A Rapid Routine Methodology Based on Chemometrics to Evaluate the Toxicity of Commercial Infant Milks Due to Hazardous Elements

Ainara Gredilla1 · Silvia Fdez-Ortiz de Vallejuelo2 · Gorka Arana2 · Alberto de Diego2 · Marcos L. S. Oliveira3,4 · Katia da Boit3 · Juan Manuel Madariaga2 · Luis F. O. Silva3

Received: 28 September 2021 / Accepted: 15 March 2022 © The Author(s) 2022

Abstract
The toxicity and the health risk assessment associated to the presence of some hazardous elements (HEs) in dried (infant formula and powdered) milks due to manufacturing and packaging process, raw materials used, environmental conditions, etc. need to be determined. With this aim, a new methodology based on the combination of health risk quotients and non-supervised (as cluster analysis (CA) and principal component analysis (PCA)) chemometric techniques is proposed in this study. The methodology was exemplified using the concentration of 27 elements, some of them HEs, measured in 12 powdered milk samples produced for children and adults in Brazil and Colombia. The concentration values were obtained by inductively coupled plasma-mass spectrometry (ICP-MS) after acid microwave digestion. Elemental concentrations vary depending upon the type of milk (initiation, growing-up, follow-on milks and adult milks). However, hazard quotients (HQ) and carcinogenic risk (CR) values showed no risk associated to the presence of HEs on milks. The methodology designed made possible to conclude that adults’ milks are more characteristic of elements naturally present in milk. Children milks present major presence of trace and minor elements. Between infant milks, sample H, designed for babies between 12 and 36 months, was identified as of poor quality. Moreover, it was possible to deduce that while the fortification process applied to children powdered milks is a probable metal and metalloid source, together with the manufacturing, the skimming process is not a contamination source for milks.

Keywords Infant milk · Powdered milk · Hazardous elements · Toxicity · Chemometrics

Introduction

Milk, considered a nearly complete food, is a good source of proteins, enzymes, minerals, fats, organic acids, carbohydrates, minerals, nutrients, and vitamins, all of which are necessary for best developmental and psychosocial outcomes for new-borns and good growth of children (Khan et al., 2014; Muhib et al., 2016). There are more than 35 micro and trace elements as inorganic ions reported to be found in raw milk (Muhib et al., 2016). Most of the components of the milk, metals or metalloids such as Ca, Cu, Zn, Mn, Mo and Fe, are considered essential elements for many physiological human functions, but they become toxic when their concentration level exceeds 40- to 200-fold their respective recommended threshold value (Koh and Judson, 1986; Rao, 2005). Some other metals, such as As, Cd, Pb and Hg, however, can be considered hazardous or toxic elements at trace level and may be accumulated along the food chain having toxic effects on human health.
Food Analytical Methods  (2022) 15:2312–2322

Metal input through powdered milk can be considered a health concern in most regions of the world. In fact, it is calculated that infants and young children absorb 50% of dietary toxic metals (compared to 10% in the case of adults) by milk consumption. Clearly, the intake of toxic metals by infants is even more dangerous than in adults, due to their high intestinal absorption capacity of toxic substances, which may cause growth problems and the risk of bad mental development. Therefore, the monitoring of these hazardous elements in the milk is essential to ensure the benefits of consuming commercial synthetic formulas (Krachler et al., 2000; Rebelo and Dutra Caldas, 2016; Saracoglu et al., 2007).

Several studies of milk samples can be found in the references (Abdulkhaliq et al., 2012; Ahmad et al., 2017; Cruz et al., 2009; Cava-Montesinos et al., 2004; Enb et al., 2009; Franco-Uría et al., 2009; Kazi et al., 2009; Kondyli et al., 2007; Krachler et al., 2000; Lutfullah et al., 2014; Martínez et al., 2019; Muñoz and Palmero, 2004; Pereira et al., 2013; Pilarczyk et al., 2013; Sager et al., 2018). Metal inputs in infant milks have also been reported, for example, by Lima de Paiva et al. (2019) for Al in milks from Brazil and by Gardener et al. (2019), for Cd and Pb in baby food from the USA, among others. However, all these studies are based on elemental concentration data recompilation, and none of them proposes an acceptable methodological tool to evaluate infant milk quality and associated risks.

For all these reasons, the objective of this work was to propose a fast, easy and conclusive analytical methodology useful for laboratories and companies working on infant milk manufacturing. The methodology proposed is based in the combination of quality guidelines, the Standardized Coefficients (SCs, proposed firstly in this study), and chemometrics applied to the elemental concentration data set. To evaluate and validate the proposed methodology, the elemental composition of some powered milk samples (for infants and adults) collected in Brazil and Colombia was used as a case study.

Materials and Methods

Sampling Collection

Thirty-six commercially available and most consumed milks (3 boxes of each of the 12 milk formulas labelled from A to L, see Table 1) were purchased from different local supermarkets in representative cities of Brazil and Colombia. All the samples were kept in their original packages and transferred to the laboratory, properly labelled and stored at room temperature. Then, samples were shipped to the IBeA laboratory located at the University of Basque Country UPV/ EHU (Bilbao, Spain) for chemical analysis. All the samples were stored at room temperature until analysis, which was
carried out within 4 months of their purchase and before the expiry date.

**Sample Preparation and Analysis**

After the reception at the IBeA laboratory, individual units, corresponding to the triplicate milk boxes of each commercial milk, were combined, homogenized and stored in glass tubes of 50 mL.

A total of 0.5 g of milk powder was exactly weighted directly into precleaned Teflon vessels and 6 mL HNO₃/1 mL of H₂O₂ mixture was added (Muhib et al., 2016). In an analytical microwave (Multiwave 3000 Microwave Oven, Perkin-Elmer), a first ramp of 600 W was applied for 5 min, then 10 min of hold, to reach the temperature established of 220 °C, and finally, it was maintained at 800 W during 5 min of ramp and 10 min of hold. After cooling, the digests were filtered (0.45 μm), transferred and diluted in polyethylene bottles to 50 mL with MilliQ water (Millipore, Bedford, MA, USA). For quality assurance, two samples of milk powder were spiked with a known amount of a multi-element standard. All samples were prepared in triplicate. Vessels containing the same acid mixture as used for the samples were also prepared and utilized as the analytical blank. The digests were stored at 4 °C until analysis.

Calibration standards were gravimetrically prepared using a Mettler-Toledo XS205 balance (0.00001 g) by dilution in water of commercial solutions of each individual element of interest at a concentration of 1000 mg L⁻¹. All the standards were acidified (1%) with sub-boiling 69% HNO₃. The argon used for the plasma was supplied by Praxair (99.99 %, Danbury, CT, USA). ⁹Be, ⁴⁵Sc, ¹¹⁵In and ²⁰⁹Bi (Specpure, Alfa Aesar, USA) were used as internal standards (10 ng g⁻¹) to correct any instrument drift during analysis. All calibration curves had regression coefficients that were greater than 0.999, showing excellent linearity over the range.

After appropriate dilution (in 1% of HNO₃), the concentrations of the 27 elements (Li, ²⁷Al, ²⁵Na, ²⁴Mg, ³⁹K, ⁴⁶Ca, ⁴⁷Ti, ⁵¹V, ⁵²Cr, ⁵⁵Mn, ⁵⁸Fe, ⁵⁹Co, ⁶⁰Ni, ⁶⁶Zn, ⁷⁵As, ⁷⁸Se, ⁸⁸Sr, ⁹⁸Mo, ¹¹⁵Cd, ¹²⁰Sn, ¹²¹Sb, ¹³³Ba, ¹⁸⁴W, ²⁰¹Tl, ²⁰⁸Pb and ²⁰⁲Hg) were measured by ICP-MS (NexION 300, PerkinElmer, Ontario, Canada) inside a clean room (class 100).

The analytical method was validated by detection limits, precision, accuracy and recovery experiments obtaining satisfactory results in all cases. Since no certified reference material was available for all the analytes, the recoveries of spiked samples were considered to assess the accuracy of the method with satisfactory results (89–105%).

The limits of detection (LOD) for each element were calculated following the IUPAC rules, defined as blank signal plus 3 SD, where SD is the standard deviation of 10 measurements of a blank. The LODs (Li: 0.013; Al: 1.4; Sr: 0.010; Mo: 0.075; Sn: 0.090; Sb: 0.00060; Ba: 0.026; W: 0.021; Hg: 0.014; Ti: 0.0036; Pb: 0.053; Na: 5.90; Mg: 0.52; K: 2.64; Ca: 0.25; Se: 0.042; Ti: 1.4; Co: 0.011; Cu: 1.6; Zn: 8.8; As: 0.66; Cd: 0.013; V: 1.5; Cr: 0.21; Mn: 0.13; Fe: 1.6 and Ni: 1.5 mg kg⁻¹) allowed the determination of minor and trace elements at the required levels in the milk samples.

The reproducibility of the method, as relative standard deviation (RSD%), was calculated after triplicate analysis of one of the powdered milk samples. The values obtained were as follow: Li: 1.3%; Al: 3.2%; Sr: 6.1%; Mo: 10%; Sn: 4.5%; Ba: 1.7%; Ti: 4.4%; Na: 6.2%; Mg: 4.1%; K: 3.8%; Ca: 2.8%; Se: 10%; Ti: 5.2%; Co: 6.7%; Cu: 0.85%; Zn: 3.7%; V: 4.5%; Cr: 13%; Mn: 6.0% and Fe: 3.6%.

**Statistical Analysis**

The data set obtained after the analysis of the samples consisted of a matrix with the concentration of all elements considered in columns (27) and the milk samples in rows (12). Concentrations below the detection limit were substituted by the half of the detection limit. Data treatment was performed by means of different chemometric tools. The cluster analysis (CA) was carried out by SPSS Statistics Software Version 20 (IBM, New York, USA). Principal component analysis (PCA) of the index data was performed by PLS-Toolbox v.7.0.2 (Eigenvector Research, USA) implemented in MATLAB 2010 software (The Mathworks, MA, USA).
Results and Discussion

Basic Statistics on Metals and Metalloids in Milk Samples

The concentrations of metal and metalloids in all the milk samples were of the same order of magnitude as reported in the literature. For example, the results for Al are in concordance with the results found by Lima de Pavia et al. (2019) in infant formulas commercialized in the city of Campinas (Brazil), and with the Zn concentrations found by Fernández-Menendez et al. (2016) in commercial formula milks of local drugstores.

In Table 2 and Fig. 1, standard deviation, variance and the box plot representation with the maximum, minimum and media value of each element are shown as basic statistics. The concentrations of major and minor elements were in the following order Ca > Na > K > Mg > Fe > Zn > Sr > Ti > Al > Cu > Mn > Ba > Pb. Elements present in trace levels were Sn, Li, Mo, W, Ti, Co, V, Cr, Ni, Se, Sb, Hg, As and Cd. Some of these heavy metals, identified as trace elements, are important inorganic contaminants with considerable risk for human health if they enter the food chain from the environment. Some others are essential elements with important nutritional value. The level of Fe, Zn, Cu, Mn, Sn and Li was higher in milks designed for infants between 0 and 6 months due to a greater variation in their composition and additional ingredients present in them. The concentration of the rest of the elements was closely similar in all varieties of infant milk studied. For adult milk samples, higher values were found for Ca, Na, K, Mg, Sr, Ti and Ba with respect to infant milks.

In order to detect if these differences were statistically significant, a Kruskal-Wallis test was carried out. This test is a nonparametric test, which is used for determining whether three or more independent samples originate from the same population. When this test leads to significant results, at least one sample differs from the others. As Fig. 2 shows, the results obtained in this case reveal that milk samples are significantly different, in a 95% of confidence level, regarding the content of Na, K, Fe, Ti, Ba and Mo. The p value was in all the cases < 0.05, which confirms that the samples are significantly different regarding these elements.

Pattern Recognition by Unsupervised Chemometric Techniques

Firstly, to obtain more information about the samples and find possible connections and groupings between the analysed milks, cluster analysis (CA) was applied. The Euclidean distance between the samples and variables fluctuated between 3 and 25 (Fig. 3a and b). The dendrograms were obtained by applying Ward’s algorithm and as it is shown in Fig. 3b, three clusters can be observed for a cut-off value of 17: one includes J, the second K and L and the third includes the rest of the milks. CA also differentiate Ca, Na and K from the rest of the elements. Therefore, CA separates infant milks from adult milks taking into account the concentration of the major elements.

Non-Carcinogenic and Carcinogenic Risks

The non-cancer risk of the investigated heavy metals through the consumption of milk was investigated by means of hazardous quotients (HQ).

Risk from metals intake through digestion was characterized using HQ as the ratio of the estimated metal dose (EDI, mg kg$^{-1}$ of body weight per day) and the reference dose (RF, mg kg$^{-1}$) values developed by USEPA and the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2005; USEPA, 2011). The EDI for each metal was firstly calculated following Eq. (1):

| Range (mg/Kg) | SD | Variance |
|---------------|----|----------|
| Ca            | 6871 | 2414 | 5,826,365 |
| Na            | 3298 | 957  | 914,947   |
| K             | 10,094 | 3003 | 9,018,511 |
| Mg            | 748  | 210  | 44,227    |
| Fe            | 124  | 34   | 1139      |
| Zn            | 61   | 17   | 302       |
| Sr            | 22   | 6    | 39        |
| Ti            | 7    | 2    | 5.6       |
| Al            | 4    | 1    | 1.36      |
| Cu            | 3    | 1    | 1.83      |
| Mn            | 1.0  | 0.4  | 0.1470    |
| Sn            | 0.21 | 0.06 | 0.0037    |
| Li            | 0.13 | 0.04 | 0.0019    |
| Mo            | 0.24 | 0.08 | 0.0057    |
| W             | 0.0168 | 0.005 | 0.0000   |
| Tl            | 0.0023 | 0.0008 | 0.0000 |
| Co            | 0.04 | 0.01 | 0.0001    |
| V             | 0.079 | 0.03 | 0.0004    |
| Cr            | 0.17 | 0.05 | 0.0023    |
| Ni            | 0.13 | 0.04 | 0.0015    |
| Se            | 0.11 | 0.04 | 0.0014    |
| Sb            | 0.004 | 0.001 | 0.0000   |
| Hg            | 0.0017 | 0.0005 | 0.0000 |
| As            | 0.09 | 0.03 | 0.0007    |
| Cd            | 0.003 | 0.001 | 0.0000   |
| Ba            | 2.4  | 0.8  | 0.5770    |
| Pb            | 0.9  | 0.2  | 0.0613    |
Here, $V_{DIR} =$ daily milk consumption rate (for infants 500 mg/day and for adults 200 mg/day); $W =$ is the average body weight (8 kg for infants and 60 kg for adults); and $C_i =$ is the experimental metal concentration in milk (mg L$^{-1}$).

Based on the EDI values, HQ values were calculated (see Eq. 2). If HQ exceeds 1.0, it indicates that there is a potential risk associated with that metal, because the EDI exceeds the Rf. As there is no a Rf value for all the elements analysed, the HQ was estimated in this case for Fe, Sr, Al, Mn, Ba, Pb, Li, Hg, Ti, Co, As, Cd, V, Cr, Ni and Se.

$$\text{EDI} = C_i \times \frac{V_{DIR}}{W}$$

(1)

$$\text{HQ} = \frac{\text{EDI}}{\text{Rf}}$$

(2)

The risk associated with the presence of metals is less harmful to the body of an adult, because of its weight, principally. Moreover, the adults’ milks are not fortified with extra nutrients; therefore, the contamination of samples through this step is usually avoided.

The possibility of cancer risk in the studied milks through intake of carcinogenic heavy metals was estimated using the carcinogenic risk (CR) (Eq. 3):

$$\text{CR} = \frac{(\text{EFr} \times \text{ED} \times \text{EDI} \times \text{CSFo})}{\text{AT}}$$

(3)

where EFr is the exposure frequency (365 days/year), ED is the exposure duration (2 years in the case of infants and 30 for adults), AT is the averaging time for carcinogens, the lifetime in this case (365 days/year × 70 years for adults and 365 days/year × 6 years for children) and the slope factor (CSFo) is used to estimate an upper-bound probability of an individual developing cancer as a result of a lifetime of exposure to a particular level of a
potential carcinogen (USEPA 2010; Zeng et al., 2015). In this case, only CR associated with the presence of As, Cd and Cr were calculated (see Table 3). USEPA considers that the CR values lower than $10^{-6}$ are negligible, and CR above $10^{-4}$ are considered unacceptable (Luo et al., 2012). The CR values obtained in this study were in all the cases below $10^{-8}$, which indicates a probability of 1 chance in 100,000,000 of an individual in developing cancer, which is considered negligible. The metal with higher CR values was As, and the sample showing the highest values was H, with a CR value for As of $1.79E-05$. However, the carcinogenic health risk due to consumption of the powdered milks analysed in this study should be neglected.

**Statistical Indexes for Nutritional Quality Characterization of Milks**

New statistical cumulative indexes called “Standardized Coefficients” (SC) were calculated according to metal and metalloid content in the milk samples as a useful tool to predict the nutritional quality of the studied milk samples. To define the SCs, first, all the concentrations were normalised by linear scaling transformation. Then, a weight was calculated for each element according to the dispersion of the concentrations of that element within the milk samples.

| Milk | CR (As)   | CR (Cd)   | CR (Cr)   |
|------|-----------|-----------|-----------|
| A    | 4.64E-06  | 3.72E-07  | 9.19E-06  |
| B    | 1.17E-06  | -         | 7.29E-06  |
| C    | 3.07E-06  | -         | 4.21E-06  |
| D    | 7.63E-07  | -         | 6.15E-06  |
| E    | 5.63E-06  | 3.44E-08  | 3.12E-05  |
| F    | 3.53E-06  | 6.97E-08  | 5.39E-06  |
| G    | 1.35E-06  | 2.69E-07  | 6.13E-06  |
| H    | 1.79E-05  | 9.85E-09  | 3.04E-06  |
| I    | 9.52E-06  | 3.30E-08  | 5.03E-06  |
| J    | 8.72E-09  | 2.37E-09  | 9.55E-08  |
| K    | 1.44E-08  | 4.00E-10  | -         |
| L    | 1.44E-08  | -         | 1.61E-08  |
When the concentrations were spread out over a large range of values a high value of weight was defined for that element, and vice versa. To do that, the relative standard deviations calculated for each element, within milk samples, were normalised by linear scaling transformation and the resulting numbers from 0 to 1 were used to weight the normalised concentrations of the elements. Finally, the normalised weighted concentrations were then averaged for each milk and, their values were normalised from 0 to 1 and multiplied by 10 (Gredilla et al., 2014). In this way, different SC values were calculated according to the elements considered for their calculation. SC_all were calculated using the concentration of the 27 elements measured in the milk samples; SC_major were determined based only on the concentration of Ca, Na and K; SC_minor with Mg, Fe and Zn; SC_toxic with Ba, Pb, Hg, As, Cd, Cr and Se; and SC_trace with the rest of elements. In Fig. 4, the values of the SCs obtained for each milk are represented. High values for SC_all, SC_minor and SC_toxic were found in H and J, and also in A for SC_toxic. For SC_trace, higher values were found for H, F and initiation milks. The pattern obtained by SC_major was slightly different, with higher coefficients for adult milks (J, K and L).

The SC values described above include several elements considered essential for humans. For example, SC_minor include Fe and Zn which are considered essential, SC_toxic includes Se and Cr also vital (although at high levels of concentration are considered toxic) and SC_trace comprises Li, Co, Mn, Mo, Ni, Cu, Sn and V, which are also essential. A new index with the mentioned essential elements was calculated called SC_ess (López-García et al., 2007). If all the milks were considered, the higher SC_ess value was found for the J sample (adult milk), but if only infant milk samples were used for the calculation of the SC_ess, samples F and H (see Fig. 5) were the samples with the highest values. Considering that several of these essentials elements at high concentration have toxic effects, a high value of SC_ess could

![Fig. 4](#) The SC values obtained for each sample

© Springer
be not a good signal of nutritional quality for milk. Given this circumstance, if the objective is to estimate the nutritional quality of the analysed sample, it is better not to focus on this index, and study the concentration values of these elements independently.

In the last step of the proposed methodology, PCA was employed. The objective in this case was to distinguish samples into groups without prior knowledge of group membership. The idea was to evaluate the potential of PCA to differentiate the nutritional level of inorganic constituents of milks by means of the SC indexes. Using the 12 powdered milks as rows and the four SC index as columns in the data matrix (variables as SCmajor, SCminor, SCtrace and SCtoxic), a PCA model was extracted whose first three PCs accounted for 94% of variance (PC1: 56%; PC2: 21% and PC3: 17%). The bi-plots (scores and loadings) of the first two PCs and PC1 vs. PC3, shown in Fig. 6, constitute the visual projection of the samples to the PCs. Figure 6a shows the scores and loading plots over the PC1-PC2 and PC1-PC3 spaces. These plots suggest that the first component (56% of variance explained) is strongly related to the average element content of the samples, in such a way that samples placed on its positive part have a higher average concentration of metals than those located on its negative part. The samples with the highest positive loading values on PC1 were H, K and L. H is more related to SCtoxic, and K and J to SCmajor, corroborating their relation with the main constituents of milk (Ca, Na and K). Taking into account the localization of H in both biplots, far away from the other infant milk samples and related to SCminor, SCtrace and SCtoxic, it could be concluded that it is not adequate for the nutritional necessities of babies. Finally, the rest of the infant samples presented low loading values, generally, on PC1 and PC2. Therefore, the elements added to milks designed for infants between 0 and 6 months due to their nutritional value do not classify them as samples of higher concern with respect to these elements. In fact, initiation milks (A, B, C and D) are placed together and presented negative loading values on PC1 and PC2. Follow-up milks (E, F and G) are also together in Fig. 6a with
positive but low loading values for PC1 and negative values for PC2. This proximity is not observed in milks designed for babies between 12 and 36 months, due to the high loading values of sample H. PC3 divided the samples due to the $SC_{\text{toxic}}$, which confirmed the higher presence of toxic elements in samples A, H and J.

Conclusions

Despite the methodology proposed here being based on the elemental composition of powdered milk samples, the results obtained after its application go far away from the simple conclusions obtained with routine studies. The combination of the elemental composition with chemometric techniques, such as CA and PCA, makes possible the identification of relations between different milks not considering the variables (metals and metalloids in this case) one by one. The use of pattern recognition techniques makes it possible to identify one of the milks made for babies between 12 and 36 months as of poor quality. This was sample H. However, it should be mentioned that this milk has no carcinogenic risk with respect to the elements considered.

This information can be completed with the one provided by the SCs. The SC indexes provide consumers, laboratories, and authorities with a simple and easy-to-understand tool to assess the nutritional quality of the samples under study. Taking into account the samples included in this study, it could be concluded that adults’ milks are more characteristic of elements naturally present in milk. The similarities between adult milks revealed that milk skimmed processed process is not a threat to the entry of contaminants into milk. On the other side, children milks present a major presence of trace and minor elements. Therefore, the addition of nutrients and the manufacturing processes should be controlled, because instead of being positive could help in the deterioration of milks designed for children.

Despite milks do not present (non)carcinogenic risks associated with the presence of As, Cd, and Cr, it is important to maintain proper measures for the reduction of metal contamination of milk samples in this area.

Taking into account the results obtained in this study on the quality of the 12 analysed milk samples, we suggest to follow the below-described methodology to apply in future studies over other kinds of foods, especially those directed to infant population.

Data Availability Data will be made available on reasonable request.

Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent is not applicable.

Conflict of Interest Ainara Gredilla declares that she has no conflict of interest. Silvia Fdez-Ortiz de Valleguelo declares that she has no conflict of interest. Gorka Arana declares that he has no conflict of interest. Alberto de Diego declares that he has no conflict of interest. Marcos L.S. Oliveira declares that he has no conflict of interest. Katia da Boit declares that she has no conflict of interest. Juan Manuel Madariaga declares that he has no conflict of interest. Luis F.O. Silva declares that he has no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Abdulkhaliq A, Swaileh KM, Hussein RM, Matani M (2012) Levels of metals (Cd, Pb, Cu and Fe) in cow milk dairy products and hen eggs from West Bank Palestine. Inter Food Res J 19:1089–1094 https://www.researchgate.net/publication/230669531
Ahmad I, Zaman A, Samad N, Ayaz MM, Rukh S, Akbar A, Ullah N (2017) Atomic absorption spectrophotometry detection of heavy metals in milk of camel, cattle, buffalo and goat from various areas of Khyber- Pakhtunkhwa (KPK). Pakistan. J Anal Bioanal Tech 8:1000367. https://doi.org/10.4172/2155-0872.1000367
ATSDR (2005) Public Health Assessment Guidance Manual (Update). U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry Atlanta, Georgia, 357.
CAC Codex Alimentarius Commission (2016) Codex Standard for infant formula and formulas for special medical purposes intended for infants. CODEX STAN 72–1981.
CAC Codex Alimentarius Commission (2017) Codex Standard for follow-up formula. CODEX STAN 156-1987.
Campillo N, Viñas P, López-García I, Hernández-Córdoba M (1998) Direct determination of copper and zinc in cow milk, human milk and infant formula samples using electrothermal atomization atomic absorption spectrometry. Talanta 46:615–622. https://doi.org/10.1016/S0039-9140(97)00306-8
Cancela S, Yebra MC (2006) Flow-injection flame atomic absorption spectrometric determination of trace amounts of cadmium in solid and semisolids milk products coupling a continuous...
Cruz GC, Din Z, Feri CD (2009) Analysis of toxic heavy metals (arsenic, lead, and mercury) in selected infant formula milk commercially available in the Philippines by AAS. Int Sci Res J 1:40–51. https://www.ymcu.com/p/S7XaM7O2uxBX4Pc

Enb A, Donia MAA, Abdi-Rabou NS, Abou-Arab AAK, El-Senayti MH (2009) Chemical composition of raw milk and heavy metals behaviour during processing of milk products. Glob Vet 3:268–275. http://www.idosi.org/gv/v3(3)/109/13.pdf

Farid S, Baloch MK (2012) Heavy metal ions in milk samples collected from animals feed with city effluent irrigated fodder. Greener J Phys Sci 2:36–43. https://api.semanticscholar.org/CorpusID:38019121

Fernández-Menéndez S, Fernández-Sánchez ML, Fernández-Colomer B, de la Flor St Remy RR, Cotallo G, Sores Freire A, Ferreira Braz B, Erthal Santelli R, Sanz-Medel A (2016) Total zinc quantification by inductively coupled plasma-mass spectrometry and its speciation by size exclusion chromatography–inductively coupled plasma-mass spectrometry in human milk and commercial formulas: importance in infant nutrition. J Chromatogr A 1428:246–254. https://doi.org/10.1016/j.chroma.2015.09.021

Fernández-Sánchez ML, de la Flor St Remy RR, González Iglesias H, López-Sastre JB, Sanz-Medel A (2012) Iron content and its speciation in human milk from mothers of preterm and full-term infants at early stages of lactation: a comparison with commercial infant milk formulas. Microchem J 105:108–114. https://doi.org/10.1016/j.micron.2012.03.016

Franco-Uría A, López-Mateo C, Roca E, Fernández-Marcos ML (2009) Source identification of heavy metals in pastureland by multivariate analysis in NW Spain. J Hazard Mater 165(1-3):1008–1015. https://doi.org/10.1016/j.jhazmat.2008.10.118

Gardener H, Bowen J, Callan SP (2019) Lead and cadmium contamination in a large sample of United States infant formulas and baby foods. Sci Total Environ 651:822–827. https://doi.org/10.1016/j.scitotenv.2018.09.026

Girard FC, Talabani M, Haimanot D (2014) Review on milk safety with emphasis on its public health world. J Dairy Food Sci World 9:166–183. https://doi.org/10.5829/idosi.wjdfs.2014.9.2.85184

Gredilla A, Fdez-Ortiz de Valdelujeo S, de Diego A, Arana G, Madriaga JM (2014) A new index to sort estuarine sediments according to the contaminant content. Ecol Ind 45:364–370. https://doi.org/10.1016/j.ecolind.2014.04.038

Hozyasz KK, Ruzyczynska A (2004) High manganese levels in milk-based infant formulas. NeuroToxicology 25:733. https://doi.org/10.1016/j.neuro.2004.03.008

Kazi TG, Jalbani N, Baig JA, Afridi HI, Sha AQ (2009) Determination of toxic elements in infant formulae by using electrothermal atomic absorption spectrometer. Food Chem Toxicol 47:1425–1429. https://doi.org/10.1016/j.fct.2009.03.025

Khan MA (2008) Nutritional adequacy of commercial infant milk formulas. Ecol Food Nutr 47:188–204. https://doi.org/10.1080/0367024070181846

Khan N, Jeong IS, Hwang JM, Kim JS, Choi SH, Nho EY, Choi JY, Park KS, Kim KS (2014) Analysis of minor and trace elements in milk and yogurts by inductively coupled plasma-mass spectrometry (ICP-MS). Food Chem 147:220–224. https://doi.org/10.1016/j.foodchem.2013.09.147

Koh TS, Judson GT (1986) Trace elements in sheep grazing near a lead-zinc smelting complex at Port Pirie South Australia. B Environ Contam Tox 37:87–95. https://doi.org/10.1007/BF01607734

Kondyli E, Katsiaris MC, Voutsinas LP (2007) Variations of vitamin and mineral contents in raw goat milk of the indigenous Greek breed during lactation. Food Chem 100:226–230. https://doi.org/10.1016/j.foodchem.2005.09.038

Krachler M, Prohaska T, Koellensperger G, Rossipal E, Stingeder G (2000) Concentrations of selected trace elements in human milk and in infant formulas determined by magnetic sector field inductively coupled plasma-mass spectrometry. Biol. Trace Elem Res 76:97–112. https://doi.org/10.1385/BTER:76:2:97

Landigran PJ, Sonawane BD, Mattison D, McCally M, Gargli A (2002) Chemical contaminants in breast milk and their impacts on children’s health: an overview. Environ Health Perspect 110:A313–A315. https://doi.org/10.1289/ehp.021100313

Leotisimidis M, Alexopoulos A, Kostopouli-Farri E (2005) Toxic and essential trace elements in human milk from Greek lactating women: association with dietary habits and other factors. Chemosphere 61:238–247. 0.1016/j.chemosphere.2005.01.084.

Lima de Paiva E, Milani RF, Morgano MA, Pavesi Arisseto-Bragotto A (2019) Aluminum in infant formulas commercialized in Brazil: occurrence and exposure assessment. J Food Compos Anal 58:1–6. https://doi.org/10.1016/j.jfca.2019.06.002

López-García I, Viñas P, Romero-Romero R, Hernández-Córdoba M (2007) Liquid chromatography–electrothermal atomic absorption spectrometry for the separation and preconcentration of molybdenum in milk and infant formulas. Anal Chim Acta 597:187–194. https://doi.org/10.1016/j.aca.2007.07.003

Luo XS, Ding J, Xu B, Wang YJ, Li HB, Yu S (2012) Incorporating bioaccessibility into human health risk assessments of heavy metals in urban park soils. Sci Total Environ 418:88–96. https://doi.org/10.1016/j.scitotenv.2012.02.053

Lutfullah G, Khan AA, Amjad AY, Perveen S (2014) Comparative study of heavy metals in dried and fluid milk in Peshawar by atomic absorption spectrophotometry. Sci World J 2014:1–5. https://doi.org/10.1155/2014/715845

Martínez MA, Casto I, Rovira J, Ares S, Nadal M (2019) Early-life intake of major trace elements, bisphenol A, tetramobisphenol A and fatty acids: comparing human milk and commercial infant formulas. Environ Res 169:246–253. https://doi.org/10.1016/j.envres.2018.11.017

Mertz W (1986) Trace elements in human and animal nutrition, 5th ed., Academic Press, New York.

Mubib MI, Chowdhury MAZ, Easha NJ, Rahman MM, Shammi M, Fardous Z, Bari ML, Uddin KH, Kurasaki M, Alam MK (2016) Investigation of heavy metal contents in cow milk samples from area of Dhaka, Bangladesh. Int J Food Cont 3:1–10. https://doi.org/10.1186/s40550-016-0039-1

Muñoz E, Palmero S (2004) Determination of heavy metals in milk by potentiometric stripping analysis using a home-made flow cell. Food Control 15:635–641. https://doi.org/10.1016/j.foodcont.2003.10.006

Pereira JSF, Pereira LSF, Schmidt L, Moreira CM, Flores EMM (2013) Metals determination in milk powder samples for adult and infant nutrition after focused-microwave induced combustion. Microchem J 109:29–35. https://doi.org/10.1016/j.microm.2012.05.010

Pilarczyk R, Wójcik J, Czerniak P, Sablik P, Pilarczyk B, Toma-Marcinik A (2013) Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. Environ Monit Assess 185:8383–8392. https://doi.org/10.1007/s10661-013-1810-9
Rao AN (2005) Trace element estimation: methods and clinical context. Online J Health Allied Sci 4:1–9. http://www.ojhas.org/issue13/2005-1-1.htm
Rebelo FM, Dutra Caldas E (2016) Arsenic, lead, mercury and cadmium: toxicity, levels in breast milk and the risks for breastfed infants. Environ Res 151:671–688. https://doi.org/10.1016/j.envres.2016.08.027
Sager M, McCulloch CR, Schoder D (2018) Heavy metal content and element analysis of infant formula and milk powder samples purchased on the Tanzanian market: international branded versus black market products. Food Chem 255:365–371. https://doi.org/10.1016/j.foodchem.2018.02.058
Saracoglu S, Saygi KO, Uluozlu OD, Tuzen M, Soylak M (2007) Determination of trace element contents of baby foods from Turkey. Food Chem 105:280–285. https://doi.org/10.1016/j.foodchem.2006.11.022
U.S.EPA Unites States Environmental Protection Agency (2011) Exposure factors handbook: National Centre for Environmental Assessment. http://www.epa.gov/ncea/efh.

Zeng F, Wei W, Li M, Huang R, Yang F, Duan Y (2015) Heavy metal contamination in rice-producing soils of Hunan Province, China and Potential Health Risks. Int J Environ Res 12:15584–15593. https://doi.org/10.3390/ijerph121215005
Zheng N, Wang Q, Zhang X, Zheng D, Zhang Z, Zhang S (2007) Population health risk due to dietary intake of heavy metals in the industrial area of Huludao City, China. Sci Total Environ 387:96–104. https://doi.org/10.1016/j.scitotenv.2007.07.044

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.