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Potential Human Health Risks Assessment through Determination of Heavy Metal Contents in Regularly Consumed Yogurts in Bangladesh

GMM Anwarul Hasan[1]*, Mohammed A. Satter[1] and Anuj Kumer Das[2]

[1]Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dr. Qudrat-E-Khuda Road, Dhaka 1205, Bangladesh.
[2]Hi-Tech Health care Ltd. Dhaka-1213, Bangladesh.

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This study estimated the concentrations of heavy metals in regularly consumed sour, sweet and homemade traditional yogurts in Bangladesh. A total of 300 yogurt samples (sour, sweet and homemade traditional); 100 yogurt samples of each category were considered for this assay. To assess the health risks, the daily yogurt consumption rate and the value of target carcinogenic and non-carcinogenic risks of these yogurt samples were also determined. In sour yogurt samples, detected heavy metal contents were 0.398±0.121 mg/kg, 0.774±0.372 mg/kg, 0.087±0.021 mg/kg, 0.131±0.026 mg/kg, 0.011±0.006 mg/kg, 0.031±0.005 mg/kg, 0.445±0.267 mg/kg and 0.029±0.024 mg/kg while in sweet yogurt samples, detected heavy metal contents were 0.410±0.146 mg/kg, 0.704±0.324 mg/kg, 0.082±0.028 mg/kg, 0.146±0.025 mg/kg, 0.010±0.005 mg/kg, 0.030±0.007 mg/kg, 0.460±0.265 mg/kg, and 0.035±0.026 mg/kg and in homemade traditional yogurt samples, detected heavy metal contents were 0.410±0.147 mg/kg, 1.502±0.367 mg/kg, 0.085±0.020 mg/kg, 0.120±0.024 mg/kg, 0.011±0.005 mg/kg, 0.020±0.005 mg/kg, 0.251±0.174 mg/kg and 0.025±0.021 mg/Kg for Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn), Lead (Pb), Cadmium (Cd), Chromium (Cr) and Arsenic (As) respectively. Among the analyzed heavy metals, highest concentration of Cu and lowest concentration of Pb was detected in all yogurt types. The values of estimated daily intake (EDI), Target hazard quotient (THQ), Total THQ (TTHQ) and Target Carcinogenic risk (TCR) confirmed that, there have no chances of any kind of carcinogenic and non-carcinogenic risks from these yogurt samples.

Keywords: Bangladesh; Carcinogenic; Heavy metals; Health risks; Non-Carcinogenic; Yogurt.

Milk products are very essential foods for human health because their mineral and protein part contributes in promoting growth. Now-a-days, these products may contain toxic elements including heavy metals because of environmental pollution. Environmental pollution is increasing day by day due to high industrial, urban, and agricultural emissions[1, 2].

Yogurt is a popular dairy food product in many parts of the world. Among various dairy products, yogurt is very popular because of their nutritional properties and potentially beneficial effects on human health[3]. The consumption of yogurt has been increased among the peoples specially women, children and diet conscious consumers because of its nutritional, therapeutic

*Corresponding author E-mail: pd-cbirmdp@bcsir.gov.bd

and health benefits[3,4,5]. In yogurt samples, several heavy metals such as Fe, Mn, Zn, Cu are very beneficial for human health while Cr, Cd, Pb and As are very harmful for human health and play negative effects on human health. There are various suspicious effects of yogurt in human health including cholesterol metabolism, immune modulation, diarrhea, Helicobacter pylori eradication, antimicrobial, anti-mutagenic, anti-cancer, and antioxidant activity 5. Despite of several beneficial effects, they would be exposed toxic health effects for consumers because, milk and dairy products might contain chemical hazards and contaminants[4]. Generally, milk and dairy products contain very less amount of heavy metals. Quality of any food may be measured in accordance to the presence of heavy metals and the quality of milk products may be affected due to poor maintenance against environmental pollution, processing conditions, sanitation, and husbandry[2]. There are some potential risk factors in milk including arsenic, lead, cadmium, chromium, copper, zinc[6]. Dairy products can be contaminated via equipment or tools used in the production line of dairy products or through packaging processes[7,8,9]. So, heavy metals present in food items can cause a serious threat to human health[10].

The presence of trace elements in any kind food is an important point of thinking because they have both suspicious and harmful consequences [1, 11, 12]. In milk products, about twenty micro nutrients are found[13]. There are various essential nutrients and minerals which are important for human health are found in milk and milk products. Heavy metals are known as co-factors in enzymatic reactions and involved in biochemical functions but in compliance with the sanitary guidelines, their excess exposure can be toxic to human health[14, 15]. Heavy metal like Zinc has a vital role in cancer biology and also in certain physical actions, but this element is recognized as a functional and structural factor of human health[4, 16]. Lead and cadmium are considered as potential carcinogens[17] which can be very dangerous especially for children and babies as they are the main consumers of milk and milk products. Heavy metal mediated toxicity can cause several problems in developmental stages for babies[18,19]. Another heavy metal, Arsenic, exposure can cause various acute problems in human health including cardiovascular, dermal, respiratory, gastrointestinal, neurological, immunological, renal, liver, hematological, mutagenic renal, developmental, reproductive, and carcinogenic effects[20,21,22]. It has been shown that, children are more susceptible than adults to Cd, Pb and As[23, 24]. Through the assessment of health risk, the potential human health risks due to exposed to the trace elements can be estimated. The results from this method can help the decision-makers to establish the significant rules and regulations to protect the population's health [20, 25]. In the current research, yogurt samples were collected from different areas of Bangladesh then quantify the concentrations of heavy metals in three types of yogurt and assessed the potential health effects of Bangladeshi peoples.

MATERIAL AND METHODS

Collection and processing of samples

Total 300 yogurt samples, sour yogurt (100 samples), sweet yogurt (100 samples) and homemade traditional yogurt (100 samples) were collected from several parts of Bangladesh. Sour and sweet yogurt samples were collected from local markets. While sampling, priyority was given to the local made yogurt samples which are not certified either GMP, HACCP or halal certifications. Homemade traditional yogurt samples were collected from the home of farmers who regularly prepare yogurts for themselves. All of those samples were kept in sterile glass bottles and started the analysis as soon as possible after collection. Sample bottles were kept away from any sources containing metals. The concentrations of Fe, Mn, Zn, Cu, Cr, Cd, Pb and As were estimated from those three types of yogurt. This research work was conducted between October, 2020 and March, 2021.

Sample Digestion

A microwave digestion system (Speed wave, Germany) was used for sample digestion and elements extraction. The digestion of yogurt was carried out by mixing 0.3 g of yogurt samples with 5 ml of Nitric Acid (65% purity, Merck, Germany) and 2 ml of H2O2 (30% purity, Merck, Germany). The samples were placed on a glass beaker after digestion and added Milli-Q water (Millipore, USA) to make it 25 ml of final volume.

Samples analysis

Atomic absorption spectroscopy (AAS)

(iCE-3000 series, Thermo-Scientific, USA) was used to investigate the concentrations of Fe, Cu, Mn, Zn, Cd, Pb, Cr and As. We prepared standard solutions at concentrations of 0.01, 0.1, 1.0, 5.0 ppm for every heavy metal. Spectral ranges were 228.67, 357.65, 324.57, 279.43, 217.35, 213.9, 248.30 and 193.7 nm for Cd, Cr, Cu, Mn, Pb, Fe, Zn and As respectively. The quality control was ensured through the certified reference materials (CRM) (Sigma Aldrich, USA).

Procedure of potential health risk measurement

Experimental samples were collected from several parts of Bangladesh. Adult peoples from studied area who regularly consume yogurt were considered as the respondents. The yogurt consumption rate was retrieved from Bangladesh Bureau of Statistics (BBS) Data[24].

Estimation of EDI

The EDI was calculated by the following equation

$$EDI = \frac{DFc \times Mc}{BW} \quad ...(1)$$

Here, DFc represents the daily consumption rate of yogurt (kgday-1) for adult peoples; the value is 27.31 g day-1 24. MC indicates the metal concentration in yogurt samples (table-1) while BW represents the average body weight of adult peoples. The average body weight for adults is 60kg[25].

Determination of THQ

The potential risk of identified heavy metals is determined through calculating THQ (USEPA, 2000) which can be expressed as:

$$THQ = \frac{EDI}{RfD} \times 10^{-3} \quad ...(2)$$

Here, reference dose expressed as RfDs; the reference doses for Fe, Cu, Mn, Zn, Pb, Cd, Cr and As are 0.7, 0.04, 0.05, 0.3, 0.004, 0.001, 1.5 and 0.0003 mg kg BW-1 day-1 respectively[26]. If THQ = 1, then, heavy metals can be considered as possible health hazardous[27].

The exposure of more than one pollutant may cause several complex effects 28. The following equation is used to determine TTHQ:

$$TTHQ = THQ\ toxicant\ 1 + THQ\ toxicant\ 2 + ... + THQ\ toxicant\ n \quad ...(3)$$

Determination of TCR

The risk of cancer development arises when the carcinogens are exposed over lifetime[29]. TCR is estimated by the following equation:

$$TCR = \frac{EFr \times ED \times EDI \times CSF^{\circ}}{BW \times AT} \times 10^{-3} \quad ...(4)$$

Here, EFr indicated exposure frequency (365 days/year), ED expresses exposure duration (70 years). BW is 60kg for Bangladeshi peoples; AT represents averaging time for carcinogens (365 days/year ×70 years) while CSF° represents the oral carcinogenic slope factor[25]. TCR was calculated for As and Pb as they are known carcinogenic heavy metals.

Statistical analysis

For statistical analysis, MS Excel 2016 and XL-stat version[17] were used to interpret the experimental data. Pearson's correlation coefficients were used to examine heavy metal concentrations in yogurt samples.

RESULTS AND DISCUSSION

Determination of heavy metals in yogurt samples

The concentrations of heavy metals were estimated in sour yogurt, sweet yogurt and homemade traditional yogurt samples which are commonly consumed by Bangladeshi peoples. The heavy metal concentrations of sour, sweet and homemade yogurt samples are presented in Table 1.. From this analysis, it was observed that, all of the analyzed yogurt samples were contaminated with heavy metals. Among the heavy metals, Cu was detected in highest concentration. Home made yogurt samples contained highest amount of Cu (1.502 ± 0.367 mg/Kg) which was followed by sour yougurt (0.774 ± 0.372 mg/Kg) and sweet yogurt (0.704 ± 0.324 mg/Kg) respectively. Pb was detected in lowest amount among the analyzed heavy metals and detected in almost similar concentration in all of the three yogurt simple types (0.011 ± 0.006 mg/Kg, 0.01 ± 0.005 mg/Kg and 0.011 ± 0.005 mg/Kg for sweet, sour and home made yogurt respectively). These three types of yogurt samples showed the same descending order in the mean value of heavy metals as Cu>Fe>Zn>Mn. The sour yogurt

Table 1. Concentration of Heavy metals in sour, sweet and yogurt samples (Results in mg/Kg). Results are presented as Mean ± SD.

	Fe	Cu	Mn	Zn	Pb	Cd	Cr	As
Sour Yogurt	0.398 ± 0.121	0.774 ± 0.372	0.087 ± 0.021	0.131 ± 0.026	0.011 ± 0.006	0.031 ± 0.005	0.445± 0.267	0.029 ± 0.024
Sweet Yogurt	0.41 ± 0.146	0.704 ± 0.324	0.082 ± 0.028	0.146 ± 0.025	0.01 ± 0.005	0.03± 0.007	0.46 ± 0.265	0.035 ± 0.026
Home made Yogurt	0.41 ± 0.147	1.502 ± 0.367	0.085 ± 0.02	0.12 ± 0.024	0.011 ± 0.005	0.02 ± 0.005	0.251 ± 0.174	0.025 ± 0.021

Table 2:-Estimated daily intake of heavy metals (mg/day) and calculated THQ values

	Fe	Cu	Mn	Zn	Pb	Cd	Cr	As
Sour Yogurt	0.000181156	0.0006	3.96E-05	5.96E-05	5.00683E-06	1.411E-05	0.00020255	1.31998E-05
THQ	2.59E-06	0.0001	7.92E-06	1.99E-06	1.25E-05	0.000141	1.35E-06	0.000439994
Sweet Yogurt	0.00018662	0.0007	3.87E-05	3.73E-05	5.01E-06	9.10E-06	0.0001143	1.14E-05
THQ	1.31E-06	3.00E-07	1.93E-08	1.12E-07	2.00E-10	9.10E-11	1.71E-06	3.41E-11
Home-made Yogurt	0.00018662	0.0007	3.87E-05	5.462E-05	5.01E-06	9.10E-06	0.000113	1.14E-05
THQ	2.67E-06	0.0002	7.74E-06	1.82E-06	1.25E-05	9.10E-05	7.62E-07	0.000379306

samples showed trend of Cr>Cd>As>Pb in average value of harmful heavy metals while the sweet and homemade yogurt samples showed the trend of Cr>As>Cd>Pb in average value of harmful heavy metals. Siginficant difference ($P < 0.05$) was observed in the Cu concentration between sour and sweet yogurt samples and sour and home made yogurt samples respectively (Figure 1). Similarly, significant difference in Cu concentration was observed between sweet and home made yogurt samples. Comparing with the other two types yogurt samples higher Cu concentration was observed in home made yogurt samples. Those yogurt samples contaminated highly with Cu might be due to direct contact of the yogurt samples with Cu bearing metals. On the other hand, Significant differences in Cr concentrations was observed among the sour, sweet and homemade yogurt samples. In that case, lower Cr concentration was detected in home made yogurt samples.

Health risk assessment

The EDI and THQ values of heavy metals in sour, sweet and homemade yogurt are shown in Table 2. As we know, EDI is expressed as the ratio of the mean heavy metal concentrations, the daily consumption rate of yogurt and the consumer's body weight[30].

Table 3.-Total target hazard quotient (TTHQ) value of sour, sweet and homemade traditional yogurt samples for non-carcinogenic health risk assessment

Individuals	Sour yogurt	Sweet yogurt	Homemade yogurt
Adult	0.000754477	3.42514E-06	0.000666757

Table 4.-Target carcinogenic risks (TCR) of heavy metals (Pb and As) due to consumption of sour, sweet and homemade traditional yogurt in the population of different administrative areas of Bangladesh

Carcinogenic metal	Sour yogurt	Sweet yogurt	Homemade yogurt
As	1.86998E-11	1.61205E-08	1.61205E-11
Pb	1.25171E-09	1.25171E-09	1.25171E-09

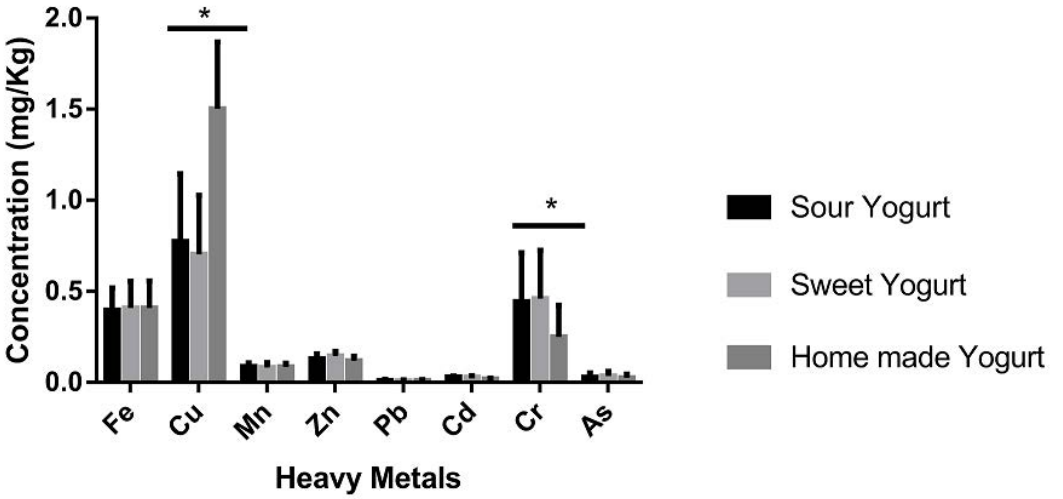

Fig. 1. Heavy metal c oncentrations in sour, sweet and home made yogurt samples (*indicates the significant values; $P<0.05$)

In this research, EDI was calculated for only adult peoples, because children have reluctance of consuming yogurt. Highest EDI value (mg/day) was detected for Cu (0.0006)while lowest EDI value was detected for Pb (5.00683E-06) in each yogurt types. The EDI values indicated that, detected elements are consumed regularly below the tolerance range.

Non- carcinogenic health risk

THQ is estimated for the purpose of evaluation of the possible non-carcinogenic risks through consumption of yogurt by Bangladeshi adult peoples. The estimated THQs of sour, sweet and homemade yogurt samples are presented in Table 2. From this study, it was identified that, THQ values of all of the heavy metals in sour, sweet and homemade yogurt samples are below 1 which indicated that there have no possibility of non-carcinogenic health threats through consumption of sour, sweet and homemade yogurt samples. The THQ values are represented as As>Cd>Cu>Pb>Mn>Fe>Zn>Cr for all of the yogurt samples. The TTHQ values of sour, sweet and homemade yogurt samples were 0.000754477, 3.42514E-06 and 0.000666757 respectively and are presented in Table 3. Although, TTHQ < 1 does not represents consumers acute health effects. The TTHQ values of these analyzed yogurt samples were smaller than 1 which indicated that there have no risk of any complex health effects.

Carcinogenic health risk

TCR values of these heavy metals were estimated to know the potential carcinogenic effects through consumption of these yogurt samples. The intake of high amount of As and Pb may expose carcinogenic risks. TCR values of As and Pb of these yogurt samples are presented in Table 4. The highest As was detected in sweet yogurt samples and the highest Pb was detected in sour and homemade yogurt samples. The values of As in sour, sweet and homemade yogurt were 1.86998E-11, 1.61205E-08 and 1.61205E-11 respectively while the values of Pb in sour, sweet and homemade yogurt samples were 1.25171E-09, 1.25171E-09 and 1.25171E-09 respectively. If , TCR < 10-6 then, there have minimal risk of cancer development; But, 10-6 >TCR>10-4 indicates the risk of cancer[26, 29]. In summary, there have no possible carcinogenic risks through consumption of sour, sweet and homemade yogurts.

A higher Cd concentration was detected in milk products in Pakistan (Mohammed et al. 2013). Several studies in Bangladesh detected higher Cr concentration in milk samples compared to the other studies in different countries[26,30]. In our study, we also detected Cr contents in yogurt samples which might be because of the milk samples were contaminated prior yogurt production while almost similar results were observed in Indian milk samples [27]. Mn contents were not detected in any studies but, lead concentration was detected in Indian study[27]. In another study from Pakistan, researchers have identified Cu, Pb and Cd in milk samples[31]. Several other studies have performed for detection of heavy metals in milk and milk products in Cyprus[32], Egyspt[33], Poland[34] and Kazakhstan[35]. The findings from our study also showed almost similar values in yogurt samples in term of heavy metal contamination.

The animal feed is an important source of their milk contamination. The previous study have identified that, milk samples which were collected from the place located near to waste water drainage, contained higher Cu and Cr contents[31]. Heavy metals are released into the environment through environmental deposition. There might be several sources of milk contamination through heavy metals including animal feed, drinking water, irrigation through waste water and long term environmental exposure[36]. If the milk is contaminated with heavy metals then, the yogurt samples must also be contaminated. If the samples are collected from environmental polluted areas then, there have potential health risks[37]. Processing including manufacturing and packaging of milk products are another important step of contamination[38]. As we know, the environmental pollution is increasing day by day and the animals are ingesting those pollutants through multiple ways. So, Proper steps should be taken to control the environmental pollution.

CONCLUSION

This study determined the concentration of heavy metals in sour, sweet and homemade yogurt samples from different areas of Bangladesh and assessed the possible health risks. Among the detected heavy metals Cu was detected in highest concentration and Pb was detected in

lowest concentration. The THQ, TTHQ and TCR values indicated no possible non-carcinogenic and carcinogenic health risks through the consumption of these yogurt samples. This study will help the consumers to access about their potential health risks through consumption of yogurt. The presence of heavy metals in the yogurt samples might be due to the presence of those heavy metals in milk samples. Proper regulations should be maintained to reduce the heavy metal contamination. Cattles should feed fresh water and contamination free feeds. Cattles should not feed the grasses that grow in the contaminated areas. Industrial wastewater should be properly treated. Moreover, milk and yogurt samples that are used for human consumption should be monitored regularly to make sure the absence of any toxic metals and presence of essential nutrients.

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Conflict of Interest

The authors declare no conflict of interest.

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