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Research Article

FOOD CONTROL

Concentration of Aflatoxin M_1 and Selected Heavy Metals in Mother Milk Samples from Pakistan

Sarah Khan[1], Amir Ismail*[1], Yun Yun Gong[2], Saeed Akhtar[1], Majid Hussain[1],

[1] Institute of Food Science and Nutrition, Bahauddin Zakariya University, Multan – Pakistan

[2] School of Food Science and Nutrition, University of Leeds - UK

ABSTRACT

Mother milk is the primary food source for neonates that if contaminated with certain toxic compounds may result in lifelong complications in the breastfeed infants. Present study was designed to evaluate the concentration of Aflatoxin M_1 (AFM_1) and the four most toxic heavy metals i.e. lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) in mother milk samples. AFM_1 was found in the range of <0.001 – 0.044 µg/L and 6.4% mother milk samples were found above the EU permissible limit (0.025 µg/L). Pb and Cd concentrations were found above the normal ranges proposed by WHO while Hg was found slightly above the WHO proposed level.

Keywords: Mother, Milk, Aflatoxin, Heavy metal, ELISA.

1. Introduction

Mother milk is considered the most important if not only source of nutrients for the neonates (Jimoh & Kolapo, 2008; Pronczuk, Moy, & Vallenas, 2004). It encompasses all the essential minerals, vitamins and high value proteins along with polyvalent characteristics that play a vital role in upkeep of a child (Mansour, Hassan, El-Aal, & Ebrahem, 2015). Moreover, mother milk also contain antibodies and numerous protective factors that help to combat diseases. Infants remain protected from many diseases if they are breastfed in early stages of their life. Mother milk consumed by a normal infant in first month of life is more than 400–500 mL and at the age of 2-6 month this amount rises to more than 760 mL per day (El-Tras, El-Kady, & Tayel, 2011).

***Corresponding Authors**
Dr. Amir Ismail
Mobile: +923136364863
E-mail address: amirismail@bzu.edu.pk

Despite of the huge benefits of mother milk, small amount of contaminants are also reported in mother milk that are related to the diet and environmental exposure of the mother (Jensen, 1983). The most frequently reported toxic xenobiotics in mother milk samples are aflatoxin M_1 (AFM_1) (Cantú-Cornelio et al., 2016; Diaz & Sánchez, 2015; Iha, Barbosa, Heck, & Trucksess, 2014) and heavy metals (Abdollahi, Tadayon, & Amirkavei, 2013; Mansour et al., 2015; Ullah, Rehman, Iqbal, Rehman, & Ahmad, 2015). However, it is pertinent to mention that breast-feeding is encouraged despite the presence of impurities in mother milk, the efforts are being made to ensure the supply of safe food to the mothers so that the neonates receive healthy mother milk.

Aflatoxins (AFs) belong to mycotoxins and are reported in food and feed items in 18 different forms. The most toxic AF is Aflatoxin B_1 (AFB_1) that is declared as group 1 category carcinogen by the International Agency for Research on Cancer (IARC). AFB_1 converts into AFM_1 inside the liver of animals through cytochrome P450 enzymes. AFM_1 is although a detoxified product of AFB_1 that is ten times less carcinogenic as compared to its parent compound but still categorized as group 2B category carcinogen (probable human carcinogen) by the IARC. Other than carcinogenicity, AFM_1 is also reported as immune suppressant, growth retardant and teratogenic. AFM_1 is not only reported in milk and milk products of animal origin but also reported in the milk of humans. European Union has set the maximum permissible level for AFM_1 in animal milk as 0.05 µg/L but for human milk it is fifty times more strict i.e. 0.025 µg/L (Ismail et al., 2016a; Ismail et al., 2017).

Heavy metals are the elements that have specific gravity higher than 5 g/cm^3 and bears the potential to trigger severe toxicity in humans even if ingested in very low doses. The most toxic heavy metals reported until now are Hg, Pb, As and Cd. Health complications mostly associated with metal toxicity are under developed nervous system, cancer of diverse types, abnormal or retarded growth, kidney failure, diarrhea and vomiting. Contamination of toxic metals in food items of human consumption are frequently reported mostly from the underdeveloped countries. Consumption of metal contaminated food items by the lactating mothers result in the residues of metals in mother milk that ultimately impacts the health of breastfeeding infants. A number of countries have reported the presence of toxic heavy metals beyond their permissible ranges in mother milk samples including Iran (Abdollahi et al., 2013), Ghana (Bentum et al., 2010), Saudia Arabia (Al-Saleh et al., 2015), Turkey (Turan, Saygi, Kiliç, & Acar, 2001) and Nigeria

(Adesiyan, Akiibinu, Olisekodiaka, Onuegbu, & Adeyeye, 2011).

To the best of our knowledge the concentration of AFM_1 in mother milk samples from Pakistan is unexplored until now. Therefore, this study was designed to assess the levels of two major toxic compound groups in mother milk samples from Pakistan i.e. AFM_1 and selected heavy metals. Current study will certainly support the health agencies of Pakistan to better understand the food safety situation and to make certain regulations against the major toxic compounds prevailing in food items that currently is missing.

2.0. Materials and methods

2.1. Study Population

Mother milk samples were collected from lactating mothers aging between 24 - 35 years. Healthy mothers were included in the study, mothers suffering from any illnesses or infections were excluded from the study. The sampling was done by the self-extraction method. A questionnaire was designed to collect the information on the subjects of the educational status, somatic measures, demographic characteristics and socioeconomic status of lactating mothers that were included in the study. To explore the effect of economic background on the concentration of toxic compounds in mother milk samples the mothers were divided into three groups based on their economic status i.e. rich (above $ 1000 per month), middle class ($ 250 - $ 1000 per month) and poor (below $ 250 per month).

2.2. Sample Collection

A total of 125 lactating mother (aged between 25 – 30 years) were selected from five districts of Punjab province of Pakistan. Their milk samples were collected in sterile plastic bottles. The samples were brought in ice boxes and stored at -80 °C until analyzed. Thawing of the samples was done at ambient temperature just prior to analysis.

2.3.0. Laboratory Testing

ELIZA kits and atomic absorption spectrophotometer were used for analyzing the concentrations of AFM_1 and heavy metals respectively, in mother milk samples.

2.3.1.1. Analysis of AFM_1 in Mother Milk Samples through ELISA Kits

Prior to analysis of AFM_1 in mother milk samples, the samples were thawed and centrifuged for 10 min at 3000 rpm (Heidolph, Germany) and the upper layer of cream was removed. The quantification of AFM_1 was performed using an ELISA kits according to the guidelines of manufacturer (Helica California, USA). Briefly, standard solutions, blanks and samples were added in their respective wells (2 µL of each) and incubated for 60 min at ambient temperature in dark. The unattached solution was discarded and the plates were washed thrice with rinsing buffer solution. Now, enzyme conjugate (100 µL) was added and again incubated for 60 minutes at 37 °C. After that washing of the wells was done to remove unbounded enzymes. The enzyme substrate and chromogen at a concentration level of 50µL each, were added in the wells and incubation was done in dark for the duration of 30 minutes at a temperature of 37 °C. The colorless chromogen converted into a blue colored product due to the action of bounded enzyme. In the next step, stop solution (100 µL per well) was added that changed the color of wells from blue to yellow. The change in color was measured on ELISA reader (Bio-Tek ELx800, Indonesia) at 450 nm.

2.3.1.2. Verification of ELISA Method Validity

AFM_1 standard solution was procured from Sigma chemicals (A6428) for verification objective. In AFM_1 free milk samples the standard solution at the level of 0.01, 0.02, 0.05 and 0.1 mg/L were added. The AFM_1 reclamation percentage was found in the range of 96.1- 98.7 % and the variation coefficient ranged between 2.1-4.3 %. The limit of detection (LOD) for AFM_1 provided by the kit manufacturing company was 0.001 µg/L. The limit of quantification (LOQ) was calculated according to the method proposed by Ismail et al. (2014). The LOQ of ELISA method was 0.003 µg/L.

2.3.2. Heavy Metals Determination

2.3.2.1. Preparation of Mother Milk Samples for Atomic Absorption Spectrophotometer

Heavy metals concentrations in mother milk samples were quantified through flame atomic absorption spectrophotometer. Digestion of samples was performed by employing wet digestion procedure as mentioned by Richards (1968). Milk sample (2 mL) was dissolved in nitric acid (10 mL) and then heated for 30 min at 100 °C. After that 5 mL per chloric acid was added and again heated and evaporated upto half of the added volume with the aid of hot plate. The digested sample was put into volumetric flask, and diluted up to 25 mL with distilled water. All samples were digested in triplicates.

2.3.2.2. Quantification of Heavy metals in mother milk samples

Mother milk samples were analyzed for Pb, Cd, Hg and As through flame atomic absorption spectrophotometer (Thermoscientific, 3000 series) by using high energy flame obtained through acetylene/nitrous and air. The analysis of blanks was done and the limits of detection for various elements were obtained as 3 time the standard deviation (SD values) of 22 technical blanks (3.3 SD/b). LOD values for Pb, Hg, Cd, and As were 0.53, 0.20, 0.62, 0.08 µg/Kg, respectively. Recovery percentages of the selected heavy metals were computed through spiking the milk samples with known concentrations of standard solutions. The recovery percentages of metals under study were found in the range of 93.8 – 97.9 %. The analysis were conducted in triplicate and the experiments were performed again if the repeatability percentage exceeded 1 %.

2.4. Ethical Consideration

The study was approved from the Bioethical society of Bahauddin Zakariya University, Multan – Pakistan. All the mothers were briefed about the study and the milk samples were taken after their consent.

2.5. Data Analysis

Statistical differences ($P < 0.05$) in AFM_1 and heavy metals concentrations of mother milk samples collected from various economic classes were computed through Statistics 8.1 software (Statistix Inc., Florida, USA). For the calculation of mean values and standard deviation (SD), Microsoft Excel (2016) version was used.

3.0. Results & Discussion

3.1. AFM_1 Occurrence in Mother Milk

Prevalence of AFM_1 in mother milk samples collected from different districts of Southern Punjab is summarized in table 1. Statistical analysis revealed significant differences in the concentration of AFM_1 among mother milk samples collected from different districts and different economic classes. The percentage of positive samples was 75 % (n=94) while overall range of AFM_1 in mother milk samples was < 0.001 – 0.044 µg/L. Comparing our results with EU permissible limit (0.025 µg/L) 6.4 % samples (n=8) were found to exceed the maximum limit. Mean maximum AFM_1 prevalence was recorded from Layyah district (0.030±0.008 µg/L) while mean minimum level was recorded from Multan district (0.017±0.002 µg/L). On economic grounds the prevalence of AFM_1 in mother milk samples was recorded in the order of Poor (0.028±0.005 µg/L) > Middle Class (0.021±0.004 µg/L) > Rich (0.018±0.004 µg/L). The educational status of mothers was found to vary from graduate (7 %) to illiterate level (48 %), while most of the mothers were house wife (59 %) and their body weights ranged between 40 – 78 Kg. A non-significant relationship ($P < 0.05$) was recorded between the level of AFM_1 in the milk of mothers and their educational, somatic as well as working status.

Prevalence of AFM_1 in mother milk samples is the indicator of aflatoxins contamination in the diet of mothers. A number of countries have reported the prevalence of aflatoxins in different food commodities and ultimately in the milk of mothers as well. Maleki, Abdi, Davodian, Haghani, & Bakhtiyari (2015) analyzed 85 mother milk samples from Iran and found 100 % samples positive for AFM_1 ranging between 0.002 – 0.010 µg/L while the mean AFM_1 level in mother milk samples was 0.005 µg/L. In Malaysia, none of the samples were reported positive for AFM_1 from a total of 45 mother milk samples Shuib, Makahleh, Salhimi, & Saad (2017). In Nigeria, 50 mother milk samples were analyzed for AFM_1 out of which 82 % samples were found positive for AFM_1 ranging between 0.003 – 0.035 µg/L, while 16 % samples were found to exceed the EU maximum limit (Adejumo et al., 2013). In Turkey, form a total of 73 mother milk samples analyzed for AFM_1, 18 samples were found positive for AFM_1 but none of the samples was found to exceed the EU maximum limit (Atasever, Yildirim, Atasever, & Tastekin, 2014). Polychronaki et al. (2007) reported 56 % samples positive for AFM_1 from a total of 443 samples ranging between 0.004 – 0.889 µg/L. Comparing these reports with our findings, it can be stated that the level of AFM_1 in mother milk samples from Pakistan is higher as compared to Malaysia and Turkey and almost in line with the findings from Nigeria, Iran and Egypt. The possible reasons behind the high incidences of AFM_1 contamination in mother milk samples from Pakistan include low literacy rate of mothers, suitable weather for the production of aflatoxins, more consumption of foods vulnerable to aflatoxins contamination by the Pakistani mothers and less implementation of rules and regulations to limit the production of aflatoxins (Ismail et al., 2016b). A number of studies from Pakistan have reported the high prevalence of aflatoxins in food commodities beyond the permissible limits such as in milk (Iqbal & Asi, 2013), in cereals (Majeed, Iqbal, Asi, & Iqbal, 2013), in spices (Iqbal, Asi, Zuber, Akhtar, & Saif, 2013) and in dry fruits (Masood, Iqbal, Asi, & Malik, 2015). The prevalence of aflatoxins in Pakistani food items beyond the permissible limits ultimately indicates the chances of AFM_1 contamination in mother milk samples and that is proved for the first time in this study.

Prevalence of AFM_1 in poor mother's milk samples indicates the consumption of moldy or poor quality foods that ultimately may lead to adverse health impacts not only on mother's health like hepatic failure, reproductive disorders and weak immunity system but also on neonates health such as impaired growth and weak immune system. The consumption of aflatoxins contaminated food more by the poorer communities is reported by a number of researchers from around the globe. In Kenya more than 200 poor people died due to the consumption of aflatoxins contaminated food (Yard et al., 2013). Highest aflatoxins contamination rate was recorded in Layyah district that might be linked with the fact that maximum number of mothers from Layyah district had rural background and depend of subsistence farming. A relation between aflatoxins intake and subsistence farming is reported by Wild & Gong (2010).

3.2. Heavy Metals in Mother Milk

Concentration of heavy metals in mother milk samples of different economic classes, collected from different districts of Punjab province are summarized in Table 3. The concentrations of different heavy metals in mother milk samples were found in the order of Pb > Cd > Hg > As. Concentrations of heavy metals on economic grounds were in the order of Rich > Middle class > Poor. Concentration of heavy metals on district basis were in the order of Muzaffargarh > Multan > DG Khan > Layyah. Pb was found as the most prevalent heavy metal in mother milk samples ranging between 0.009 – 0.440 mg/L while the mean Pb level was 0.095 mg/L. Significant differences were recorded for cadmium concentration in mother milk samples from different districts of Punjab collected from mothers belonging to different economical classes ($P < 0.05$). Cadmium concentration in milk mother milk samples were found in the range of 0.0002 – 0.301 mg /L, while the mean contamination level was 0.052 mg/L. Hg concentration in mother milk samples was recorded in the range of 0.203 – 3.981 µg/L, while the mean level was 0.614 µg/L. Concentration of As in mother milk samples was found in the range of 0.092 – 1.240 µg/L, while the mean As level was 0.504 µg/L. Furthermore, a non-significant relationship ($P < 0.05$) was recorded between the tested heavy metals and AFM_1 indicating the different natures of these two classes of contaminants.

The reported levels of Pb in mother milk samples from Nigeria (Adesiyan et al., 2011), Egypt (Saleh, Ragab, Kamel, Jones, & El-Sebae, 1996), Mexico (Namihira, Saldivar, Pustilnik, Carreón, & Salinas, 1993) and Turkey (Gürbay et al., 2012) were 0.009, 0.101, 0.460, 0.361 mg/L, respectively. The findings of our study are in line with the reported level of Pb in mother milk samples from different countries. The acceptable range of Pb in different food commodities proposed by WHO is 2 - 5 µg/L (World Health Organization, 1989), comparing this limit with our results 100 % samples were found to exceed the acceptable limit. Prevalence of Pb above the permissible limits in animal milk and vegetable samples from Pakistan are also reported by us in earlier studies (Ismail et al., 2014, 2015).

The maximum permissible level for Cd proposed by WHO is 1 µg/L (World Health Organization, 1989), comparing this level with our results 87 % samples were found to exceed the limit. The mean level of Cd in mother milk samples from Spain (García-Esquinas et al., 2011) and Cyprus (Kunter et al., 2017) are 0.45 and 1.31 µg/L, respectively and these values are much lower than our findings. Mansour, Hassan, El-Aal, & Ebrahem (2015) reported mean Cd level in mother milk samples from Egypt as 0.025 mg/L (0.001 - 0.061 mg/L) that is almost in line to our findings. (Goudarzi, Parsaei, Nayebpour, & Rahimi (2013) reported Cd level in mother milk samples from Iran (Isfahan) in the range of 0.45–5.87 mg/L, indicating a higher level of Cd in mother milk samples from Iran as compared to Pakistan. Prevalence of Cd above permissible limit in mother milk samples from Pakistan is a serious concern for the health of neonates and mothers, as it may damage the kidney and bones of the newborn and also disturbs Ca and Zn absorption in the body.

The permissible range for Hg in mother milk samples proposed by WHO is 1.4 -1.7 µg/L (World Health Organization, 1989), comparing this limit with our findings 7% samples were found to exceed the maximum permissible level. Mean reported level of Hg from Spain by García-Esquinas et al. (2011) was 0.53 µg/L that is almost in line with our findings. Mean reported level of Hg in mother milk samples from Saudi Arabia was 0.884 µg/L and 43.2% samples were reported to have Hg above 1 µg/L (Al-Saleh et al., 2015), the reported level of Hg in Saudi mothers is slightly higher than our findings.

Maximum allowable daily intake of As proposed by WHO is 15 µg/kg/week. The concentration of As in mother milk samples from this study seems in safe limits. Concentration of As from Cyprus (Kunter et al., 2017) and Taiwan (Chao et al., 2014) were 0.72 and 0.16 – 1.6 µg/L and these values are almost in line with our study. Mean As concentration in mother milk samples from Ghana reported by Bentum et al. (2010) is 1.54 µg/L that is slightly higher than our studies. A review of As concentration in mother milk samples from different countries indicates that the As level found in this study seems in the safe ranges. Although a higher prevalence rate of As is reported in food and water of Pakistan by a number of researchers (Rasheed, Kay, Slack, Gong, & Carter, 2017; Rasheed, Slack, Kay, & Gong, 2017) but its low level in mother milk samples might be due to the toxin filtering role of mother's body.

4.0. Conclusion

This study is the first report on the prevalence of AFM_1 and heavy metals in mother milk samples from Pakistan. AFM_1 was fund positive in 75 % samples while 6.4 % samples were found to have contamination level above the EU maximum permissible level of AFM_1 in mother milk i.e. 0.025 µg/L. Poor mothers and ultimately the poor breastfeed infants appeared to be on the highest risk side. Among the four tested heavy metals i.e. Pb, Cd, Hg and As, the concentrations of Pb and Cd were found above the safe levels and thus require strict monitoring and preventive measures to protect the health of mothers and infants. Prevalence of toxic metals and aflatoxins in the mother milk samples is the indicator that food supplied to the mothers is not safe for human consumption. Strict actions are needed to ensure the supply of safe food to the consumers especially the lactating mothers. Government agencies working in food and agriculture sector must create awareness and should adopt strict regulatory measures to control the exposure of these contaminants. It is pertinent to mention that despite the prevalence of some toxic substances still mother milk is the best source of nutrition for the infants due to its matchless nutritional significance.

Conflict of Interest

The authors declare no conflict of interest.

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References

Abdollahi, A., Tadayon, F., & Amirkavei, M. (2013). Evaluation and determination of heavy metals (mercury, lead and cadmium) in human breast milk. In E3S Web of Conferences (Vol. 1, pp. 3–5). https://doi.org/10.1051/e3sconf/20130141037

Adejumo, O., Atanda, O., Raiola, A., Somorin, Y., Bandyopadhyay, R., & Ritieni, A. (2013). Correlation between aflatoxin M1 content of breast milk, dietary exposure to aflatoxin B1 and socioeconomic status of lactating mothers in Ogun State, Nigeria. Food and Chemical Toxicology, 56, 171–177. https://doi.org/10.1016/j.fct.2013.02.027

Adesiyan, A. A., Akiibinu, M. O., Olisekodiaka, M. J., Onuegbu, A. J., & Adeyeye, A. D. (2011). Concentrations of some biochemical parameters in breast milk of a population of Nigerian nursing mothers using hormonal contraceptives. Pakistan Journal of Nutrition, 10(3), 249–253.

Al-Saleh, I., Abduljabbar, M., Al-Rouqi, R., Eltabache, C., Al-Rajudi, T., Elkhatib, R., & Nester, M. (2015). The extent of mercury (Hg) exposure among Saudi mothers and their respective infants. Environmental Monitoring and Assessment, 187(11). https://doi.org/10.1007/s10661-015-4858-y

Atasever, M., Yildirim, Y., Atasever, M., & Tastekin, A. (2014). Assessment of aflatoxin M1 in maternal breast milk in Eastern Turkey. Food and Chemical Toxicology, 66, 147–149. https://doi.org/10.1016/j.fct.2014.01.037

Bentum, J. K., Sackitey, O. J., Tuffuor, J. K., Essumang, D. K., Koranteng-Addo, E. J., & Owusu-Ansah, E. (2010). Lead, cadmium and arsenic in breast milk of lactating mothers in Odumanse-Atua community in Manya Krobo district of eastern region of Ghana. Journal of Chemical and Pharmaceutical Research, 2(5), 16–20.

Cantú-Cornelio, F., Aguilar-Toalá, J. E., de León-Rodríguez, C. I., Esparza-Romero, J., Vallejo-Cordoba, B., González-Córdova, A. F., … Hernández-Mendoza, A. (2016). Occurrence and factors associated with the presence of aflatoxin M1 in breast milk samples of nursing mothers in central Mexico. Food Control, 62, 16–22. https://doi.org/10.1016/j.foodcont.2015.10.004

Chao, H.-H., Guo, C.-H., Huang, C.-B., Chen, P.-C., Li, H.-C., Hsiung, D.-Y., & Chou, Y.-K. (2014). Arsenic, cadmium, lead, and aluminium concentrations in human milk at early stages of lactation. Pediatrics and Neonatology, 55(2), 127–34. https://doi.org/10.1016/j.pedneo.2013.08.005

Diaz, G. J., & Sánchez, M. P. (2015). Determination of aflatoxin M 1 in breast milk as a biomarker of maternal and infant exposure in Colombia. Food Additives & Contaminants: Part A, 32, 1192–1198. https://doi.org/10.1080/19440049.2015.1049563

El-Tras, W. F., El-Kady, N. N., & Tayel, A. A. (2011). Infants exposure to aflatoxin M 1 as a novel foodborne zoonosis. Food and Chemical Toxicology, 49(11), 2816–2819. https://doi.org/10.1016/j.fct.2011.08.008

García-Esquinas, E., Pérez-Gómez, B., Fernández, M. A., Pérez-Meixeira, A. M., Gil, E., Paz, C. de, … Aragonés, N. (2011). Mercury, lead and cadmium in human milk in relation to diet, lifestyle habits and sociodemographic variables in Madrid (Spain). Chemosphere, 85(2), 268–276. https://doi.org/10.1016/j.chemosphere.2011.05.029

Goudarzi, M. a., Parsaei, P., Nayebpour, F., & Rahimi, E. (2013). Determination of mercury, cadmium and lead in human milk in Iran. Toxicology and Industrial Health, 29(9), 820–823. https://doi.org/10.1177/0748233712445047

Gürbay, A., Charehsaz, M., Eken, A., Sayal, A., Girgin, G., Yurdakök, M., … Aydın, A. (2012). Toxic metals in breast milk samples from Ankara, Turkey: assessment of lead, cadmium, nickel, and arsenic levels. Biological Trace Element Research, 149(1), 117–122. https://doi.org/10.1007/s12011-012-9400-2

Iha, M. H., Barbosa, C. B., Heck, A. R., & Trucksess, M. W. (2014). Aflatoxin M1 and ochratoxin A in human milk in Ribeir??o Preto-SP, Brazil. Food Control, 40(1), 310–313. https://doi.org/10.1016/j.foodcont.2013.12.014

Iqbal, S. Z., & Asi, M. R. (2013). Assessment of aflatoxin M1 in milk and milk products from Punjab, Pakistan. Food Control, 30(1), 235–239. https://doi.org/10.1016/j.foodcont.2012.06.026

Iqbal, S. Z., Asi, M. R., Zuber, M., Akhtar, J., & Saif, M. J. (2013). Natural occurrence of aflatoxins and ochratoxin A in commercial chilli and chilli sauce samples. Food Control, 30(2), 621–625. https://doi.org/10.1016/J.FOODCONT.2012.09.003

Ismail, A., Akhtar, S., Levin, R. E., Ismail, T., Riaz, M., & Amir, M. (2016a). Aflatoxin M1: Prevalence and decontamination strategies in milk and milk products. Critical Reviews in Microbiology, 42(3), 418–427. https://doi.org/10.3109/1040841X.2014.958051

Ismail, A., Levin, R. E., Riaz, M., Akhtar, S., Yun, Y., & Oliveira, C. A. F. De. (2017). Effect of different microbial concentrations on binding of a fl atoxin M 1 and stability testing. Food Control, 73, 492--496. https://doi.org/10.1016/j.foodcont.2016.08.040

Ismail, A., Riaz, M., Akhtar, S., Ismail, T., Ahmad, Z., & Hashmi, M. S. (2015). Estimated daily intake and health risk of heavy metals by consumption of milk. Food Additives & Contaminants. Part B, Surveillance, 8(4), 260–5. https://doi.org/10.1080/19393210.2015.1081989

Ismail, A., Riaz, M., Akhtar, S., Ismail, T., Amir, M., & Zafar-ul-Hye, M. (2014). Heavy metals in vegetables and respective soils irrigated by canal, municipal waste and tube well waters. Food Additives & Contaminants: Part B, 7(3), 213–219. https://doi.org/10.1080/19393210.2014.888783

Ismail, A., Riaz, M., Levin, R. E., Akhtar, S., Gong, Y. Y., & Hameed, A. (2016b). Seasonal prevalence level of aflatoxin M1 and its estimated daily intake in Pakistan. Food Control, 60, 461–465. https://doi.org/10.1016/j.foodcont.2015.08.025

Jensen, A. A. (1983). Chemical contaminants in human milk. In Residue Reviews (pp. 1–128). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4612-5601-4_1

Jimoh, K., & Kolapo, A. (2008). Mycoflora and aflatoxin production in market samples of some selected Nigerian foodstuffs. Research Journal of Microbiology, 3(3), 169–174.

Kunter, İ., Hürer, N., Gülcan, H. O., Öztürk, B., Doğan, İ., & Şahin, G. (2017). Assessment of aflatoxin M1 and heavy metal levels in mothers breast milk in Famagusta, Cyprus. Biological Trace Element Research, 175, 42–49. https://doi.org/10.1007/s12011-016-0750-z

Majeed, S., Iqbal, M., Asi, M. R., & Iqbal, S. Z. (2013). Aflatoxins and ochratoxin A contamination in rice, corn and corn products from Punjab, Pakistan. Journal of Cereal Science, 58(3), 446–450. https://doi.org/10.1016/j.jcs.2013.09.007

Maleki, F., Abdi, S., Davodian, E., Haghani, K., & Bakhtiyari, S. (2015). Exposure of infants to aflatoxin M1 from mother's breast milk in Ilam, Western Iran. Osong Public Health and Research Perspectives, 6(5), 283–287. https://doi.org/10.1016/j.phrp.2015.10.001

Mansour, N. A., Hassan, N. M., El-Aal, S. A. A., & Ebrahem, M. H. (2015). Quantitative analysis of lead, cadmium, heavy metals and other toxic elements in some human breast milk samples. Asian Journal of Chemistry, 27(12), 4443–4448. https://doi.org/10.14233/ajchem.2015.19163

Masood, M., Iqbal, S. Z., Asi, M. R., & Malik, N. (2015). Natural occurrence of aflatoxins in dry fruits and edible nuts. Food Control, 55, 62–65. https://doi.org/10.1016/J.FOODCONT.2015.02.041

Namihira, D., Saldivar, L., Pustilnik, N., Carreón, G. J., & Salinas, M. E. (1993). Lead in human blood and milk from nursing women living near a smelter in Mexico City. Journal of Toxicology and Environmental Health, 38(3), 225–232. https://doi.org/10.1080/15287399309531714

Polychronaki, N., West, R. M., Turner, P. C., Amra, H., Abdel-Wahhab, M., Mykkänen, H., & El-Nezami, H. (2007). A longitudinal assessment of aflatoxin M1 excretion in breast milk of selected Egyptian mothers. Food and Chemical Toxicology, 45(7), 1210–1215. https://doi.org/10.1016/j.fct.2007.01.001

Pronczuk, J., Moy, G., & Vallenas, C. (2004). Breast milk: an optimal food. Environmental Health Perspectives, 112(13), 722–723. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15345351

Rasheed, H., Kay, P., Slack, R., Gong, Y. Y., & Carter, A. (2017). Human exposure assessment of different arsenic species in household water sources in a high risk arsenic area. Science of The Total Environment, 584–585, 631–641. https://doi.org/10.1016/J.SCITOTENV.2017.01.089

Rasheed, H., Slack, R., Kay, P., & Gong, Y. Y. (2017). Refinement of arsenic attributable health risks in rural Pakistan using population specific dietary intake values. Environment International, 99, 331–342. https://doi.org/10.1016/J.ENVINT.2016.12.018

Richards, L. A. (1968). Agriculture Handbook No. 60: Diagnosis and improvement of saline and alkaline soils.

Saleh, M. A., Ragab, A. A., Kamel, A., Jones, J., & El-Sebae, A. K. (1996). Regional distribution of lead in human milk from Egypt. Chemosphere, 32(9), 1859–1867. https://doi.org/10.1016/0045-6535(96)00079-3

Shuib, N. S., Makahleh, A., Salhimi, S. M., & Saad, B. (2017). Natural occurrence of aflatoxin M1 in fresh cow milk and human milk in Penang, Malaysia. Food Control, 73, 966–970. https://doi.org/http://dx.doi.org/10.1016/j.foodcont.2016.10.013

Turan, S., Saygi, S., Kiliç, Z., & Acar, O. (2001). Determination of heavy metal contents in human colostrum samples by electrothermal atomic absorption spectrophotometry. Journal of Tropical Pediatrics, 47(2), 81–85. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11336140

Ullah, N., Rehman, H. U., Iqbal, A., Rehman, A., & Ahmad, I. (2015). Estimation of Toxic Metals in Milk Collected from Lactating Mothers in Karak , Khyber Pakhtunkhwa Pakistan. International Journal of Basic Medical Sciences and Pharmacy (IJBMSP), 5(1), 24–27.

Wild, C. P., & Gong, Y. Y. (2010). Mycotoxins and human disease: A largely ignored global health issue. Carcinogenesis, 31(1), 71–82. https://doi.org/10.1093/carcin/bgp264

World Health Organization. (1989). Minor and trace elements in breast milk. Report of a joint WHO/IAEA collaborative study. Retrieved from http://www.who.int/iris/handle/10665/39678

Yard, E. E., Daniel, J. H., Lewis, L. S., Rybak, M. E., Paliakov, E. M., Kim, A. A., … Sharif, S.

K. (2013). Human aflatoxin exposure in Kenya, 2007: a cross-sectional study. Food Additives & Contaminants: Part A, 30(7), 1322–1331. https://doi.org/10.1080/19440049.2013.789558