen in Table 7, the EDI values for the three elements studied for adults and children in cocoa products are below the RfD (Ni=20, Pb=3.5, Cd=3, μg kg^{-1} day^{-1}), suggesting that consumers may not be exposed to levels of these elements throughout their lives that could be harmful to health. Considering the element concentrations of all the investigated samples, for adults the maximum estimated daily consumption (4.3 μg kg^{-1} day^{-1}) was for Ni in a white chocolate (WH 1), followed by Pb (3.1 μg kg^{-1} day^{-1}) in a dark sample (DK 9), Cd show the lowest value (0.22 μg kg^{-1} day^{-1}) quantified in a gianduia chocolate, while for children the maximum estimated daily consumptions was for Ni in two white chocolate (WH 1 and WH 2) (20 μg kg^{-1} day^{-1}), for Pb (15 μg kg^{-1} day^{-1}) in a dark sample (DK 9) while for Cd was (1.0 μg kg^{-1} day^{-1}) quantified in a gianduia chocolate (GI 6).

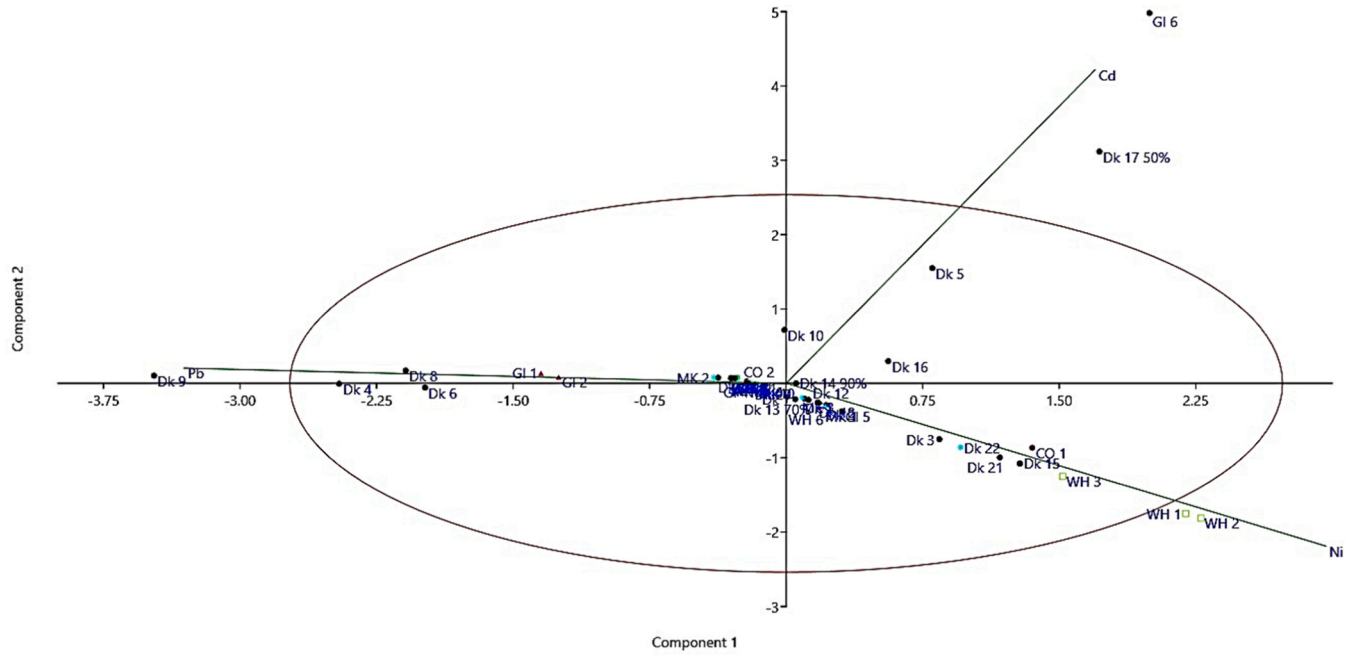
4.2. Target Hazard Quotient (THQ)

As suggested by the US-Environmental Protection Agency (US-EPA) (Castorina and Woodruff, 2003), also, we calculated the Target Hazard Quotient, particularly, to evaluate the probability of adverse health effects in humans exposed to certain hazardous trace element, in this case, through chocolate and cacao products.

THQ is the ratio of exposure to the hazardous element to the dose at which adverse health effects are expected to occur (Ain et al., 2023). The reference dosage is the highest level at which this is predicted.

This parameter describes the non-cancer risk of hazardous substance by the ratio between exposure dose (i.e. dietary intake) and the reference dose (RfD) (US-EPA). Reference doses (RfDs, in mg kg^{-1} day^{-1}) of an element is an estimate of a daily oral exposure to the population

a)



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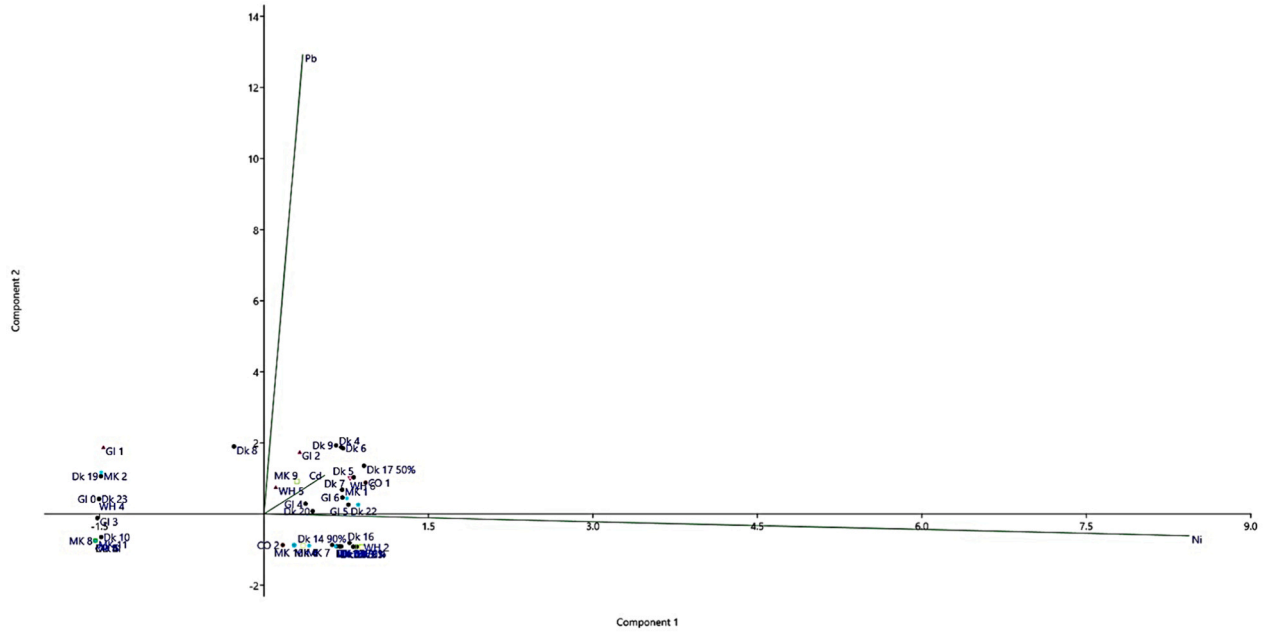


Fig. 5. Biplot of two principal components obtained from scaled data a) and for data pretreated by boxcox approach 5b.

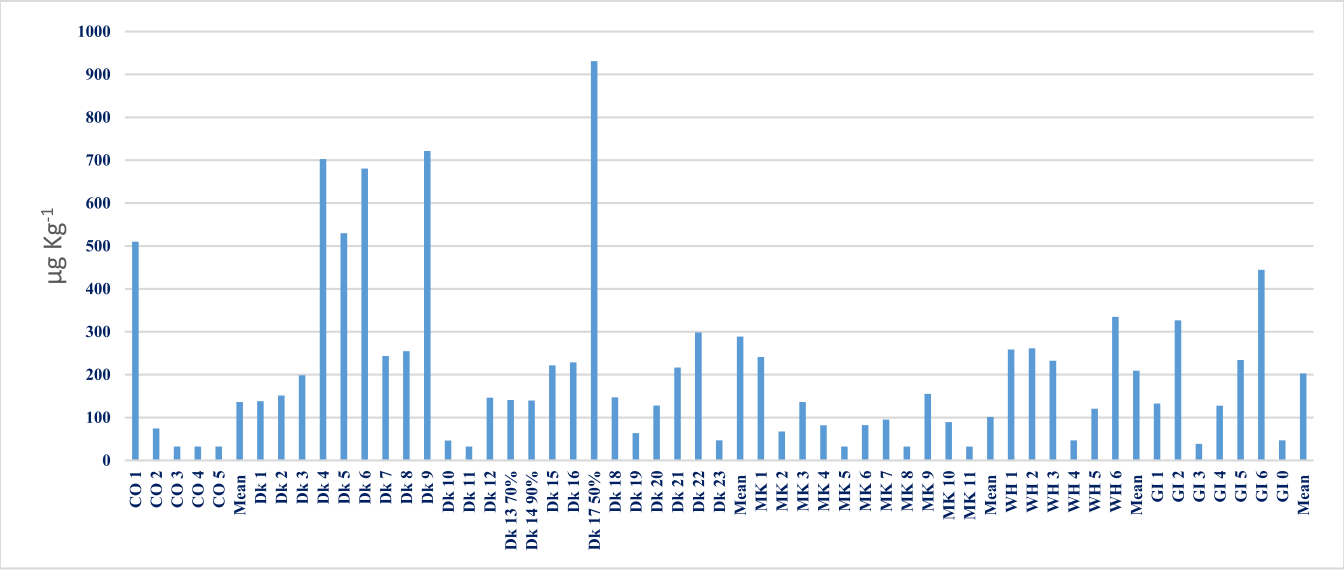


Fig. 6. Metal pollution index.

(including sensitive subgroups) that is likely to produce no appreciable risk of deleterious effects during a lifetime (USEPA-IRIS, 2002) (Castorina and Tracey, 2003; U.S.E.P.A. (EPA), 2005; U.S.E.p. AgencY). RfDs are determined by their potential toxic risk for daily exposure.

THQ values were calculated using the equation:

$$THQ = \frac{E_{FR} \times Ed \times F_{IR} \times C}{RfD \times BW_a \times AT_n}$$

E_{FR} corresponds to the exposure frequency to the trace element (350 days), Ed is the exposure duration (70 y for adults and 6 for children) (Antoine et al., 2017; Gao et al., 2020), F_{IR} is the chocolate ingestion rate in grams per day (25 g), C is the concentration of the trace element in the given food (µg kg^{-1}), RfD is the oral reference dose of the trace element in µg $kg^{-1}day^{-1}$, BW_a is the reference body weight of 70 kg for adults and 19 for children and AT_n is the averaged exposure time (25500 days for adults and 2190 for children).

The average THQ values for different types of cocoa products are shown in the Table 7, for adults, values for Ni ranged from $4.0{\cdot}10^{-4}$ to $2.1{\cdot}10^{-1}$ with a mean value of $3.6{\cdot}10^{-2}$, while for children are in the range $1.8{\cdot}10^{-3}$ - $9.7{\cdot}10^{-1}$, indicating that all samples show non-carcinogenic health risks. For lead, THQ values for adults ranged from $3.9{\cdot}10^{-3}$ to $8.5{\cdot}10^{-1}$ and from $1.8{\cdot}10^{-2}$ to 4.0 for children, in particular, for the latter consumers, the consumption of four dark chocolates (Dk 4, Dk 6, DK 8, DK 9) and two gianduia (GI 1 and GI 2) products from renowned industries is relatively harmful. Similarly, for cadmium, THQ values ranged from $4.2{\cdot}10^{-3}$ to $7.0{\cdot}10^{-2}$ and from $2.0{\cdot}10^{-2}$ to $3.2{\cdot}10^{-2}$ for adults and children, respectively.

4.3. Hazard Index (HI)

The Hazard Index (HI) is calculated as the addition of the individual target hazard quotients relative to the investigated elements. Even if single THQ for an element is lower than unity, the cumulative effect may result in adverse health effects. A THQ value < 1 indicates that the trace hazardous metal concentration is no harmful, while a THQ value > 1 indicates that it has potential harm to the human body (US-EPA). The HI equation is:

$$HI = \sum_{N=1}^{i} THQ_n$$

Cumulative risk (HI) for Ni, Pb and Cd ranged from $2.1{\cdot}10^{-1}$ to $8.8{\cdot}10^{-1}$ for adults and from $4.0{\cdot}10^{-2}$ to 4.1 for children (Table 7). For children HI > 1 was found in 6 samples (DK 4, DK 6, DK 8, DK 9, G 1 and G 2) produced by important companies.

4.4. Target cancer risk (TCR)

Target Cancer Risk (TCR) is employed to evaluate the potential risk associated with exposure to carcinogens over a lifetime exposure period. In this case, instead of the oral reference dose, as is used for the determination of THQ, the oral slope factor (CPS_o) is employed. This factor determines, together with the dose of the carcinogen, the probability of an excess cancer risk over the lifetime of the exposed individual. TCR is calculated as follows:

$$TCR = \frac{E_{FR} \times Ed \times F_{IR} \times C \times CPS_0}{BW_a \times AT_c}$$

Where E_{FR} is the exposure frequency to the element (350 days), Ed is the duration (70 yrs) of exposure, F_{IR} is the food ingestion rate in Kg per day (0.025 g), C is the concentration of the element in the sample (µg kg^{-1}), CPS_o is the oral cancer slope factor for the specific element (Pb = 0.0085, Cd = 0.38, Ni = 1.7 mg kg^{-1} day^{-1}) (Şeker, 2023), BW_a is the reference body weight of 70 kg, AT_c is the averaged exposure time to the carcinogen (350 days·70 yrs).

For all types of cocoa products investigated by us, the average TCR values are shown in the Table 8.

The USEPA has established safety limits for TCR of $1{\cdot}10^{-6}$ or less. The values from $1{\cdot}10^{-6}$ to $1{\cdot}10^{-4}$ are classified as moderate risk, while a critical value for people health is considered to be $1{\cdot}10^{-4}$. Values of $1{\cdot}10^{-4}$ and above are risky values that are important for public health and need attention (Antoine et al., 2017; Shaheen et al., 2016; U, 2015).

For Ni, TCR values concerning adults ranged from $1.3{\cdot}10^{-5}$ to $7.1{\cdot}10^{-3}$, the latter relating to a white chocolate (WH 2) (Table 8), also in this case produced by a great company. Only in 15 of the analysed samples this parameter is lower than 10^{-4}, while for children the values are in the range $1.1{\cdot}10^{-7}$ to $3.3{\cdot}10^{-2}$ and in 13 samples is lower 10^{-4}. For lead and cadmium, the above parameter slightly exceeds the value 10^{-4} for children in few samples.

5. Conclusions

The main aim of this investigation was to optimize voltammetric methods to quantify three elements of toxicological interest (lead, cadmium and nickel) in chocolate and cocoa-based food samples in which

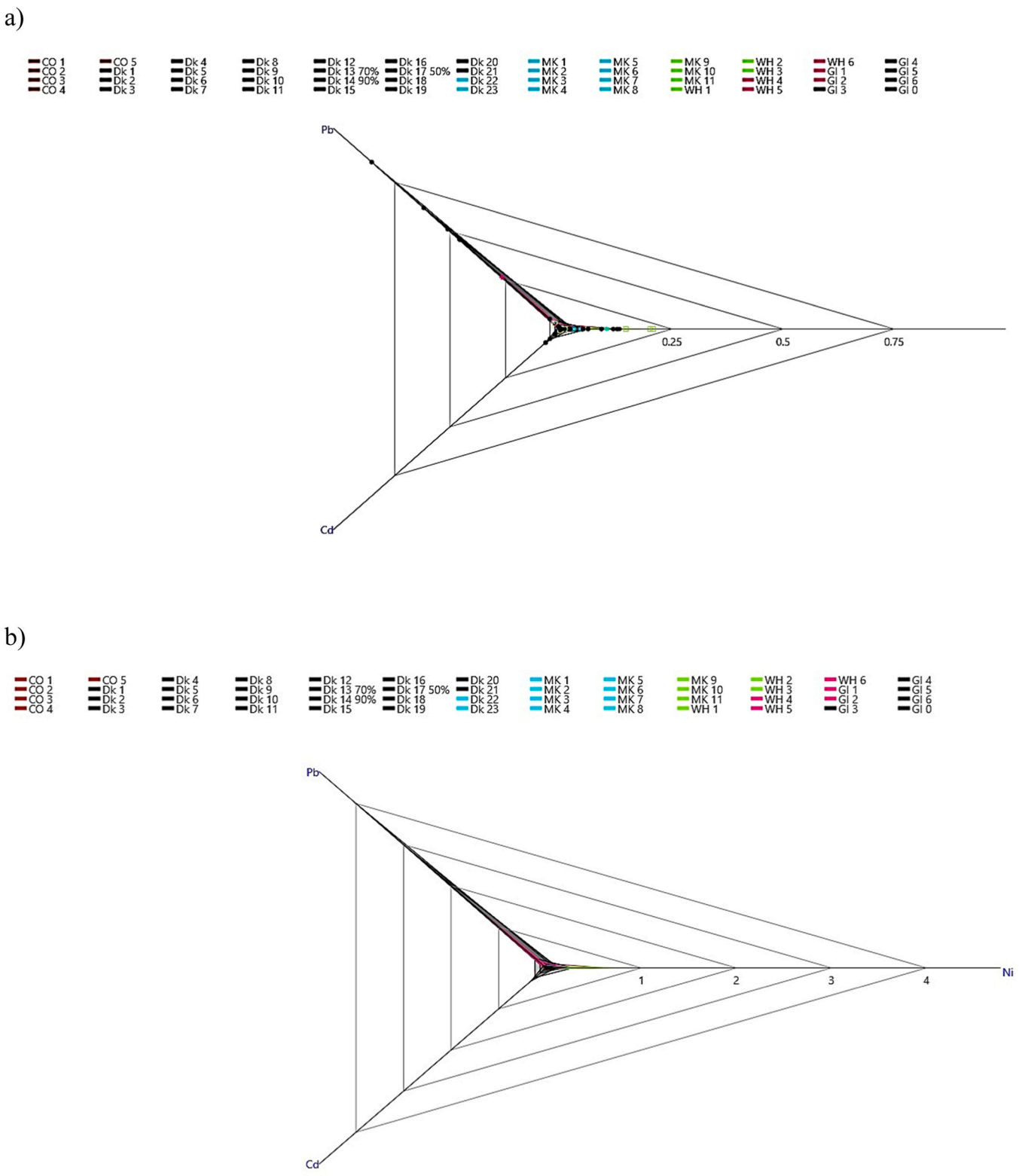
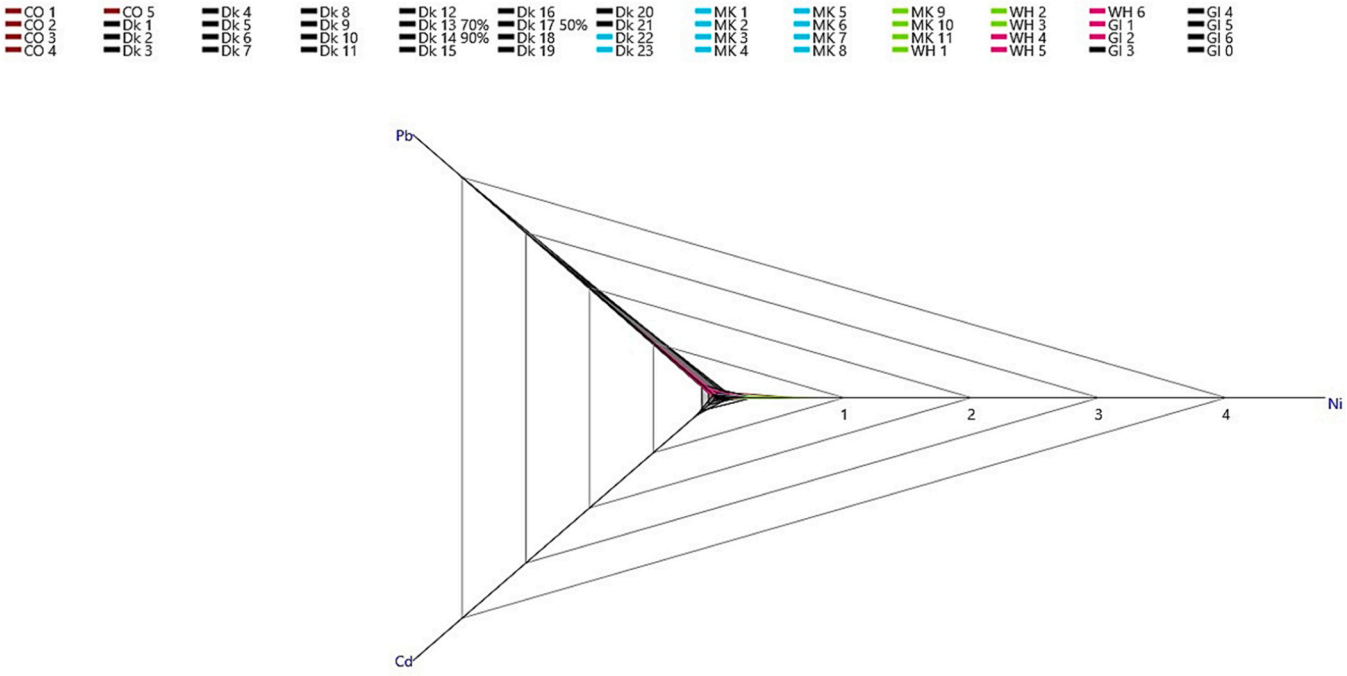


Fig. 7. Target hazard quotient (THQ) values for analysed metals for adults (a) and Children (b) due to the consumption of chocolate and cacao products. The values are less than 1 and no significant risks have been identified.

concentrations should be, theoretically, at ppb levels. Fifth two samples of Italian chocolate and cacao products were investigated. After acid mineralization by microwave, metal quantification was performed using anodic re-dissolution and differential pulse voltammetric techniques. It has been found that voltammetry is very advantageous to quantify trace elements in these food matrices due to their high sensitivity derived from the electrochemical pre-concentration of the element at the electrode surface also is a fast and low-cost technique. From the evaluation of the quality parameters, it is clear that the optimized methods used for chocolate and cocoa-based food samples are quite satisfactory to assess any risks due to the consumption of the above sweets; however, it should be considered that, given their high sensitivity, they can be used for

a)



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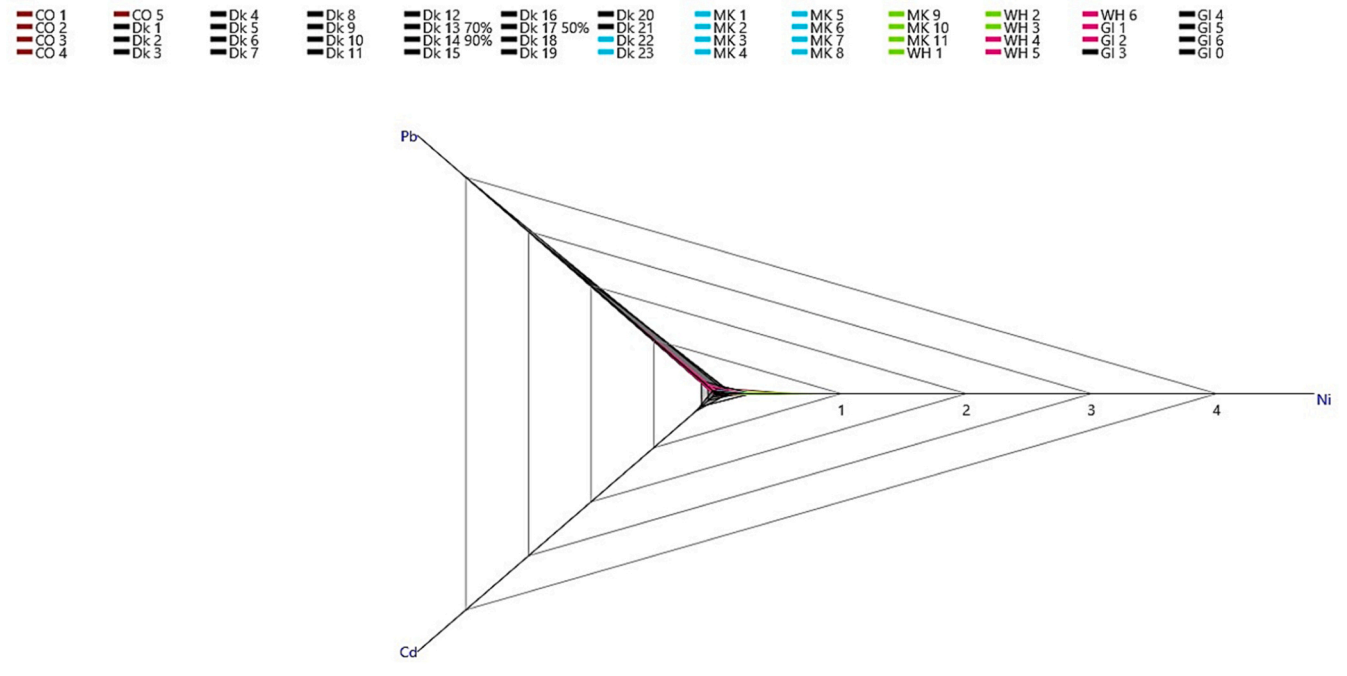

Fig. 8. Target Cancer Risk (TCR) values for analysed metals for adults (a) and Children (b) due to the consumption of chocolate and cacao products. The values are less than 1 and no significant risks have been identified.

Table 6
Contamination categories based on EF values.

EF < 2	Little or no enrichment
2 ≤ EF < 5	**Moderate enrichment**
5 ≤ EF < 20:	**Significant enrichment**
20 ≤ EF < 40:	**Very high enrichment**
EF ≥ 40	**Extremely high enrichment**

other complex matrices of environmental interest, for example for the soils where cocoa plants are grown and for other foods.

The results show that most of the concentrations of the three metals in the different samples are, even of some orders, lower than those of the earth's crust (EF<1). Only in six of the analysed samples the EF, relative to cadmium and nickel, are greater than 1 and this suggests that, in these cases, the enrichment could derive from anthropogenic sources. The highest value of the Metal Pollution Index was found in a dark chocolate sample produced by an important Italian company. Furthermore, the data showed that the cocoa and chocolate samples contain cadmium concentrations compliant with the limits established by law and with the recommended daily and weekly doses. In light of this, we can assume that cadmium comes from the roots of the cocoa tree, therefore, in the finished product the concentration of this element is relatively low.

As regards lead, it is present at higher concentrations in some dark chocolate samples and in 6 samples it exceeds the legal limit (Rankin et al., 2005). However, the daily and weekly intake amount of the metal remain below the recommended daily dose for adult people while the limit is exceeded if children eat two of the analysed samples. In all cases, it should not be overlooked that many people, especially children, consume much higher daily and weekly quantities than those considered. The concentrations of Ni, in five samples, exceed the legal limit established and the recommended daily and weekly dose. The presence of nickel could come from contamination of the cultivation soil, from the use of low-purity chemical fertilizers or, most likely, from the stainless steel machinery used to process the cocoa paste. Some metals (nickel, chromium, vanadium, etc.) are present in stainless steels used in the production of equipment necessary in all stages of food preparation. Stainless steels are a group of iron-based alloys that contain several elements with at least 11 % Cr, (Liu et al., 2023), to increase corrosion resistance, during the steel production process is also possible to add other alloying elements in addition to chromium, including nickel, and creating the Ni-Cr alloys, such as AISI 304 steel (18–20 % Cr; 8–10 % Ni). In particular, it can be assumed that one of the sources of nickel in chocolate is the chromium-nickel balls of mills used in chocolate production. These mills reduce the components of the chocolate mass to micrometer size so that the chocolate achieves the desired consistency and fineness (Godebo et al., 2024). Therefore, the machinery used could release metals if damaged or worn (Addai et al., 2024). It cannot be excluded that, with normal wear and tear or corrosion, they may contaminate the processed materials when they come into contact with various fluids. Contamination is expected to be greater in foods that require many preparation stages (for example cocoa products) as has been found by some authors in flour samples (Liu et al., 2023; Telloli et al., 2025).

To limit the release of some elements during the manufacture phase, it would be necessary to optimize processes at lower temperatures and having shorter contact times.

The highest nickel concentrations were found in white chocolate samples in whose composition there are significant percentages of hydrogenated fats (cocoa butter) to obtain products with desired organoleptic properties and with more viscous or solid consistencies. In the production of the aforementioned fats, due to the reaction of the nickel catalyst and the fatty components, the formation of lipophilic nickel salts also occurs, such as Ni(fatty acid)$_2$ salts (Dohnalova et al., 2017). Given the American Environmental Safety Institute (AESI) claims that chocolate manufacturers are not taking appropriate measures to remove potentially dangerous levels of several metals from their chocolate products or informing consumers of the health risks (BBC NEWS, 2002) (Rehman and Husnain, 2012) and in light of the above by us, chocolate producers should carry out quality controls on the raw materials used, in particular by quantifying the concentrations of nickel and any other dangerous substances on the hydrogenated fats used. In addition to the nutritional values reported by law on the label, the maximum quantities of chocolate that can be eaten daily or weekly by adults and children should be reported.

The high variability on the content of the three elements and the enrichment values of chocolate samples can be explained by the cocoa content and its origin, mainly because cocoa beans accumulate metals from the soil where the trees were growing while contamination during production processes is limited.

Table 7
Health risk parameters (mean values).

	Adults			Children			Adults				Children			
	Ni	Pb	Cd	Ni	Pb	Cd	Ni	Pb	Cd	HI	Ni	Pb	Cd	HI
Sample type	**EDI** ($\mu g\ kg^{-1}\ day^{-1}$)			**EDI** ($\mu g\ kg^{-1}\ day^{-1}$)			**THQ**			**ΣTHQ**	**THQ**			**ΣTHQ**
Cocoa	**0.57**	**0.030**	**0.015**	**2.7**	**0.14**	**0.071**	**0.027**	**0.0084**	**0.0049**	**0.041**	**0.13**	**0.04**	**0.023**	**0.19**
Dark	**0.81**	**0.46**	**0.025**	**3.8**	**2.0**	**0.12**	**0.039**	**0.11**	**0.0080**	**0.16**	**0.18**	**0.54**	**0.037**	**0.76**
Milk	**0.21**	**0.036**	**0.013**	**1.0**	**0.17**	**0.062**	**0.010**	**0.010**	**0.0042**	**0.024**	**0.047**	**0.046**	**0.020**	**0.11**
White	**2.1**	**0.049**	**0.013**	**9.8**	**0.23**	**0.062**	**0.10**	**0.013**	**0.0042**	**0.12**	**0.47**	**0.06**	**0.020**	**0.55**
Gianduia	**0.25**	**0.31**	**0.042**	**1.2**	**0.90**	**0.20**	**0.012**	**0.084**	**0.014**	**0.11**	**0.057**	**0.39**	**0.063**	**0.51**
Mean	**0.74**	**0.24**	**0.022**	**3.5**	**1.4**	**0.10**	**0.036**	**0.065**	**0.0072**	**0.11**	**0.17**	**0.30**	**0.033**	**0.50**

Table 8
Target Cancer Risk.

	Adults				Children			
	Ni	Pb	Cd	HI	Ni	Pb	Cd	HI
Sample type	**TCR**			**Σ TCR**	**TCR**			**Σ TCR**
Cocoa	**9.3E−04**	**2.5E−07**	**5.5E−06**	**9.4E−04**	**4.4E−03**	**1.2E−06**	**2.6E−05**	**4.4E−03**
Dark	**1.3E−03**	**3.4E−06**	**9.2E−06**	**1.3E−02**	**1.1E−07**	**5.5E−08**	**3.3E−09**	**1.6E−07**
Milk	**3.4E−04**	**3.0E−07**	**4.8E−06**	**3.5E−04**	**1.6E−03**	**1.4E−06**	**2.2E−05**	**1.6E−03**
White	**3.4E−03**	**4.0E−07**	**4.8E−06**	**3.4E−03**	**1.6E−02**	**1.9E−06**	**2.2E−05**	**1.6E−00**
Gianduia	**4.2E−04**	**2.5E−06**	**1.7E−05**	**4.3E−04**	**1.9E−03**	**1.2E−05**	**7.2E−05**	**2.2E−03**
Mean	**1.2E−02**	**1.9E−06**	**1.5E−06**	**1.2E−02**	**5.5E−03**	**8.7E−06**	**3.8E−05**	**5.6E−03**

Chemometric data suggest significant differences in the contamination profiles of the three metals. Ni appears to be influenced by a wider variety of factors, while Pb and Cd show more consistent behaviour with sporadic inputs. These results highlight the need for further investigation into the sources and patterns of metal contamination, especially in cases where extreme outliers are observed.

Considering the TCR values of the three elements taken into consideration and their sum, in many cases the consumption of 25 g of chocolate per day, especially by children, can be considered risky for their health and for the probable onset of cancer.

CRediT authorship contribution statement

Orecchio Silvia: Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Amorello Diana:** Investigation, Formal analysis. **Gioè Francesca:** Formal analysis. **Orecchio Santino:** Writing – original draft, Validation, Investigation, Conceptualization. **Barreca Salvatore:** Writing – original draft, Data curation.

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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