South African Journal of Animal Science 2021, 51 (No. 5)

Concentrations of metal residues in domestically produced and imported milk in Kosovo

B. Dragusha[1], H. Aliu[1#] & S. Dizman[2]
[1]Faculty of Agriculture and Veterinary, University of Pristina, 10000 Pristina, Kosovo
[2]Faculty of Arts and Sciences, Recep Tayyip Erdogan University, 53 100 Rize, Turkey

(Submitted 15 March 2021; Accepted 24 July 2021; Published 19 October 2021)

Abstract

The aim of this study was to investigate the concentration of metal residues in cows' milk and the health risks to humans from its consumption. In total 37 milk samples were analysed, namely 32 raw milk samples from domestic rural milk collection centres, and 5 imported sterilized milk samples from markets. The concentrations of chromium (Cr), manganese (Mn), nickel (Ni), and copper (Cu) in the milk samples were from 0.06 to 20.3 µg/kg, 4.7 to 64.8 µg/kg, 2.16 to 65.99 µg/kg and 0.21 to 44.7 µg/kg, respectively. Concentrations of iron (Fe), zinc (Zn), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) ranged from 157.52 to 989.95 µg/kg, 1429.63 to 5718.71 µg/kg, 0.12 to 2.01 µg/kg, 0.22 to 2.28 µg/kg, 0.00 to 0.29 µg/kg, and 0.17 to 4.29 µg/kg, respectively. The concentrations of Mg, Fe and Zn were slightly higher in domestic milk samples than in imported milk. The concentrations of Cr, Ni, Cu, As, Cd, Hg, and Pb were higher in imported milk samples than in the samples of domestic milk. Overall concentrations of minor elements had good nutritional values and the levels of the heavy toxic metals including As, Cd, Hg, and Pb were lower than the recommended limits and did not pose any threat to consumers.

Keywords: health metals, milk, toxicity
[#]Corresponding author: hamdi.aliu@uni-pr.edu

Introduction

Milk and milk products are high-value foodstuffs that are important in the human diet. Milk is a source of proteins, fats, vitamins, and minerals in well-balanced proportions and is considered a valuable source of nutrients for humans.

Milk is also an indicator of environmental contamination (Enb *et al.*, 2009; Qin *et al.*, 2009; Yüzbaşi *et al.*, 2009). Milk and milk products made from may contain heavy metals as chemical contaminants. Heavy metals are commonly defined as those metals that have a specific density of more than 5 g/cm^3. The main threats to human health from heavy metals are associated with exposure to Cd, Pb, Hg, and As (Järup, 2003). Obviously, the human body needs a certain amount of metal elements for regular function, as long as they are not present in overly large amounts (Singh *et al.*, 2011).

Industry, agriculture, domestic effluents, combustion, decomposition of chemical fertilizers, and pesticides are some of the main sources of heavy metal pollution. Contamination of water resources and agricultural lands with heavy metals has increased around the world as a result of rapid economic development and remains a critical environmental issue that affects plants, animals, and human health negatively (Fang *et al.*, 2016; Rezania *et al.*, 2016). Heavy metals are not biodegradable in the environment, but accumulate in the food chain through biotransformation and bioaccumulation (Aslam *et al.*, 2011).

The frequent occurrence of heavy metals in dairy products may be attributed to the contamination of milk, which results from cows consuming contaminated hay, grain, and water. Keeping in mind that milk is a route for the passage of heavy metals from soil to humans, the concentration of heavy metals in milk represents a concern for the health of consumers (Boudebbouz *et al.*, 2021). The continuous intake of heavy metals from consuming contaminated milk can disrupt many biological and biochemical processes in humans (Balkhair & Ashraf, 2016).

Heavy metals such as Cr, Mn, Fe, Ni, Cu, and Zn are essential to metabolic activity in living organisms. Deficiency in any of these metal nutrients will result in the onset of characteristic clinical

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v51i5.8

symptoms. Other metals such as As, Cd, Hg, and Pb are not essential and have no biological function. Even with the essential heavy metals there is a narrow gap between the concentration at which they are required and the concentration at which they are deemed toxic (Belete *et al.*, 2014; Higham & Tomkins, 1993). The toxicity of heavy metals depends on their speciation and soluble metal species are generally the most toxic (de Paiva Magalhães *et al.*, 2015)

Heavy metals such as As, Cd, Hg, and Pb are highly toxic, with infants and children being most vulnerable. Depending on the amount and duration of exposure, these metals can affect the nervous system, hormonal system, cardiovascular system, liver, kidneys, and the reproductive system (Meshref *et al.*, 2014). Chronic exposure to Pb and Hg may cause anaemia, hypertension, kidney disease, autoimmune diseases such as rheumatoid arthritis, damage to the foetal brain, tremors, anxiety, restlessness, depression, and sleep disturbances (Barakat, 2011; Järup, 2003). Lead is nephrotoxic, even at levels lower than 5 mg/dL, especially in patients with diabetes, hypertension and kidney problems (Ekong *et al.*, 2006). Exposure to inorganic metals such as As can cause cancer in various organs of the human body, whereas intake of Cd may cause nausea, vomiting and kidney damage (Järup, 2003; Ng *et al.*, 2003). Numerous studies demonstrated that low dose exposure to As, Cd, Hg, and Pb interactions in mixtures was synergistic and these metals showed higher toxicities than individual heavy metals (Cobbina *et al.*, 2015).

Therefore, it is essential to analyse the level of heavy metals in milk and to suggest possible pathways by which they migrate from soil to the human populace. To address this concern, the first intention of this study was to analyse milk produced in the territory of Kosovo and to compare its heavy metal content with that of imported milk.

Material and Methods

In 2018 and 2019, a total of 37 milk samples were collected across Kosovo. The first 32 raw milk samples were taken from rural milk collection centres and five additional sterilized imported milk samples were selected from various markets. Each sample of 1000 mL was placed in a labelled polyethylene bottle. Afterwards, each sample was kept in an ice cooled box at 2 - 8 °C, and transported to the laboratory for analysis. A portable GPS was used to record the geographical coordinates at which each milk sample was collected. Heavy metal concentrations in milk samples were measured with inductively coupled plasma mass spectrometry in high sensitivity mode (Bruker 820, Karlsruhe, Germany), which has been used successfully to determine the concentrations of multiple elements in an assay (Herrero-Latorre *et al.*, 2017). The instrument was set to measure plasma flow at 18.00 l/min, auxiliary flow at 1.80 l/min, sheath gas flow at 0.20 l/min, nebulizer flow at 1.01 l/min, sampling depth at 6.50 mm, power at 1.40 kW, pump rate at 4 rpm, and stabilization delay 10 seconds. The method validation and calibrations for measuring heavy elements in milk samples are described below (Herrero-Latorre *et al.*, 2017).

For the quantitative analysis, an external calibration technique and quality control was used for the ten heavy elements under scrutiny. First, for the background measurement, a blank sample was recorded. Then, the calibration for heavy metals such as Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb was performed at five levels from 10 to 500 ppb. For quality control of measuring system, a standard with various heavy metals concentrations was used. Calibration curves and QC for cadmium and lead are presented in Figure 1. The same procedures were applied to the other heavy metals.

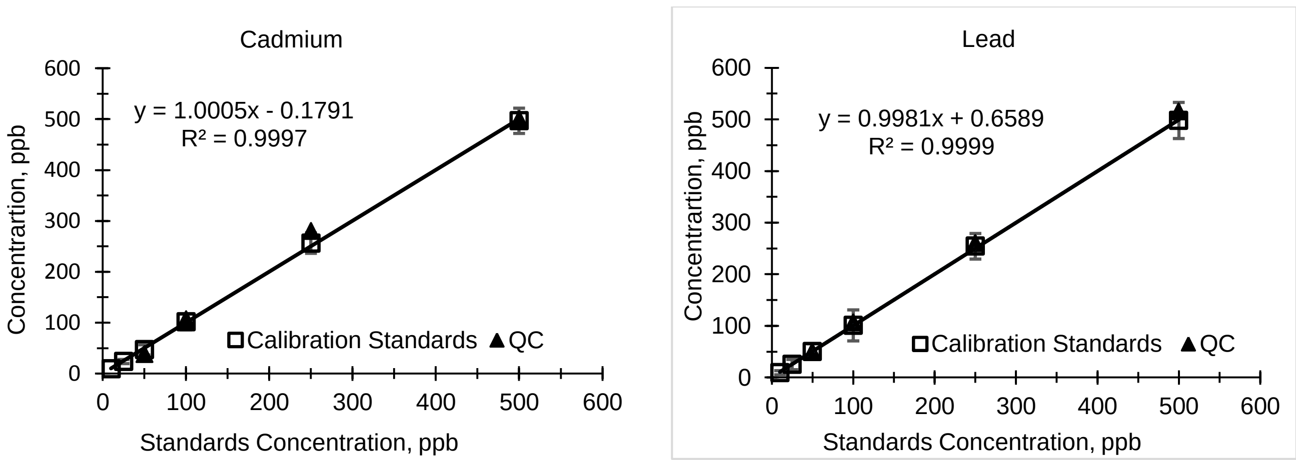

Figure 1 Calibration curves with standards and quality control for cadmium and lead

Concentrations of the heavy metals were reported as mean ± relative standard deviation. One way analysis of variance was used for comparisons of datasets of milks that originated from rural collection centres and imported milk samples, with significance level P <0.05. The statistical calculations were carried out with MS Excel (Microsoft Inc., Redmond, Washington, USA).

To find the estimated daily intake (EDI), the method of Cano-Sancho *et al*. (2010) was used. The EDI depends on the quantity of milk consumed and the concentration of heavy metals in the milk. The body mass of humans can influence their tolerance of contamination. The following formula was used to calculate EDI in µg/kg bodyweight (BW)/day):

$$EDI = \frac{M_{Intake}\ x\ C_{metal}}{\overline{BW}_{individual}}$$

where: M_{Intake} is the daily average of milk intake (L/day),
C_{metal} represents the average of specific heavy metals in milk (µg/L), and
$\overline{BW}_{individual}$ represents the average body mass of a person.

The average daily consumption of milk per adult person was regarded as 0.2 L and the average mass for an adult person as 60 kg BW (Al-Ashmawy, 2011). The daily intake of heavy metals was compared with the recommended dietary allowance (RDA) values (Institute of Medicine, 2001).

Results and Discussions

This section reports findings for 10 heavy metals in 37 milk samples, 32 taken from rural fresh milk collection centres and 5 from markets as imported milk. The heavy metals assayed in this study and descriptive statistics characterizing their distributions for samples from the domestic marketplaces are reported in Table 1. Similar characterizations of the heavy metals found in the samples of imported milk are shown in Table 2. Residues of Zn, Fe, Mn, Cu and Cr were detected in all of the milk samples analysed in this study. The average concentrations indicated Zn > Fe > Mn > Cu > Cr. The elements Ni, As, Cd, and Pb were present in 40% to 60% of the samples. Mercury was usually detected only in trace amounts and was encountered only in three samples that were obtained domestically. The ratio of metal concentrations in the samples from domestic milk collection centres to those in imported milk was from 0.01 to 1.7 (Table 2). The average concentrations of Hg, Mn, Fe, and Zn from domestic milk were higher compared with the imported milk, whereas the concentrations of As, Pb, Cd, Ni, Cr, and Cu were higher in the imported milk than in domestic milk. The differences between milk samples from these sources were significant for Cr, Fe, As, and Cd.

Table 1 Concentration of metal elements in milk samples from domestic marketplaces (n = 32)

Heavy metal	Minimum, µg/kg	Maximum, µg/kg	Mean, µg/kg	Standard deviation, µg/kg	Estimated daily intake, µg/kg bw/day
Chromium	0.06	20.34	9.93	6.51	3.31E-02
Manganese	4.76	64.86	22.54	11.30	7.52E-02
Iron	157.5	989.9	426.1	225.9	1.42E+00
Nickel	2.16	65.99	16.86	17.81	5.62E-02
Copper	0.21	44.75	18.34	12.22	6.12E-02
Zinc	1429	5718	3151	830	1.05E+01
Arsenic	0.12	1.11	0.42	0.28	1.43E-03
Cadmium	0.22	1.78	1.25	0.45	4.17E-03
Mercury	0.01	0.03	0.02	0.01	6.67E-05
Lead	0.17	4.29	1.73	1.69	5.80E-03

Table 2 Concentration of heavy metals in imported milk samples (n = 5) and the ratio of concentrations from domestic and imported samples

Heavy metal	Minimum, µg/kg	Maximum, µg/kg	Mean, µg/kg	Standard deviation, µg/kg	Estimated daily intake, µg/kg bw/day	Ratio
Chromium	15.48	19.21	17.56	1.37	5.86E-02	0.56*
Manganese	9.19	23.76	17.14	6.26	5.71E-02	1.31
Iron	171.5	314.2	244.0	59.3	8.13E-01	1.74*
Nickel	10.84	35.52	16.34	10.74	5.45E-02	1.03
Copper	10.23	42.33	27.81	14.10	9.27E-02	0.66
Zinc	1757	4019	3011	915	1.00E+01	1.05
Arsenic	0.65	2.01	1.18	0.69	3.94E-03	0.36*
Cadmium	1.87	2.28	2.08	0.29	6.92E-03	0.60*
Mercury	Tr	Tr	Tr	Tr	Tr	
Lead	1.33	3.86	1.73	1.85	5.78E-03	1.00

Tr: trace amount
*Difference between domestic and imported samples was not zero with probability P <0.05

Manganese was slightly more concentrated in the imported samples, whereas Cr, Ni and Cu were much more concentrated in the domestic milk samples. Similar results were reported for Cr, Mn and Cr concentrations in raw milk samples (Dobrzañski *et al.*, 2005; Yasotha *et al.*, 2020). Graphically, the whisker plots in Figure 2 illustrate the distributions for Ni, Cr, Mn, Cu, Fe and Zn for all samples combined. Higher average concentrations for Fe and Zn were detected in domestic milk in contrast to imported milk, with a ratio 1.74 and 1.04, respectively. High levels of Zn in dairy products had been observed (Khan *et al.*, 2014).

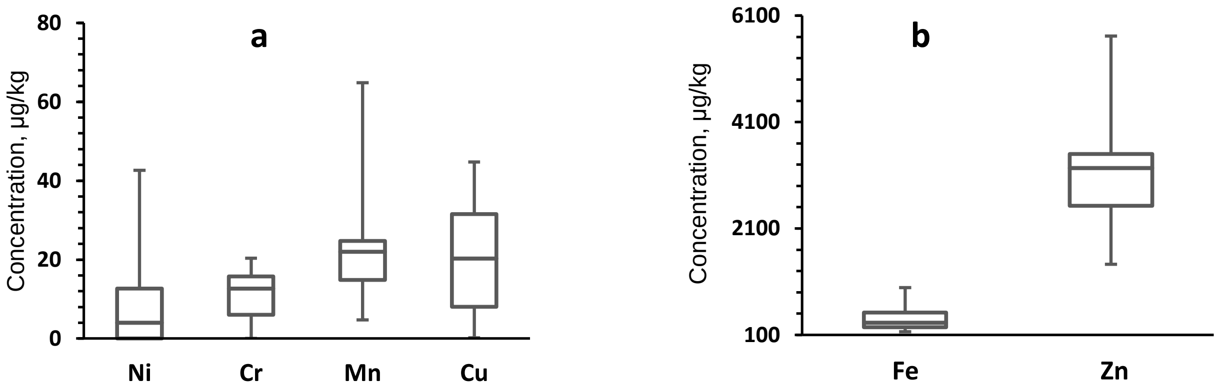

Figure 2 Concentrations of nickel, chromium, manganese, copper; iron and zinc in domestic and imported milk samples

Ni: nickel; Cr: chromium; Mn: manganese; Cu: copper; Fe: iron; ZN: zinc

Arsenic is among the most toxic elements with cumulative effects on the human body. The presence of As has been connected with certain types of cancer in humans, including lung, liver, skin and bladder cancer (Martinez *et al.*, 2011). Also, non-carcinogenic effects of chronic As exposure include neurobehavioral and neuropathic effects (Kapaj *et al.*, 2006). In the present study, As was encountered on 41% of domestic milk samples in the range between 0.12 and 1.11 µg/kg, whereas its presence in the imported milk samples was from 0.65 to 2.01 µg/kg. Nonetheless, the mean of As levels observed in the current study was lower than had been reported in milk samples from Croatia (Bilandžić *et al.*, 2011). The maximum permissible limit for As in milk established by the European Commission is 10 µg/l or approximately 10 µg/kg (Commission Regulation (EC) No. 1881, 2006).

Cadmium is another highly toxic element that affects primarily the skeleton system, kidneys and liver (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2006). The limit for Cd of 2.6 µg/l in milk has been proposed by the International Dairy Federation (Hitchins, 1996). This limit remains the only documented maximum limit of Cd level in milk. The mean level of Cd in the domestic and imported milk samples from this study was between 1.25 and 2.08 µg/kg, respectively.

Exposure to Hg, even in small amounts, is a danger to humans and animals (Ye *et al*., 2016). In the current study, Hg residues were found only in 8% of domestic milk samples, with a mean of 0.002 µg/kg, and it was not detected in any of the imported milk samples. Similar results were reported for Italian domestic milk with an average level of 0.002 µg/kg (Caggiano *et al.*, 2005).

Lead was present in 41% of domestic and 60% of imported samples. The EU regulation for Pb in unheated and heat-treated milk is 20 µg/kg (Commission Regulation (EC) No. 1881, 2006). Pollution of the environment with heavy metals such as Pb continues to be a worldwide problem (Enb *et al.*, 2009).

Conclusions

The present study provided important information about heavy metal concentrations in imported and domestic milk in Kosovo. On average, none of the heavy metal residues in the milk samples that were assayed in this study reached internationally established safety limits and EDI values were within acceptable ranges. The current study was the first presentation of heavy metal concentrations in milk sampled from the whole of Kosovo. It could be used by policy makers to manage potential health risks.

Acknowledgements

The authors received no specific funding for this work.

Author' Contributions

Conceptualization: HA, formal analysis: SD, methodology: SD and BD, software: SD and HA, validation: SD, investigation: BD, writing original draft: HA, writing, review and editing: BD, SD, and HA

Conflict of Interest Declaration

The authors declare that they have no conflicts of interest.

References

Al-Ashmawy, M.A.M., 2011. Prevalence and public health significance of aluminum residues in milk and some dairy products. J. Food Sci. 76, T73-T76. doi.org/10.1111/j.1750-3841.2011.02064.x

Aslam, B., Javed, I. & Khan, F.H., 2011. Uptake of heavy metal residues from sewerage sludge in the milk of goat and cattle during summer season. Pak. Vet. J. 31, 1, 75-77.

Balkhair, K.S. & Ashraf, M.A., 2016. Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. Saudi J. Biol. Sci. 23, S32-S44. doi.org/10.1016/j.sjbs.2015.09.023

Barakat, M.A., 2011. New trends in removing heavy metals from industrial wastewater. Arab. J. Chem. 4, 361-377. doi.org/10.1016/j.arabjc.2010.07.019

Belete, T., Hussen, A. & Rao, V.M., 2014. Determination of concentrations of selected heavy metals in cow's milk: Borena Zone, Ethiopia. J. Health Sci. 4, 105-112.

Bilandžić, N., Đokić, M., Sedak, M., Solomun, B., Varenina, I., Knežević, Z. & Benić, M., 2011. Trace element levels in raw milk from northern and southern regions of Croatia. Food Chem. 127, 63-66. doi.org/10.1016/j.foodchem.2010.12.084

Boudebbouz, A., Boudalia, S., Bousbia, A., Habila, S., Boussadia, M.I. & Gueroui, Y., 2021. Heavy metals levels in raw cow milk and health risk assessment across the globe: A systematic review. Sci. Total Environ. 751, 141830. doi.org/10.1016/j.scitotenv.2020.141830

Caggiano, R., Sabia, S., D'Emilio, M., Macchiato, M., Anastasio, A., Ragosta, M. & Paino, S., 2005. Metal levels in fodder, milk, dairy products, and tissues sampled in ovine farms of Southern Italy. Environ. Res. 99, 48-57. doi.org/10.1016/j.envres.2004.11.002

Cano-Sancho, G., Marin, S., Ramos, A.J., Peris-Vicente, J. & Sanchis, V., 2010. Occurrence of aflatoxin M1 and exposure assessment in Catalonia (Spain). Rev. Iberoam. Micol. 27, 130-135. doi.org/10.1016/j.riam.2010.05.003

Cobbina, S.J., Chen, Y., Zhou, Z., Wu, Xueshan, Zhao, T., Zhang, Z., Feng, W., Wang, W., Li, Q., Wu, X. & Yang, L., 2015. Toxicity assessment due to sub-chronic exposure to individual and mixtures of four toxic heavy metals. J. Hazard. Mater. 294, 109-120. doi.org/10.1016/j.jhazmat.2015.03.057

Commission Regulation (EC) No. 1881, 2006. Setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Inion 364, 5-24.

de Paiva Magalhães, D., da Costa Marques, M.R., Baptista, D.F. & Buss, D.F., 2015. Metal bioavailability and toxicity in freshwaters. Environ. Chem. Lett. 13, 69-87. doi.org/10.1007/s10311-015-0491-9

Dobrzañski, Z., Kolacz, R., Gorecka, H., Chojnacka, K. & Bartkowiak, A. 2005. The content of microelements and trace elements in raw milk from cows in the Silesian Region. Pol. J. Environ. Stud. 14, 5, 685-689.

Ekong, E.B., Jaar, B.G. & Weaver, V.M., 2006. Lead-related nephrotoxicity: A review of the epidemiologic evidence. Kidney Int. 70, 2074-2084. doi.org/10.1038/sj.ki.5001809

Enb, A., Donia, M.A.A., Abd-Rabou, N.S., Abou-Arab, A.A.K. & El-Senaity, M.H., 2009. Chemical composition of raw milk and heavy metals behavior during processing of milk products 8. Glob. Vet. 3, 268-275.

Fang, H., Huang, L., Wang, J., He, G. & Reible, D., 2016. Environmental assessment of heavy metal transport and transformation in Hangzhou Bay, China. J. Hazard. Mater. 302, 447–457. doi.org/10.1016/j.jhazmat.2015.09.060

Herrero-Latorre, C., Barciela-García, J., García-Martín, S. & Peña-Crecente, R.M., 2017. The use of honeybees and honey as environmental bioindicators for metals and radionuclides: A review. Environ. Rev. 25, 463-480. doi.org/10.1139/er-2017-0029

Higham, A., Tomkins, R., 1993. Determination of trace quantities of selenium and arsenic in canned tuna fish by using electroanalytical techniques. Food Chem. 48, 85-93. doi.org/10.1016/0308-8146(93)90226-6

Hitchins, A.D., 1996. The International Dairy Federation's procedure for the validation of microbiological analytical methods for dairy foods. Food Control 7, 13-18. doi.org/10.1016/0956-7135(96)00006-0

Institute of Medicine, 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academies Press, Washington, D.C. doi.org/10.17226/10026

IARC (International Agency for Research on Cancer), 2006. Inorganic and organic lead compounds. IARC Monographs on The Evaluation of Carcinogenic Risks to Humans. IARC, Lyon, France.

Järup, L., 2003. Hazards of heavy metal contamination. Br. Med. Bull. 68, 167-182. doi.org/10.1093/bmb/ldg032

Kapaj, S., Peterson, H., Liber, K. & Bhattacharya, P., 2006. Human health effects from chronic arsenic poisoning – A review. J. Environ. Sci. Health, Part A 41, 2399-2428. doi.org/10.1080/10934520600873571

Khan, N., Jeong, I.S., Hwang, I.M., Kim, J.S., Choi, S.H., Nho, E.Y., Choi, J.Y., Park, K.S. & Kim, K.S., 2014. Analysis of minor and trace elements in milk and yogurts by inductively coupled plasma-mass spectrometry (ICP-MS). Food Chem. 147, 220-224. doi.org/10.1016/j.foodchem.2013.09.147

Martinez, V.D., Vucic, E.A., Becker-Santos, D.D., Gil, L. & Lam, W.L., 2011. Arsenic exposure and the induction of human cancers. J. Toxicol. 2011, 431287. https://doi.org/10.1155/2011/431287

Meshref, A.M.S., Moselhy, W.A. & Hassan, N.E.-H.Y., 2014. Heavy metals and trace elements levels in milk and milk products. J. Food Meas. Charact. 8, 381-388. doi.org/10.1007/s11694-014-9203-6

Ng, J.C., Wang, J. & Shraim, A., 2003. A global health problem caused by arsenic from natural sources. Chemosphere 52, 1353–1359. doi.org/10.1016/S0045-6535(03)00470-3

Qin, L.-Q., Wang, X.-P., Li, W., Tong, X. & Tong, W.-J., 2009. The minerals and heavy metals in cow's milk from China and Japan. J. Health Sci. 55, 300–305. doi.org/10.1248/jhs.55.300

Rezania, S., Taib, S.M., Md Din, M.F., Dahalan, F.A. & Kamyab, H., 2016. Comprehensive review on phytotechnology: Heavy metals removal by diverse aquatic plants species from wastewater. J. Hazard. Mater. 318, 587-599. doi.org/10.1016/j.jhazmat.2016.07.053

Singh, R., Gautam, N., Mishra, A. & Gupta, R., 2011. Heavy metals and living systems: An overview. Indian J. Pharmacol. 43, 246. doi.org/10.4103/0253-7613.81505

Yasotha, A., Dabadé, D.S., Singh, V.P. & Sivakumar, T., 2020. Risk assessment of heavy metals in milk from cows reared around industrial areas in India. Environ. Geochem. Health. doi.org/10.1007/s10653-020-00758-1

Ye, B.J., Kim, B.G., Jeon, M.J., Kim, S.Y., Kim, H.C., Jang, T.W., Chae, H.J., Choi, W.J., Ha, M.N. & Hong, Y.S., 2016. Evaluation of mercury exposure level, clinical diagnosis and treatment for mercury intoxication. Ann. Occup. Environ. Med. 28, 5. https://doi.org/10.1186/s40557-015-0086-8

Yüzbaşi, N., Sezgin, E., Yildirim, Z., Yildirim, M., 2009. Changes in Pb, Cd, Fe, Cu and Zn levels during the production of Kaşar cheese. J. Food Qual. 32, 73–83. doi.org/10.1111/j.1745-4557.2007.00167.x