HEALTH RISK ASSESSMENT OF SOME HEAVY METALS AND TRACE ELEMENTS IN SEMI-SWEET BISCUITS: A CASE STUDY OF ROMANIAN MARKET

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In recent decades, interest in bakery products has increased due to their nutritional properties. But in addition to this important aspect, they can also contain significant amounts of heavy metals and trace elements that can affect human health. Metals are found everywhere in nature and their appearance in food products can be due both, to the accumulation from the technological flow and from the raw materials used for their production. This study aims to determine the concentrations of some heavy metals and other trace elements (lead, cadmium, chromium, copper, iron, zinc and manganese) in 15 brands of Petit Beurre semi - sweet biscuits on the Romanian market, with different countries of origin (Romania, Republic of Moldova, Turkey, Germany, France and the Netherlands). The samples prepared by dry digestion, were then analyzed by inductively coupled plasma mass spectrometer (ICP – MS). The concentrations found were between < 0.00009 and 0.029 mg/kg for Pb, 0.004 and 0.010 mg/kg for Cd, 0.004 and 0.065 mg/kg for Cr, 0.520 and 0.881 mg/kg for Cu, 1.162 and 2.896 mg/kg for Zn, < 0.00009 mg/kg for Mn, 9.004 and 21.779 mg/kg for Fe. The values found were below the maximum allowed limits for all metals. Based on the results, a risk analysis was performed, by determining the following parameters: Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Total Target Hazard Quotient (TTHQ), for 2 age groups - children and adults. For both groups, THQ values were lower than 1 for the tested metals except iron. TTHQ values were also higher than 1 for both children and adults.

Keywords: contamination, risk assessment, heavy metals, biscuits, trace elements

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1. Introduction

Interest in bakery products is growing by the day, because of their nutritional value. The word biscuit comes from the Latin "*panis biscoctus*" which represented a twice baked bread, used by sailors in long expeditions due to their long shelf life. Over time these bakery products have developed, and today they are used as gifts, snacks, but most often in children's diet. They can have various shapes, fillings, and glazes [1]. The classification of biscuits is diverse, and it depends on the appearance, sugar and fat content or even glazes and creme used [2]. The general classification of these bakery products is according to the sugar and fat content, being divided into four main categories: crackers, semi-sweet, short (high fat and high sugar) and soft biscuits [3].

Biscuit consumption is an important sector in Europe, with the highest consumption volume being recorded in the United Kingdom (13.6 kg per capita), followed by Italy (10.5 kg per capita), France (7.6 kg per capita), Czech Republic and Slovakia (5.5 kg per capita), Germany (5 kg per capita), Hungary (4.7 kg per capita). Poland (3.8 kg per capita) and on the last place, registering the lowest consumption is Romania (3.4 kg per capita) [4].

In recent decades, global industrialization has intensified and thus increased the diversity of contamination in most industries, especially in the food industry. Thus, issues related to food safety and security has become a global concern, being associated with the safety of human health [5].

Bakery products are widely used, they can have not only the content of minerals beneficial to the body, but also the intake of heavy metals, which lead to disorders or intoxications, following a long consumption. Metals such as zinc, iron, copper, cobalt, chromium, manganese etc. are essential to the body, while metals, like lead, nickel, cadmium are toxic even in low concentrations [6]. The body's essential metals are constituents of some enzymes and are of major importance in the involvement of proteins in the metabolic pathways, their deficiency leading over time to dysfunctions of these metabolic pathways and can cause diseases. There are no known biological functions in the body that require the intake of toxic metals, on the contrary, these even in very low concentrations, lead to intoxications [7].

Among the effects of lead on the human body, we can list adverse effects of the nervous, renal, reproductive, hepatic systems, blood, musculo-skeletal, development [8, 9]. Also, Pb have carcinogenic action, which can lead to tumors, being classified in carcinogenic risk group 2B [9]. These effects may occur especially in infants, children and pregnant women [8]. Even at very low concentrations, Cd can be toxic for human health, and can cause disorders of the reproductive system, embryonic development, renal filtrate system, immune system, nervous system, DNA changes, anemia, bone, lung cancer, cell death [8, 9]. Cd is also an environmental pollutant, with effects on soil and food quality [9]. Cr has important functions in the human body, such as biosynthesis of factors (glucose tolerance factor), metabolism of lipids, protein, carbohydrate, in high concentrations can promote skin cancer, dementia, but also diseases of the kidneys, stomach, respiratory system, liver, growth depression [8, 10]. Trivalent chromium has a lower toxicity than hexavalent chromium [10]. Cu is present in the human body, being involved in redox reactions, having an antioxidant role in eliminating free radicals, hemoglobin synthesis, various metabolic processes, protection of nerve cells, prevention of cell degradation, but also a component of several important enzymes [8–10]. Symptoms of Cu deficiency include hypochromic anemia, poor pigmentation of hair and skin, osteoporosis, low immunity, bone deformities, mental retardation, cardiovascular disease, colon cancer [8, 11]. Excess Cu can produce oxidative stress, DNA damage, reduced cell proliferation, diarrhea, nausea, liver disease, kidney damage [12, 8]. Zn is an essential element of the human body, with a catalytic role for a large number of enzymes, regulatory and structural, being involved in development during pregnancy, childhood, adolescence, metabolism, normal growth, general well-being, proper sense of taste and smell. It also has an important role in the immune system, protein synthesis, DNA synthesis, cell division [8-9, 11]. Mn is very important for various biochemical processes, for bone development, metabolism regulation, cellular protection from free radical species, for reproduction, proper functioning of the nervous system, protein, lipid carbohydrate metabolism, glycosaminoglycan formation, immune system improvement, protein synthesis, vitamins and enzymes, activator of some enzymes [8–11, 13]. Adverse effects due to chronic exposure to this element include the prevalence of metabolic disorders, obesity, hepatic steatosis, oxidative stress, insulin resistance, atherosclerosis, hyperlipidemia, impaired reproduction, poor growth, apathy, tremors [8, 9, 13]. Fe, the most abundant microelement in the human body has countless functions, of which, more important are the synthesis of hemoglobin, the transfer of oxygen and electrons in the body, is part of many enzymes, participates in energy production reactions, metabolic processes, necessary for the synthesis of collagen, antibodies, RNA [8, 11]. The deficiency of this element is manifested by anemia, irritability, hair loss, loss of attention, bleeding, severe fatigue, dyspnea, lowered memory [8, 11]. However, exposure to high concentrations of Fe can have side effects, such as abdominal pain, diarrhea, conjunctivitis, vomiting, liver failure, free radical formation, cell death [8, 11, 14].

The aim of this study is to determine the levels of some heavy metals and trace elements (Pb, Cd, Cr, Cu, Zn, Mn, Fe) in 15 biscuit brands available on the Romanian market. Also, for the results obtained, a comparative study was performed with other sources in the literature to see if there are differences in the levels of metals tested.

Table 1

Samples description

Sample	Country	Ingredients
P1	**RO**	Wheat flour, glucose syrup – fructose, sugar, non-hydrogenated vegetable fat (palm), aeration agents (ammonium carbonates, sodium carbonates, pyrophytes), wetting agent (glycerol), emulsifier (soy lecithin), iodized salt, flavors, preservative (sodium metabisulphite), enzyme (protease).
P2	**RO**	White wheat flour 62.1%, sugar, vegetable fat (palm), glucose syrup, pasteurized whole milk 1.2%, invert glucose syrup, aeration agents (sodium hydrogen carbonate, ammonium acid carbonate, sodium pyrophosphate), iodized salt (salt, potassium iodate), whey powder, flavor, preservative (sodium metabisulphite).
P3	**MD**	Wheat flour, sugar, vegetable fats (palm), invert syrup (sugar, water, acidity regulators (citric acid, sodium carbonates)), loosening agents (sodium carbonates, ammonium carbonates), iodized food salt, acidity regulator (citric acid), vanillin flavor, flour tartar agent (sodium metabisulphite).
P4	**TR**	Wheat flour 69.5%, sugar, vegetable oil (palm), fresh pasteurized milk 2.4%, invert sugar syrup, aeration agents (ammonium acid carbonate, sodium acid carbonate, sodium acid pyrophosphate), salt, whey powder (milk), flavor, preservative (sodium metabisulphite).
P5	**RO**	Wheat flour, water, sugar, invert sugar, non-hydrogenated palm vegetable oil, loosening agents (ammonium bicarbonates, sodium bicarbonate, sodium acid pyrophosphate), corn starch, iodized salt, emulsifier (soy lecithin), flour-missing agent (sodium metabisulphites), acidifying (citric acid), flavoring
P6	**MD**	Wheat flour, water, sugar, invert sugar glucose, palm vegetable oil, aeration agents (sodium acid carbonate, ammonium acid carbonate, sodium acid pyrophosphate), wetting agent (glycerol), food iodized salt, natural vanilla flavor, emulsifier (soy lecithin).
P7	**RO**	White wheat flour, glucose syrup – fructose, sugar, non-hydrogenated vegetable fat of palm, aeration agents (ammonium carbonates, sodium carbonates, diphosphates), wetting agent (glycerol), emulsifier (soy lecithin), iodized salt, preservative (sodium metabisulphite), flavors
P8	**RO**	Wheat flour 57%, sugar 11%, butter 5.5%, palm oil 3.5%, invert sugar, loosening agents (E503 ammonium bicarbonate, baking soda E500), glucose syrup, whey powder, malt extract, iodized salt, flavors, protease, preservative (sodium metabisulphite E223).
P9	**TR**	Wheat flour 68.7%, sugar, vegetable oil (palm), fresh pasteurized milk 2.4%, honey 1.1%, syrup invert sugar, aeration agents (ammonium acid carbonate, sodium acid carbonate, sodium pyrophosphate), salt, whey powder (milk), flavor, preservative (sodium metabisulphite).
P10	**FR**	Wheat flour 70%, sugar, butter 12.8% (milk) (with 15% reconstituted butter), milk powder 2.5%, semi-skimmed milk, aeration agents (ammonium bicarbonates, baking soda, diphosphates), egg powder, salt, flavors.
P11	**DE**	White wheat flour, glucose syrup – fructose, sugar, non-hydrogenated palm vegetable fat, aeration agents (ammonium carbonates, sodium carbonates, diphosphates), iodine, iodine, wetting agent (glycerol), emulsifier (rapeseed lecithin), flavors, preservative (sodium metabisulphite).
P12	**RO**	White wheat flour, isolucose (glucose syrup and fructose), non-hydrogenated vegetable fats from palm trees, sugar, corn starch, aeration agents (baking soda,

Sample	Country	Ingredients
		ammonium bicarbonate), iodized salt, preservative (sodium metabisulphite), flavors.
P13	**NL**	Wheat flour (gluten), sugar, butter 11.3% (milk), glucose syrup, skimmed milk powder, aeration agent: ammonium carbonates, salt, emulsifier: lecithins.
P14	**RO**	White wheat flour, fructose glucose syrup, sugar, vegetable fat (palm), aeration agents (ammonium carbonates, sodium carbonates, diphosphates), wetting agent (glycerol), emulsifier (soy lecithin), iodized salt, preservative (sodium metabisulphites), flavors.
P15	**RO**	Wheat flour, water, sugar, vegetable oil (palm), invert sugar, aeration agents (ammonium acid carbonate, sodium hydrogen carbonate), corn starch, iodized salt, stabilizer (sodium pyrophosphate), emulsifier (soy lecithin), flavors, flour tartar agent (sodium metabisulphites), acidifying (citric acid).
RO-Romania; MD-Republic of Moldova; TR-Turkey; FR-France; DE-Germany; NL-Netherlands		

2. Materials and method

Sample collection

In this study, 15 different brands of semi-sweet biscuits, commercially available, were purchased from local markets and hypermarkets and then analyzed. Comprehensive information about tested samples, regarding the country of origin and the ingredients used, are presented in Table 1.

Reagents

All reagents and chemicals used were of analytical grades. Ultrapure nitric acid (HNO_3 65%) was purchased from Merck (Merck Co., Darmstadt, Germany). A Multielement Standard Solution 6 for ICP-MS were purchased from Sigma Aldrich (Missouri, USA). Dilutions and solutions were performed with ultrapure water (18.2 MΩ.cm). All the glassware were cleaned and decontaminated with 10 % HNO_3 solution (v/v).

Equipment

The quantification of the heavy metals and trace elements was performed using an inductively coupled plasma mass spectrometer (ICP–MS), model NexION300Q (Perkin Elmer Inc., Waltham, USA). An analytical balance with an accuracy of 0.0001 g, OHAUS Adventurer Pro, model AV 264 and a GFL water bath, type 1032, were used for weighing the samples, respectively for evaporating the 1:1 HNO_3 (v/v) solution, used at ash treatment. The operating conditions are listed in Table 2.

Sample preparation

To determine the content of heavy metals in the biscuit samples, they were subjected to dry digestion. Thus, 5 grams of the well-crushed and homogenized sample were weighed with a precision of 0.0001 grams, in porcelain crucibles. They were placed in a calcination furnace and subjected to calcination at a temperature of 550^0C for 4 hours until a white ash is obtained. After 6 hours, the crucibles are removed from the oven, left to cool, then treated with 2.5 mL 1:1 HNO_3 (v/v), which is evaporated to dryness on a water bath at 100^0C. If the ash still has uncalcined matter, put the crucibles in the calcination again until a white ash is obtained. If the resulting ash is white, it is treated with 2.5 mL 1:1 HNO_3 (v/v), cover with a watch bottle and leave on the water bath for 15 min. Then, the sample is transferred to 50 mL volumetric flasks with ultrapure water made up to the mark and analyzed by ICP – MS Spectrometry. All samples were performed in triplicate, the value mentioned being the mean of the 3 replicates.

Table 2

ICP – MS operating conditions

Parameter description	Current value
Radio Frequency Generator	Free running type, 40 MHz
Radio Frequency power	1000 W
Nebulizer Gas Flow	0.89 L/min
Auxiliary Gas Flow	1.20 L/min
Plasma Gas Flow	16 L/min
Sample Uptake Flow	1.0 mL/min
Dual Detector Mode	Pulse
Number of sweeps/readings	3
Number of readings/replicates	3
Number of replicates	3
Wash	Time (45), speed (± rpm) – 24
Sample flush	Time (35), speed (± rpm) – 24
Read delay	Time (15), speed (± rpm) – 20
Ba, Co, Cu, Zn, Mn, Ni, Li, Fe, Pb, Cd, Cr, Sb mass	138, 59, 63, 66, 55, 27, 60, 7, 57, 208, 111, 52, 121

Calibration

The calibration curves for each element were performed with the Multielement Standard Solution 6 consisting of 5 points, 10 µg/L, 20 µg/L, 30 µg/L, 40 µg/L and 50 µg/L. Among the performance parameters of the method used, the linearity for this concentration range proved to be very good. Except Fe which had a regression coefficient of 0.9963, all other metals had a regression coefficient over 0.9990. The recovery of the method was between 91.5% - 108.44%. The detection limits of tested elements obtained after 10 repeated measurements of the blank solution were 0.07 µg/L for Cr, 0.08 µg/L for Cu, 0.09 µg/L for Pb, Cd and Mn, 0.12 µg/L for Zn and 0.18 µg/L for Fe.

Health risk assessment

The risk analysis was performed to determine if the metals tested were present in the samples at a level that could endanger the health of consumers. To evaluate this parameter, it is necessary to determine the estimated daily intake of metals (EDI), target hazard quotients for noncarcinogenic risk and total target hazard quotient [8]. The risk assessment was performed for 2 age categories, namely, for children with an average weight of 15 kg, and for an adult with an average weight of 70 kg. An average biscuit daily consumption of 50 g for children and 100 g for adults was approximated.

Estimated daily intake (EDI)

The estimated daily intake was determined by estimating the levels of heavy metals ingested by the two age groups following the consumption of biscuits. This indicator was calculated for each metal and for each sample. EDI was calculated using Equation 1 [8].

$$EDI = \frac{C\ metal\ x\ D\ intake}{BW}, \quad (1)$$

where EDI is Estimated daily intake, in µg/kg bw/day, C_{metal} is the concentration of metal in sample, in mg/kg D_{intake} is Daily food intake, in kg/person/day and BW is body weight, in kg.

Target hazard quotient (THQ)

This indicator was determined to estimate the level of concern of heavy metals and trace elements found in samples, after ingestion of biscuits. For its estimation, only the ingestion pathway was taken into account. This indicator was calculated for each metal and for each sample. THQ was calculated using Equation 2 [8].

$$THQ = \frac{EDI}{RFD}, \quad (2)$$

where THQ is Target Hazard Quotient (unitless), EDI is Estimated Daily Intake, in µg/kg bw/day and RFD is reference dose for oral exposure. The values of RFD are 0.0036 for Pb, 0.0005 for Cd, 0.003 for Cr, 0.0037 for Cu, 0.3 for Zn, 0.046 for Mn and 0.007 for Fe [15 – 17].

Based on THQ values, it was estimated Total Target Hazard Quotient, TTHQ, using Equation 3 [8], which represents the sum of all THQ. This parameter was estimated for each sample.

$$TTHQ = THQ_{Pb} + THQ_{Cd} + THQ_{Cr} + THQ_{Cu} + THQ_{Zn} + THQ_{Mn} + THQ_{Fe}, \quad (3)$$

where TTHQ is Total Target Hazard Quotient (unitless) and THQ is Target Hazard Quotient (of each metal).

3. Results and discussion

The content of heavy metals and trace elements are presented in Table 3. Results are presented as mean ± SD of the 3 measurements replicate. Regarding their detection in the tested samples, Pb was detected in 86.7% of the samples, Cd, Cr, Cu, Zn and Fe were detected in all samples.

Table 3

Heavy Metal Concentration in tested brands of semi-sweet biscuits

Sample	Heavy metal (mg/kg) ± SD						
	Pb	**Cd**	**Cr**	**Cu**	**Zn**	**Mn**	**Fe**
P1	0.012 ± 8.73	0.010 ± 5.77	0.065 ± 3.45	0.881 ± 0.21	2.896 ± 9.21	< LOD	16.980 ± 3.57
P2	0.017 ± 7.47	0.008 ± 9.17	0.044 ± 7.40	0.601 ± 0.95	1.630 ± 2.28	< LOD	11.011 ± 3.88
P3	0.029 ± 1.47	0.004 ± 9.65	0.042 ± 2.50	0.722 ± 9.46	2.018 ± 0.10	< LOD	21.779 ± 9.26
P4	0.004 ± 0	0.005 ± 8.48	0.034 ± 2.87	0.740 ± 0.11	2.573 ± 7.13	< LOD	18.700 ± 2.30
P5	0.012 ± 9.18	0.004 ± 8.32	0.026 ± 5.18	0.641 ± 8.80	1.469 ± 5.94	< LOD	10.654 ± 8.23
P6	0.008 ± 8.0	0.008 ± 5.59	0.021 ± 3.44	0.693 ± 9.56	1.787 ± 0.14	< LOD	12.147 ± 8.09
P7	0.004 ± 9.64	0.008 ± 1.81	0.012 ± 0.58	0.680 ± 3.41	1.674 ± 1.77	< LOD	12.294 ± 0.68
P8	0.004 ± 5.67	0.007 ± 9.30	0.026 ± 5.39	0.618 ± 8.58	1.989 ± 0.26	< LOD	14.001 ± 9.84
P9	0.002 ± 7.08	0.005 ± 8.50	0.010 ± 7.58	0.752 ± 1.56	2.513 ± 0.78	< LOD	16.320 ± 2.85
P10	< LOD	0.005 ± 8.47	0.007 ± 9.41	0.520 ± 6.87	1.549 ± 2.10	< LOD	14.137 ± 1.16
P11	0.004 ± 8.94	0.005 ± 9.98	0.009 ± 0.81	0.820 ± 5.07	2.798 ± 2.06	< LOD	14.565 ± 0.73
P12	0.007 ± 9.56	0.005 ± 3.07	0.012 ± 1.09	0.800 ± 4.41	1.970 ± 5.34	< LOD	16.306 ± 3.48
P13	0.005 ± 8.66	0.009 ± 6.08	0.008 ± 8.94	0.550 ± 0.71	1.788 ± 0.71	< LOD	17.819 ± 2.52
P14	< LOD	0.008 ± 8.43	0.008 ± 9.05	0.705 ± 3.26	1.819 ± 2.61	< LOD	12.130 ± 0.34
P15	0.001 ± 5.67	0.004 ± 7.08	0.004 ± 8.74	0.568 ± 0.43	1.162 ± 1.0	< LOD	9.004 ± 4.14
CV	108.21	31.35	79.60	15.41	25.37	-	23.76
FAO/WHO $^{(a)}$ **Maximum permissible limit** a – [8]							
	0.3 mg/kg	0.2 mg/kg	2.3 mg/kg	73.3 mg/kg	99.4 mg/kg	500 mg/kg	426 mg/kg

However, Mn was not detected in any sample. Levels of potentially toxic metals presented in the tested samples varied between < LOD and 2.896 mg/kg. The concentrations were calculated using Equation 4.

$$Concentration\ (mg/kg) = \frac{Concentration\ (\mu g/L)\ x\ V}{W}, \quad (4)$$

where V = Final volume (50 ml) of solution, and W = Initial weight (5 g) of sample measured [18].

As concerning Coefficient of Variation (CV), which measure relative variability between different measurements, it was 108.21% for Pb, 31.35% for Cd, 79.58% for Cr, 15.41% for Cu, 25.37% for Zn, 23.76% for Fe. In the case of Mn, because it was not found in any sample, the coefficient of variation was not calculated. The differences registered between the tested brands can be due to the degree of contamination of the raw material (wheat flour), technological processes (baking), food contact materials, environmental contamination, differences in production methods. It is considered that the main source of contamination of biscuits is wheat flour, obtained from wheat with a high content of heavy metals [18]. The lead levels found in the samples ranged from 0.00009 – 0.029 mg/kg. The concentrations found did not exceed the maximum permissible limit of 0.3 m /kg.

In the case of cadmium, quantified amounts varied from 0.004 – 0.010 mg/kg, being less than the maximum allowable limit of 0.2 mg/kg.
Regarding chromium, the values found were between 0.004 – 0.065 mg/kg, being much lower compared to the maximum permissible limit of 2.3 mg/kg.
Copper levels varied between 0.520 – 0.881 mg/kg, being lower than maximum permissible limit of 73.3 mg/kg. The zinc concentrations found vary between 1.162 – 2.896 mg/kg, being much lower than the maximum allowed limit of 99.4 mg/kg. Table 3 shows that Mn was lower than detection limit (0.09 µg/L), so implicitly lower than the maximum permissible value of 500 mg/kg.
Regarding iron, the levels varied between 9.004 – 21.779 mg/kg, being lower than maximum permissible value of 426 mg/kg.

As it can be seen in Table 4, the concentrations found in the present study are comparable to the concentrations found in other research papers [6]. However, lower or higher [19 - 25] concentrations were also found. The differences between these values may be due to the degree of contamination of the product itself, or the method of analysis used to detect them.

Health risk assessment
Estimated Daily Intake (EDI)

Table 5 summarizes the calculated Estimated Daily Intake (EDI) values for consumption of biscuits, for children (5a) and adults (5b). For children EDI varied between 0.00 – $9{,}67 \cdot 10^{-5}$, $1.33 \cdot 10^{-5}$ – $3.33 \cdot 10^{-5}$, $0.13 \cdot 10^{-4}$ – $2.20 \cdot 10^{-4}$, $1.73 \cdot 10^{-3}$ – $2.94 \cdot 10^{-3}$, $3.87 \cdot 10^{-3}$ – $9.65 \cdot 10^{-3}$, 0.00, $3.00 \cdot 10^{-2}$ – $7.27 \cdot 10^{-2}$, for Pb, Cd, Cr, Cu, Zn, Mn and Fe. For adults EDI were between 0.00 – $41.4 \cdot 10^{-6}$ for Pb, $5.71 \cdot 10^{-6}$ – $14.3 \cdot 10^{-6}$ for Cd, $0.57 \cdot 10^{-5}$ – $9.43 \cdot 10^{-5}$ for Cr, $0.74 \cdot 10^{-3}$ – $1.26 \cdot 10^{-3}$ for Cu, $1.66 \cdot 10^{-3}$ – $4.14 \cdot 10^{-3}$ for Zn, 0.00 for Mn and $1.29 \cdot 10^{-2}$ – $3.11 \cdot 10^{-2}$ for Fe.

THQ values for children and adults are presented in the Table 6, respectively Table 7. If the THQ value is less or equal to 1, repeated exposure to the contaminant no side effects occur, but if the THQ value is greater than 1, some side effects may occur, but not carcinogenic [26 – 27].

In case of children, the values of THQ for Pb, Cd, Cr, Cu, Zn, Mn and Fe, ranged between 0.00 – $26.9 \cdot 10^{-3}$, $2.67 \cdot 10^{-2}$ – $6.67 \cdot 10^{-2}$, $0.44 \cdot 10^{-2}$ – $7.33 \cdot 10^{-2}$, $4.68 \cdot 10^{-1}$ – $7.94 \cdot 10^{-1}$, $1.29 \cdot 10^{-2}$ – $3.22 \cdot 10^{-2}$, 0.00 and 4.29 – 10.4. Regarding adults, the values of THQ were between 0.00 – $11.5 \cdot 10^{-3}$ for Pb, $1.14 \cdot 10^{-2}$ – $2.86 \cdot 10^{-2}$ for Cd, $1.90 \cdot 10^{-3}$ – $31.4 \cdot 10^{-3}$ for Cr, $2.01 \cdot 10^{-1}$ – $3.40 \cdot 10^{-1}$ for Cu, $5.53 \cdot 10^{-3}$ – $13.8 \cdot 10^{-3}$ for Zn, 0.00 for Mn, 1.84 – 4.45 for Fe.

As it can be seen in Table 6 (for children), the values of THQ were less than 1, except for Fe, in which all samples had a THQ greater than 1. This implies a potential risk in case of a chronic consumption of these samples. The same can be seen for the THQ values in Table 7 (for adults).

The values of TTHQ are presented in Tables 6 and Table 7. If TTHQ is greater than 1, consumers are subject to side effects, but not carcinogenic and if TTHQ is less than or equal to 1, no side effects can occur [26 – 27]. The values varied between 4.84 – 11.2 for children and 2.08 – 4.78, being greater than 1, which means a potential risk after chronic ingestion. The mean values of TTHQ were 7.63 for children and 3.27 for adults, which shows that the levels at which heavy metals are found in the tested samples can cause more health problems in children than in adults, in case of repeated consumption.

Table 4

Comparative study on heavy metals and trace elements in biscuits

Analyte	Country	Concentration (mg/kg)	Analysis method	Reference
Pb, Cd, Cr, Cu, Zn, Mn, Fe	Romania	< 0.00009 – 0.029, 0.004 – 0.010, 0.004 – 0.065, 0.520 – 0.881, 1.162 – 2.896, < 0.00009, 9.004 – 21.779	ICP – MS	Present study
Ca, Cr, Cu, Fe, Pb, Cd	Nigeria	1.4 – 10.1, 0.12 – 0.25, 0.08 – 0.51, 1.02 – 2.07, ND* – 0.08, 0.003 – 0.09	F – AAS	[19]
B, Cr, Cu, Fe, Mn, Ni, Zn	Turkey	0.0 – 15.38, 0.26 – 0.66, 0.0 – 2.47, 0.0 – 6.81, 0.0 – 11.85, 0.27 – 0.67, 0.19 – 9.04	ICP – OES	[20]
Zn, Cu, Pb, Cd	Nigeria	1.77 – 2.95, 0.11 – 0.44, 0.10 – 1.19, 0.02 – 0.10	F – AAS	[21]
Zn, Fe, Cr, Mn, Ca, Mg, Ni, Pb, Cu, Co, Cd	Nigeria	21.8 – 49.3, 33.9 – 109.0, 0.1 – 0.7, 0.01 – 2.90, 205.6 – 395.3, 119.0 – 121.3, 2.2 – 4.9, < 0.001 – 1.1, 0.5 – 5.0, < 0.001 – 1.30, 0.03 – 0.05	F – AAS	[22]
Cd, Co, Cr, Cu, Fe, Mn, Zn	Serbia	0.1204, 0.2602, 2.366, 5.015, 102.1, 27.7, 15.58	ISP - AES	[23]
Cd, Pb, Cu, Zn	Egypt	0.013 – 0.122, 0.126 – 0.127, 0.787 – 1.386, 2.347 – 4.749	AAS	[24]
Pb, Cd, Mb, Ni, Se, Cu, Zn, Mn	Spain	0.0256, 0.00791, 0.0963, 0.0634, 0.0179, 0.99, 3.95, 3.42	GF – AAS	[25]

*ND – Not Detected

ICP–MS: Inductively Coupled Plasma–Mass Spectrometry; ICP–AES: Inductively Coupled Plasma–Atomic Emission Spectrometry; F–AAS: Flame Atomic Absorption Spectrometry; ICP–OES: Inductively Coupled Plasma–Optical Emission Spectrometry; AAS: Atomic Absorption Spectrometry; GF–AAS: Graphite Furnace Atomic Absorption Spectrometry

The 15 samples analyzed in this study were used as a comparison with other results from the literature.

Table 5a

Estimated Daily Intake (μg/kg bw/day) values for children

Sample	Pb (x 10^{-5})	Cd (x 10^{-5})	Cr (x 10^{-4})	Cu (x 10^{-3})	Zn (x 10^{-3})	Mn	Fe (x 10^{-2})
Sample 1	4,00	3,33	2,20	2,94	9,65	NA	5,66
Sample 2	5,67	2,67	1,50	2,00	5,43	NA	3,67
Sample 3	9,67	1,33	1,40	2,41	6,73	NA	7,27

Sample 4	1,33	1,67	1,17	2,47	8,58	NA	6,23
Sample 5	4,00	1,33	0,86	2,14	4,90	NA	3,55
Sample 6	2,67	2,67	0,70	2,31	5,96	NA	4,05
Sample 7	1,33	2,67	0,40	2,27	5,58	NA	4,10
Sample 8	1,33	2,67	0,87	2,06	6,63	NA	4,67
Sample 9	0,67	1,67	0,33	2,51	8,38	NA	5,44
Sample 10	0,00	1,67	0,23	1,73	5,16	NA	4,71
Sample 11	1,33	1,67	0,30	2,73	9,33	NA	4,86
Sample 12	2,33	1,67	0,43	2,67	6,57	NA	5,44
Sample 13	1,67	3,00	0,27	1,82	5,96	NA	5,94
Sample 14	0,10	2,67	0,27	2,35	6,06	NA	4,04
Sample 15	0,33	1,33	0,13	1,89	3,87	NA	3,00
NA – Not applicable							

Table 5b

Estimated Daily Intake (μg/kg bw/day) values for adults

Sample	**Pb (x 10^{-6})**	**Cd (x 10^{-6})**	**Cr (x 10^{-5})**	**Cu (x 10^{-3})**	**Zn (x 10^{-3})**	**Mn**	**Fe (x 10^{-2})**
Sample 1	17,1	14,3	9,43	1,26	4,14	NA	2,43
Sample 2	24,3	11,4	6,43	0,86	2,33	NA	1,57
Sample 3	41,4	5,71	6,00	1,03	2,88	NA	3,11
Sample 4	5,71	7,14	5,00	1,06	3,68	NA	2,67
Sample 5	17,1	5,71	3,71	0,92	2,10	NA	1,52
Sample 6	11,4	11,4	3,00	0,99	2,55	NA	1,74
Sample 7	5,71	11,4	1,71	0,97	2,39	NA	1,76
Sample 8	5,71	11,4	3,71	0,88	2,84	NA	2,00
Sample 9	2,86	7,14	1,43	1,07	3,59	NA	2,33
Sample 10	0,00	7,14	1,00	0,74	2,21	NA	2,02
Sample 11	5,71	7,14	1,29	1,17	4,00	NA	2,08
Sample 12	10,0	7,14	1,86	1,14	2,81	NA	2,33
Sample 13	7,14	12,9	1,14	0,78	2,55	NA	2,55
Sample 14	0,43	11,4	1,14	1,01	2,60	NA	1,73
Sample 15	1,43	5,71	0,57	0,81	1,66	NA	1,29
NA – Not applicable							

Table 6

Target Hazard Quotient and Total Target Hazard Quotient values for children

Sample	**Pb (x 10^{-3})**	**Cd (x 10^{-2})**	**Cr (x 10^{-2})**	**Cu (x 10^{-1})**	**Zn (x 10^{-2})**	**Mn**	**Fe**	**TTHQ**
Sample 1	11,1	6,67	7,33	7,94	3,22	NA	8,00	9,06
Sample 2	15,7	5,33	5,00	5,41	1,81	NA	5,24	5,92
Sample 3	26,9	2,67	4,67	6,50	2,24	NA	10,4	11,2
Sample 4	3,70	3,33	3,89	6,67	2,86	NA	8,90	9,68
Sample 5	11,1	2,67	2,89	5,77	1,63	NA	5,07	5,73
Sample 6	7,41	5,33	2,33	6,24	1,99	NA	5,78	6,51
Sample 7	3,70	5,33	1,33	6,13	1,86	NA	5,85	6,56
Sample 8	3,70	5,33	2,89	5,57	2,21	NA	6,67	7,33
Sample 9	1,85	3,33	1,11	6,77	2,79	NA	7,77	8,52
Sample 10	0,00	3,33	0,78	4,68	1,72	NA	6,73	7,26

Sample 11	3,70	3,33	1,00	7,38	3,11	NA	6,94	7,75
Sample 12	6,48	3,33	1,44	7,21	2,19	NA	7,76	8,56
Sample 13	4,63	6,00	0,89	4,91	1,99	NA	8,49	9,07
Sample 14	0,28	5,33	0,89	6,35	2,02	NA	5,78	6,49
Sample 15	0,93	2,67	0,44	5,12	1,29	NA	4,29	4,84

NA – Not applicable

Table 7

Target Hazard Quotient and Total Target Hazard Quotient values for adults

Sample	**Pb (x 10^{-3})**	**Cd (x 10^{-2})**	**Cr (x 10^{-3})**	**Cu (x 10^{-1})**	**Zn (x 10^{-3})**	**Mn**	**Fe**	**TTHQ**
Sample 1	4,76	2,86	31,4	3,40	13,8	NA	3,47	3,88
Sample 2	6,75	2,29	21,4	2,32	7,76	NA	2,25	2,54
Sample 3	11,5	1,14	20,0	2,79	9,61	NA	4,45	4,78
Sample 4	1,59	1,43	16,7	2,86	12,3	NA	3,82	4,15
Sample 5	4,76	1,14	12,4	2,47	7,00	NA	2,17	2,46
Sample 6	3,17	2,29	10,0	2,68	8,51	NA	2,48	2,79
Sample 7	1,59	2,29	5,71	2,63	7,97	NA	2,51	2,81
Sample 8	1,59	2,29	12,4	2,39	9,47	NA	2,86	3,14
Sample 9	0,79	1,43	4,76	2,90	12,0	NA	3,33	3,65
Sample 10	0,00	1,43	3,33	2,01	7,38	NA	2,89	3,11
Sample 11	1,59	1,43	4,29	3,16	13,3	NA	2,97	3,32
Sample 12	2,78	1,43	6,19	3,09	9,38	NA	3,33	3,67
Sample 13	1,98	2,57	3,81	2,10	8,51	NA	3,64	3,89
Sample 14	0,12	2,29	3,81	2,72	8,66	NA	2,48	2,78
Sample 15	0,40	1,14	1,90	2,19	5,53	NA	1,84	2,08

NA – Not applicable

4. Conclusions

In this study, an inductively coupled plasma mass spectrometry technique was used to detect levels of heavy metals and trace elements in biscuit samples prepared by a dry digestion method.

The concentrations found varied between < 0.00009 – 0.029 mg/kg, 0.004 – 0.010 mg/kg, 0.004 – 0.065 mg/kg, 0.520 – 0.881 mg/kg, 1.162 – 2.896 mg/kg, < 0.00009 mg/kg, 9.004 – 21.779 mg/kg for Pb, Cd, Cr, Cu, Zn, Mn, respectively Fe. The values found were below the maximum allowed limits for all metals tested. Also, the levels found are comparable to those found in other research studies. THQ values for children and adults were lower than 1 for all metals except iron, where THQ values were higher than 1 for the 15 biscuit samples.

Also, the TTHQ values were higher than 1, both for children and adults. In contrast, the TTHQ values calculated for the age group of children were higher than those of adults, children being prone to more health problems caused by daily consumption of 50 g of biscuits. Among the sources of contamination of these samples, the most important would be the contamination of the raw material used to obtain biscuits, but also from the packaging materials used, contamination on the technological flow, or environmental contamination.

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