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Investigation of contamination pathway and human health risk assessment from metals in milk from the cows grazing in an industrial area: a mass balance approach

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Abstract

The water bodies within the industrial areas are often used for the disposal of effluents leading to heavy metal contamination in water, soil, and vegetation. However, the impact of this metal enrichment on the food web has not been much explored. The present study investigates the food chain contamination of eight metals (Al, Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in the milk from the cattle grazing on a shallow lake bed within the industrial town of Ranipet, India, and associated health risk from the consumption in adults and children. The average concentrations were found to be 24.93, 7.08, 3.31, 0.18, 0.12, 0.08, 0.014, and 0.008 in mg/L for Zn, Al, Fe, Cr, Pb, Mn, Cu, and Cd, respectively. The hazard indices ranged from 0.55 to 1.85 for children; the Incremental Lifetime Cancer Risk (ILCR) values of Cd and Cr were above 10-4 for consumption of milk in both adults and children, which signify serious health risk. The mass balance evaluates the primary intake of all the metals, except Al, are from forage; where for Al it is from the soil. The existing milk consumption patterns projected that 531 children and 1279 adults, drinking contaminated milk are at considerable risk. The analyses of tail switch hair samples indicated the cattle are also environmentally exposed to metals indicating their subclinical effect. Hence the study alerts the elevated and often overlooked risk associated with the food chain contamination from milk in the industrial belt and recommends stringent quality control and monitoring.

Keywords: Cancer risk; Cow milk; Hazard index; Metal contamination; Shallow Lake.

Declarations

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Availability of data and Material

The switch-tail hair metal concentrations and risk estimation are not included in the manuscript can be found at: https://doi.org/10.6084/m9.figshare.14479476.v1. All other data are available from the authors upon reasonable request.

Code availability

Not applicable

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Ethics approval

All of the experiments were carried out in compliance with the standards of international, state, and/or institutional animal care and use guidelines. There are no experiments involving human participants in this article. The manuscript has not been submitted or published in any form, in part or in whole.

Consent to participate

All the samples were collected with the consent of the owners to use in this study. No animal was harmed during the collection of the samples.

Consent for publication

All the authors have read and approved the paper for submission of journal.

Introduction

Rapid urbanization coupled with industrial and agricultural activities have resulted in the exponential increase of pollutants released into the environment. Among them, metals pollution are significant due to their bioaccumulation and prolonged persistence in the environment (Lin et al. 2016). However, several metals such as chromium, copper, iron, manganese, zinc etc. are essential for human metabolism in small quantity, but excess concentrations can cause chronic health effects (Al osman et al. 2019; Giri and Singh 2020). Other metals like cadmium, lead, mercury, etc. aren't essential and cause detrimental effects on human health even at lower levels (Zhang et al. 2017). Metals can be easily accumulated into plants grown on contaminated soils. Thus, significant amount of metals can be transferred from contaminated soil to plants and grass, causing accumulation in grazing ruminants, especially cattle. Moreover, accumulation of these metals can cause toxic effects in cattle; but also in humans consuming metal contaminated milk and meat (Pilarczyk et al. 2013; Giri and Singh 2020; Pšenková et al. 2020).

The milk and its products are basic foods with essential source of nutrients in daily human diet, especially infants. Among them the cow milk is considered as one of the greatest nutritional valued food as being vital in children's diet (Castro Gonzalez et al. 2017). So, the metal content in the milk can be a significant indicator of food hygiene as well as degree of contamination (Licata et al. 2012). Further, the children are more vulnerable than adults to acute toxicity at lower doses as their defence mechanisms aren't completely developed. Due to their undergoing developmental processes, the acute or chronic exposures can lead to serious anomalies which can either persist or develop at later age (Scheuplein et al. 2002). Hence the contamination of cattle milk is a matter of increasing concern and need to be addressed without any tolerance.

The metal contamination of milk has been reported earlier in many parts of the world; especially due to anthropogenic activities (Castro Gonzalez et al. 2017; Yasotha et al. 2020; Boudebbouz et al. 2021). Zhou et al. (2019) reported that the milk samples were contaminated with Pb from industrially polluted area in Tangshan province, China. Iqbal et al. (2020) reported that metals like Cd, Cr, Cu, Mn and Pb were present in buffalo milk samples collected from agricultural farms irrigating with industrial wastewaters in Multan city, Pakistan. Further, significant carcinogenic risk was reported for all age groups (adults and children) by consumption of the milk from this area. There are several studies of milk contamination from India especially from industrial and mining areas. Giri and Singh. (2019) reported significant health risks by consumption of milk from locally rearing cows in vicinity of copper mining and processing industries in Singhbhum of Jharkhand state. Raghu (2015) reported that the milk samples from a barite mining site in Kadapa District, Andhra Pradesh were significantly contaminated with Ba, Co, Sr and Zn. Yasotha et al. (2020) reported that the milk samples collected from the cows reared in industrial areas of Erode District, Tamil Nadu were contaminated with Cd, Cr, Pb and Zn. Hence, from the literature, it was evident that metals pave their way through contaminated forage/water to the cattle; eventually into the milk and subsequent food chains.

Soil was considered as sink of metals and the exact anthropogenic metal contribution was due to discharge of industrial effluents. The ruminants grazing in the industrially influenced areas has different sources for metal intakes such as contaminated water, forage and soil (Zhou et al. 2019; Iqbal et al. 2020). The soil ingestion while grazing was out looked by most of the studies as they often consider milk contamination and associated health risks; which need to be explored. Also, the quantified contribution of metals from the different intake sources to the milk is not apprehended properly. Hence, for the detailed understanding, mass balance needs to be evaluated. This aids to distinguish the appropriate sources of metals to the cattle and in identification of intake and output route of metals which helps to formulate mitigation strategies. For the best of the knowledge, the mass balance concept for the milk contaminated by the metal including soil as potential intake has not been studied before.

Ranipet is an industrial hub in southern India mainly known for its leather production, contributing 37% of country's export leather and finished products. There are other medium to small-scale industries in Ranipet, mostly engaged in chemical, leather and tool making. Several publications reported about the widespread pollution in water and soil due to the effluents and waste discharged from various industries in the surrounding waterbodies (Srinivasa Gowd and Govil 2008). The present study area, Puliyanthangal Lake is one among the water bodies situated in this area, receiving partially treated and untreated wastes from near-by industries and untreated domestic wastes from the surrounding villages (Government of India, Ministry of MSME 2016; Thangarajan, 1999). Recent publication reported about the multiple metal enrichment in the soil and vegetation in the lake bed and suspected that the milk of the cattle raised from the lake area may also have elevated level of metals (Sai Chaithanya et al. 2021).

Based on that finding, the present study attempted to investigate the metal concentrations in the milk of the cattle grazing in the lake bed. Human health risk has been assessed among adults and children who are consuming milk. The mass balance approach has been adopted to perceive the most important intake and output route for individual metals along with their percentage of accumulation in the cattle.

Materials and methods

Study area

Ranipet industrial area spreads over 54 km^2 and mushroomed with numerous tanneries, ceramics, pigments and paints, pharmaceuticals, refractories, chemicals and industrial hubs (Tamma Rao et al. 2013). This is also the location of Tamil Nadu Chromate and Chemicals Limited (TCCL); which is reported to have 0.15 million tons of open dumps of Chromium wastes for decades; contaminating groundwater and soil in its vicinity to considerable extent (TNPCB 2010). This site is considered to be one among the world's worst ten toxic threat (Blacksmith institute 2013). Most of the small-scale industries in this area discharges partially treated and untreated wastes into the surrounding water bodies such as Palar river, Puliyanthangal Lake, Vanapadi Lake, Narasingapuram Lake, Puliyangannu Lake and Chettithangal Lake (Srinivasa Gowd and Govil 2008). Moreover, these lakes are shallow and grazing by cattle has been observed on the unsubmerged parts of the lake beds throughout the year. The study area, Puliyanthangal Lake (Fig. 1) is one among these lakes (Latitude 12° 57' 53.12"; Longitude 79° 17' 36.99") located in Ranipet industrial area of Tamil Nadu. The lake area is of 21.6 ha and the submerged area fluctuating from 0.80 ha (pre-monsoon) to 14.70 ha (post monsoon). Topographically, this area is covered by metamorphic rocks; mostly gneiss and granite of Archean age. The unsubmerged part of the lake area is used as grazing ground for cattle from the surrounding villages. It has been observed that the cows are the only cattle grazing and ingesting lake water throughout the year in the study area.

Collection of samples

The present study was conducted to identify the metal contamination transfer from forage, soil and water to the cattle reared in Puliyanthangal Lake. Also, the transfer of metals through food chain by consumption of milk by adults and children was evaluated. Hence a systematic procedure was adopted in selection of various types of samples. The methodology adopted is shown in the Fig. 2.

All the samples were collected during the month of April, 2018. Ten healthy cows among the cattle reared in the study area were randomly selected to maintain uniformity in sampling and their respective milk, urine and dung were collected. All the samples were collected with the consent of the owners to use in this study. The cattle were never stimulated or forced to excrete dung and urine.

The water samples (n=7; W-1 to W-7) were collected from different corners of the lake without disturbing the sediments in pre-washed sterile plastic containers and they were transferred to laboratory in icebox.

Then they were filtered through Millipore 0.45µm filter paper; acidified to pH less than 2 and stored in refrigerator until analysis at 4º C (Miclean et al. 2019).

The lake area is predominated by *Cynodon dactylon* (Bermuda grass) and limited amount of *Sida acuta* (common wireweed) and *Eclipta prostrata (false daisy).* All three types of plants are being consumed by the cattle were considered in this study. As per the area preliminary investigation, the daily weighted consumption by cattle was considered as 80:10:10 for *Cynodon dactylon*, S*ida acuta, and Eclipta prostrata* respectively. The plant samples (n=7; P-1 to P-7) were collected along with their root from the accessible parts of lake area where cattle grazing was observed. Among them, three samples were from *Cynodon dactylon and two samples each from* S*ida acuta and Eclipta prostrata.* The samples were taken out carefully and brought back to laboratory in air-tight zip lock pouches. They were cleaned properly with double distilled water; air dried and oven dried at 50 °C until they obtained constant weight; crushed by using mortar and pestle and stored in a vacuum desiccator until analysis (Almeida et al. 2004) .The soil samples (n=14; S-1 to S-14) were considered from the study Chaithanya et al. (2021) as the samples were collected in the similar time frame from the same study area.

Milk samples (n=10; M-1 to M-10) were collected in triplicates in the acid pre-washed sterile plastic containers during evening milking hours. Then, samples were transferred to laboratory in ice box and immediately digested for metal analysis on the same day. Urine samples (n=10; U-1 to U-10) were collected in triplicates; directly upon excretion from the cattle to prevent contamination from the contact of the ground surface. All the samples were packed separately in acid pre-washed sterile plastic containers and transferred to laboratory in ice box and immediately digested for metal analysis on the same day. Similarly, the cattle-dung (CD) samples (n=10; CD-1 to CD-10) were collected directly upon excretion into sterile containers. After transferring, the samples were air dried; then they were crushed by using mortar and pestle and stored in vacuum desiccator until analysis (Zhang et al. 2012).

Microwave digestion

All the samples were digested in Multiwave Pro 24-HVT50 (Make-Anton Paar). The milk and urine samples were subjected to digestion by accurately transferring 1 ml of sample to Teflon vessels by using 8 mL Nitric acid 65% and 2 mL Hydrogen peroxide 30% (Miclean et al. 2019). For cattle-dung samples, 0.5 g of sample with same mixture was considered (Zhang et al. 2012). For plant samples, 0.5 g of sample with 6ml Hydrochloric acid and 2ml Nitric acid were used as aqua-regia (Almeida et al. 2004). Blanks were also subjected to same conditions for quality control. After digestion, Teflon vessels were carefully opened under fume hood, transferred to vacuum filtration unit, filtered through Millipore 0.45µm filter paper; filtered samples were refrigerated at 4°C in sterile sample containers prior to analysis (Mohammed et al. 2017).

Analysis and quality control

In the present study eight metals (Al, Cd, Cr, Cu, Fe, Mn, Pb and Zn) have been selected for water, plant, milk, urine and dung samples analysis. All the metals were analysed by inductively-coupled plasma optical emission spectrometry (ICPOES, Model Avio-200, Perkin Elmer) (Antoniadis et al. 2019). The operational parameters of ICPOES are mentioned in the Supplementary Table S1. The multi-elemental standards of Perkin Elmer were utilized for obtaining calibration; by serial dilution of 100 mg/L. For quality control purpose, all the samples were measured in triplicates. Two method blanks were used for a batch of 24 samples and the analysis was repeated for 15% of the samples. The standard deviation for the measured analytes and samples were less than 10%. The variations in the concentration of repeat samples were below 10%. Also, the Relative Standard Deviations (RSD) of each set of replicate samples were below 15% for all the metals analysed.

Survey to estimate average daily self-consumption and total milk yield from cattle owners

The owners of the cattle (CO) grazing in the study area are from the surrounding Puliyanthangal and Periyanthangal villages. The information regarding the average self-consumption of milk by the owners (children and adult) and total milk yield per day from all the cattle were collected to estimate the amount of milk directed for sale.

Market survey to estimate average daily intake of milk buyers

To identify the average daily intake of milk consumption among children and adults, market surveys were conducted among ten milk buyers (MB) in the study area. The data regarding consumption patterns and average daily intake of milk along with the number of adults and children in their families were collected.

Survey to estimate average daily intake, milk yield and excretion of cattle from cattle herds and owners

To identify the average daily dietary intakes and average daily excretion details of the cattle, surveys were conducted among the cow herds and owners of the cattle rearing in the study area. Details covering daily intake of forage and water, total milk yield, urine and faecal excretion patterns of cattle (n=10; C-1 to C-10) were asked personally the responses were considered for estimating them.

Mass balance calculations

The mass balance study was conducted to identify the daily average metal intake, excretion and respective bio-accumulations by the cattle. The mass balance study was carried out on the basis of following considerations:

1. The input information such as daily intake of water and forage by cattle and output information such as daily yield of milk, excretion of urine and dung were based on single time sampling.
2. The cattle daily ingestion rate of soil while grazing was considered from the literature (Healy 1972; Mayland et al. 1975).
3. The information for calculating mass balance equation was based on the average of randomly selected ten cattle among the total cattle grazing regularly in the study area.
4. The metabolism of cattle was considered to be constant; the parameters such as cattle intake, milk yield, quantity of urine and dung are constant throughout this period. Hence, mean intake and output were taken into the account in these calculations.

It was performed by calculating the total intake and output of metals by using the following equations:

$$Metal_{input} = Metal_{output} + Metal_{accumulation} \tag{1}$$

$$Metal_{input} = Metal_{water} + Metal_{soil} + Metal_{forage} \tag{2}$$

$$Metal_{output} = Metal_{milk} + Metal_{urine} + Metal_{dung} \tag{3}$$

Where, $Metal_{input}$ was total intake of metal by cattle through water, soil and forage in mg/day/cow; $Metal_{output}$ was total output by cow through milk, dung and urine in mg/day/cow; $Metal_{accumulation}$ into cattle was the difference between input and output in mg/day/cow. The quantity of intake by water and forage; and the quantity of output by milk, dung and urine for ten cattle was estimated from the survey conducted among cattle herds and owners from the study area. The soil ingestion rate of cattle per day by grazing was considered from the literature.

Risk determination

The daily intake of metal considered to be chronic can be calculated by using the following equation

$$Chronic\ Daily\ Intake\ (CDI) = C_{metal}\ D_{milk\ intake}/B_{average\ weight} \tag{4}$$

Where, C is the metal concentration in the milk in mg/kg, D is the amount of milk consumed in kg and B is the body weight in kg (Castro Gonzalez et al. 2017). Average Indian adult body weight was considered as 52 kg as per Giri and Singh (2019) and 15 kg for children. The following risk assessment indices were considered for evaluating the associated health risk to humans by consumption of milk.

Hazard Quotient (HQ)

HQ is the ratio of chronic daily intake of a metal to its oral reference dose and calculated by using the following equation

$$\mathrm{HQ} = CDI/R_fD \tag{5}$$

Where, R_fD is reference oral dose; considered as 1, 0.001, 1.5, 0.04, 0.7, 0.14, 0.0036 and 0.3 mg/kg/day for Al, Cd, Cr, Cu, Fe, Mn, Pb and Zn respectively. (Integrated Risk Information System (IRIS) 2015, Castro Gonzalez et al. 2017) The value of HQ greater than unity indicates probable harmful health effects for the exposed population.

Hazard Index (HI)

The HI is the summation of individual HQ of studied metals. HI provides the collective potential risk of all the metals. The value of HI greater than unity indicates possible detrimental effect on human health.

$$HI = \sum HQ_i \tag{6}$$

Where, HQ_i is Hazard Quotient of individual metal and HI is the total hazard index for all the metals considered (Castro Gonzalez et al. 2017).

Incremental lifetime cancer risk (ILCR)

The risk of cancer from consumption of milk from the study area can be calculated by using the following equation

$$ILCR = \mathrm{CDI} \times \mathrm{SF} \tag{7}$$

Where, CDI is the daily consumption and SF is the slope factor of the metals which can be carcinogenic (Cd, Cr and Pb). The SF values for Cd, Cr and Pb were 0.38, 0.05 and 0.0085 (Castro Gonzalez et al. 2017; Ukah et al. 2019). The ILCR can be considered as "acceptable" when ILCR is less than 1.0×10^{-6} and it is "serious" when the value exceeds 10^{-4}.

The summation of the individual cancer risks from these metals gives the total cancer risk (Castro

Gonzalez et al. 2017); can be calculated by using the following equation

$$Cancer\ risk_{total} = \sum ILCR_i \tag{8}$$

Where, $ILCR_i$ is lifetime cancer risk for individual metal (Cr. Cd, and Pb) and Cancer Risk $_{total}$ is the total lifetime cancer risk for all the three metals.

Statistical application

To identify possible sources of contamination in the study area, Principal Component Analysis (PCA) was performed by using factor extraction by IBM SPSS Statistics 23. All graphs were plotted by using Origin Pro, 2017. Data handling and mean were calculated by using Microsoft Excel, 2010 (Giri and Singh 2020).

Results and Discussion

Metal concentrations in the water, plant and soil samples

The water samples collected from the lake showed significant concentrations of metals such as Al, Cr, Fe and Mn (Table 1). The metals in lake water samples were in the range of 0.04 – 2.86 mg/L for Al, 0.05 – 0.18 mg/L for Cd, 0.84 – 6.73 mg/L for Cr, 0.01 – 0.78 mg/L for Cu, 0.31 – 1.70 mg/L for Fe, 0.23 – 1.84 mg/L for Mn and 0.20 – 0.66 mg/L for Zn. The Cr was predominated in the water samples as its largest input to the lake water was due to the discharge of partially treated and untreated effluents from nearby tanneries (Srinivasa Gowd and Govil 2008). Also, as the lake receives runoff from nearby agricultural fields and domestic wastes from the surrounding villages, the lake water was contaminated to significant extent and it exceeded BIS (BIS 2012) limits of drinking water except for Zn. Further, the lake water being the primary source of drinking for the cattle reared in this region, the cattle intake metal contaminated water throughout their life time; increasing their probability of risk to metal accumulation.

The plant samples collected from the study area showed extreme concentrations of Al, Cr, Fe, Mn and moderate concentrations of Cu, Pb, Zn; their mean concentrations are shown in Table 1. The metals in plant samples were in the range of 254.91 – 402.15 mg/kg for Al, 0.36 – 0.96 mg/kg for Cd, 97.92 – 198.97 mg/kg for Cr, 3.73 – 10.89 mg/kg for Cu, 232.25 – 1132.24 mg/kg for Fe, 17.34 – 48.38 mg/kg for Mn, 0.58 – 3.16 mg/kg for Pb and 5.77 – 25.68 mg/kg for Zn. The huge concentrations of metals observed in the plant samples were due to the transfer of metals from the lake bed sediments. Puliyanthangal Lake was reported for its chromium contamination in the sediments (Srinivasa Gowd and Govil 2008; Mandal et al. 2011) ; but our previous study on the lake-bed sediments indicated significant anthropogenic multi-metal enrichment. Further, these metals were present in bio-available forms; hence aiding in accumulation of metals in the vegetation growing on the lake bed (Sai Chaithanya et al. 2021). Thus, the cattle reared locally in the study area ingests contaminated vegetation on daily basis; opening the gateway for these metals into the food chain. The soil samples were considered from the study Chaithanya et al. (2021); mean metal concentration of soil samples is shown in Table 1.

Metal concentrations in the milk samples

The metal analysis of milk samples collected showed variations in the metals analysed (Table 1); metals like Al, Fe and Zn showed higher concentrations than others. The metals in milk samples were in the range of 0.62 – 19.49 mg/L for Al, 0.006 – 0.014 mg/L for Cd, 0.06 – 0.30 mg/L for Cr, 0.026 – 0.044 mg/L for Cu, 0.39 – 8.31 for Fe, 0.024 – 0.282 mg/L for Mn, 0.05 – 0.39 mg/L for Pb and 10.02 – 36.25 mg/L for Zn. The Indian standards for metal contaminants in milk are only available for Pb; the metal concentrations observed for Pb was higher than the permissible limit (0.02 mg/L) in most of the samples (Food Safety and Standards Regulations 2011).

Metal concentrations in the urine and cattle-dung samples

To understand the extent of exposure of cattle by grazing forage and by ingestion of lake water, metal concentrations of their urine and dung samples was determined (Table 1). The metals in urine samples were in the range of 1.53 – 13.32 mg/L for Al, 0.005 – 0.012 mg/L for Cd, 0.08 – 0.31 mg/L for Cr, 0.006 – 0.076 mg/L for Cu, 0.38 – 10.83 mg/L for Fe, 0.019 – 0.605 mg/L for Mn, 0.06 – 0.26 mg/L for Pb and 0.04 – 1.06 mg/L for Zn. The highest metal concentrations observed was Al with 13.2 mg/L and Fe with 10.83 mg/L. The Cd was below detection level in most of the samples with 0.012 mg/L as maximum concentration. The metals in cattle-dung samples were in the range of 1079.07 – 5536.25 mg/kg for Al, 0.12 – 0.84 mg/kg for Cd, 16.48 – 420.62 mg/kg for Cr, 29.31 – 99.48 mg/kg for Cu, 1244.11 – 4198.80 mg/kg for Fe, 142.61 – 263.07 mg/kg for Mn, 5.62 – 12.67 mg/kg for Pb and 62.35 – 119.82 mg/kg for Zn. Chromium was found as high as 420.62 mg/kg, highest concentrations of Cu, Mn and Pb were 99.48, 263.07 and 12.67 mg/kg. As cattle were observed to be in lake for most of the time in a day, the excretion of dung on lake bed can be considered as secondary source of metal contamination.

Surveys to estimate average daily self-consumption and total milk yield from cattle owners

The cattle from Puliyanthangal village and Periyanthangal village were usually fed on the pastures and water of Puliyanthangal Lake. Total number of cattle which are grazing regularly in the study area were counted as ninety-one from thirty-eight owners. The self-consumption of milk by these thirty-eight families was calculated and averaged. Apart from this, the total milk yield from the ninety-one cattle were estimated to be 339 L per day with an average yield of 3.72 L of milk per day per cow.

The results from the survey showed that, the average daily consumption of milk per family was around 0.56 L (Table 2). As the total milk yield from the cattle were estimated to be 339 L per day, thirty-eight families of owners consume 21.2 L of milk per day. The remaining 317.8 L of milk was sold to the houses in the surrounding villages. The survey results showed that 60% of adults were consuming the milk whereas in case of children, it was 100%. Hence children in the study area have higher probability of contaminated milk intake than adults.

Market surveys to estimate average daily intake of milk by consumers

Market survey was conducted to understand the dietary habits and consumption patterns among the local consumers of this milk from ten families (Table 3). The daily intake of milk by adults and children were calculated separately by measuring the volumes of their respective containers used for the consumption of milk on daily basis. The mean volume was considered and applied in estimation of CDI. It was observed that the daily intake of adult and child was 206.5 mL and 145 mL per day respectively.

Surveys to estimate average daily intake, milk yield and excretion of cattle from cattle herds and owners

The survey was conducted among the cattle herds and owners to identify the average daily intake of feed and water of cattle along with their average daily milk yield. Also, the data regarding average daily excrements of urine and dung were collected. The intake of forage by cattle was in the range of 20 – 30 kg/day whereas the water intake was 30 – 40 L in a day (Table 4). The yield of milk from a single one ranged from 2 – 6 L per day. Also, the daily excrements from cattle in the form of urine and dung were 5 – 8 L per day and 6 – 10 kg per day respectively. Hence, for the mass balance study, the mean of these values collected from ten cattle were utilized.

Mass balance

To investigate the primary source and sink of metals in the cattle, mass balance study was conducted by calculating total metal intakes and outputs. The difference between the input and output provides insights about the accumulation patterns of different metals into the cattle. Also, the cattle ingest soil due to grazing of root crops to ground level. Hence, ingestion of soil while grazing was considered as one of the input of metals to the cattle. The soil ingestion rate was estimated to be in the range of 0.1 to 1.5 kg/animal per day; with a median value of 0.5 kg/animal per day. Hence, this value was adopted for calculating soil ingestion rate of cattle per day in the mass balance study (Healy 1972; Mayland et al. 1975). Also, the mean metal concentrations of soil from the study area were considered from Chaithanya et al. (2021).

The Fig. 3 shows the mass balance of metals in percentages and concentrations of input and output. The highest input of all the metals except Al to the cattle were due to the grazing of contaminated forage. The ingestion of soil while grazing is the major source of Al. The estimated percentages of accumulation of metals in cattle were in the order of Cd>Cr>Fe>Al>Pb>Mn>Zn>Cu. This can be further supported by the identified concentrations of Al, Cr, Fe, Mn and Zn accumulated in the tail switch hair of these cattle (Supplementary Table S2). Also, organs like liver and kidneys were prone to metal bio-accumulations, detail insight regarding bio-accumulation capacities of different metals can be obtained by considering these samples for further studies (Miranda et al. 2009).

The output of mass balance study indicated that the estimated concentrations of Al, Cu, Mn, Pb and Zn leaving through excretion through dung were significantly higher than other metals. It indicates substantial concentrations of these metals in their consumed feed (Zhang et al. 2012). The metals excreted through urine were considerably less; even though the estimated concentrations by mass balance approach showed ample amount of Al and Fe, the overall percentage of these metals leaving through urine were negligible. The percentages of Pb and Zn leaving through milk were higher than other metals. Thus, Pb and Zn pave their way into the food chain from contaminated forage and water; which reflected in the substantial HQ and HI values.

Risk assessment of metals due to milk consumption

To identify the risk associated to the human health by consumption of milk from the study area, health risk assessment was performed. Chronic daily intake (CDI), hazard quotient (HQ), hazard index (HI) and incremental lifetime cancer risk (ILCR) was evaluated. The mean CDI values of metals for adult and child through the milk consumption are mentioned in Table 5. Moreover, the mean CDI values were compared with the provisional tolerable daily intake (PTDI) values suggested by Food and agriculture organisation of the United States (FAO) and World Health Organisation (WHO); mentioned in Table 4 (JECFA 2003). The mean CDI values for adults and children were lesser than the PTDI values.

The HQ values for the metals for adult and child through milk consumption are mentioned in Table 6. For adults, the individual metals may not pose any considerable risk as none of the metals has HQ value greater than unity. However, for children, Pb and Zn showed few values greater than 1, indicating significant risk by these metals through milk consumption. The HI provides the overall risk by combining the effects of all the metals; for adults, HI values ranged from 0.23 to 0.76 with mean of 0.54 (Table 6). For children, HI values ranged from 0.55 to 1.85 with mean of 1.31; which is higher than 1, indicating significant risk to them. The Pb and Zn are the highest contributors with the HI values of 1.03 and 1.16 respectively. The permissible limit for Pb in milk is limited to 0.2 ppm according to Food Safety and Standards Regulations (2011). The children under six years are more vulnerable to the adverse effects even at lower threshold levels than adults; Pb contamination by food can cause neurological disorders and can be carcinogenic. Even though the Zn is not as toxic as Pb, excess Zn intake can cause nausea, vomiting, diarrhoea and fever. Hence, the children are more likely to be affected than adults by consuming the cattle milk from the study area.

The ILCR values of Cd and Cr were above 10^{-4} for consumption of milk in both adults and children (Fig. 4). The ILCR levels were highest in children (Cd = 0.0020 and Cr = 0.0014) than in adults (Cd = 0.0008 and Cr = 0.0006). Hence, there is considerable cancer risk for the adults and children by consuming the cattle milk from the study area. Also, the combined cancer risk due to Cd, Cr and Pb indicated that there is significant risk for both adults and children. The Cd can cause lung and renal cancers in humans; Cr present in Cr (+6) form (chromate) can be toxic and carcinogenic. The Cr can cause lung, gastrointestinal and breast cancers. Moreover, when children are habituated for daily intake of contaminated food; leads to the progressive accumulation of metals in the organs, can lead to the health complications when they become older (Castro Gonzalez et al. 2017). Hence it is advised to quit the consumption of milk from the cattle rearing in the study area.

Estimating the number of people potentially at risk from drinking metal contaminated milk

To estimate the number of people consuming the metal contaminated milk from the cattle reared in the study area, the cattle owners were identified and their mean self-consumption of milk per family per day was estimated to be 0.56 L/day. Also, the total milk yield from the ninety-one cattle per day was estimated to be 339 L/day. Hence, 21 L of milk per day was self-consumed by the cattle-owners. The rest of the remaining milk i.e., 318 L/day was sold to the consumers. From the market survey conducted among the consumers of the milk, the average daily intake of milk per adult and child was estimated to be 206.5 mL and 145 mL per day respectively. Also, from the surveys conducted among thirty-eight families, it was approximated that the number of children consuming milk per family was 1 and the number of adults consuming milk per family was 2.4. From this data, the number of adults and children at potential risk from consuming this metal contaminated milk was approximated to be 1279 adults and 531 children. The calculation regarding this estimation is mentioned under the section 'Risk estimation'; refer to Supplementary Information.

Source identification

To identify sources of the metals identified in milk samples of the study area, multivariate Principal Component Analysis (PCA) was applied by using SPSS. The VARIMAX rotation along with Kaiser Normalization was used to normalise the data. The PCA reduced number of variables to two principal components (PCs) (Table 7) which explained 79.53 % of data variance. The first principal component contributed 40.87 % of total variance influenced by metals such as Al, Cr and Fe. The presence of Al and Fe can be of natural origin as both are main components of rock forming minerals (Lin et al. 2016). The anthropogenic sources of Fe in the study area are unknown. The sources of Cr can be anthropogenic as the study area is mushroomed with tanneries; hence their effluent discharges can be the only source (Srinivasa Gowd and Govil 2008). Hence the first principal component is combination of both natural and anthropogenic sources.

The second principal component contributed 38.66 % of variance influenced by Cd, Mn and Pb. The Cd can be due to the agricultural activities surrounding the study area. The presence of Mn can be due ceramic industrial activities in the vicinity of lake as Mn is widely used in ceramic industries. Also, there is a possibility of Mn and Zn enrichment from receiving runoff during storms from near-by agricultural fields, where they can be used as fertilizers. The possible source for Pb in the study area can be possibly from pigments and paint industrial activities. The presence of Pb and Zn can also be due to the vehicular activities of the area. Hence, the second principal component seems to be associated with anthropogenic input.

Conclusions

1. The mass balanced study reveals that the major source of the metals is from grazing. Hence, grazing of the contaminated forage needs to be controlled and alternative feed can be provided for the cattle.

2. Soil is the most important route for the Al due to its elevated concentration. This indicates that soil cannot be overlooked as major source for ingestion when the soil concentration is high.

3. The milk of the cattle grazing in the study area is having elevated level of metals specifically Al, Fe, Pb and Zn. The Ranipet industrial area has total surface water of 138 ha which is mostly shallow and being

used for similar purposes. Considering the combined health risk associated with the contaminated milk produced in this area will be alarmingly high, mostly in the children.

4. The milk produced is also sold among the floating customers which makes the situation more critical and difficult to estimate.

5. The dung samples have elevated concentrations of Al, Cr, Fe, Mn and Zn which can act as secondary sources of contamination as these cattle were observed to be in the lake area for most of the day time. As cow dung is the major sink of metals, the burning of cow dung cakes for domestic purposes may further lead to indoor air pollution (Pal et al. 2007).

6. The major fraction of the metals like Al, Cd, Cr, and Fe are retaining in the cattle which was confirmed from the analysis of the tail-switch hair samples from the cattle (Supplementary Table S2). This indicate that the cattle are severely exposed to metal toxicity. Apart from that the human health risk associated with the consumption of cattle meat also needs to be evaluated.

7. Finally, most of the cattle reared within the industrial areas graze in the shallow basins/waterbodies, due to the unavailability of grazing lands. These areas are being used as unauthorized sites for the waste/effluent disposal and are most susceptible to metal contamination. Hence the cattle milk and meat can be a potential source of metal contamination in the food chain which need to be emphasized with detail monitoring and integrate into policy implication.

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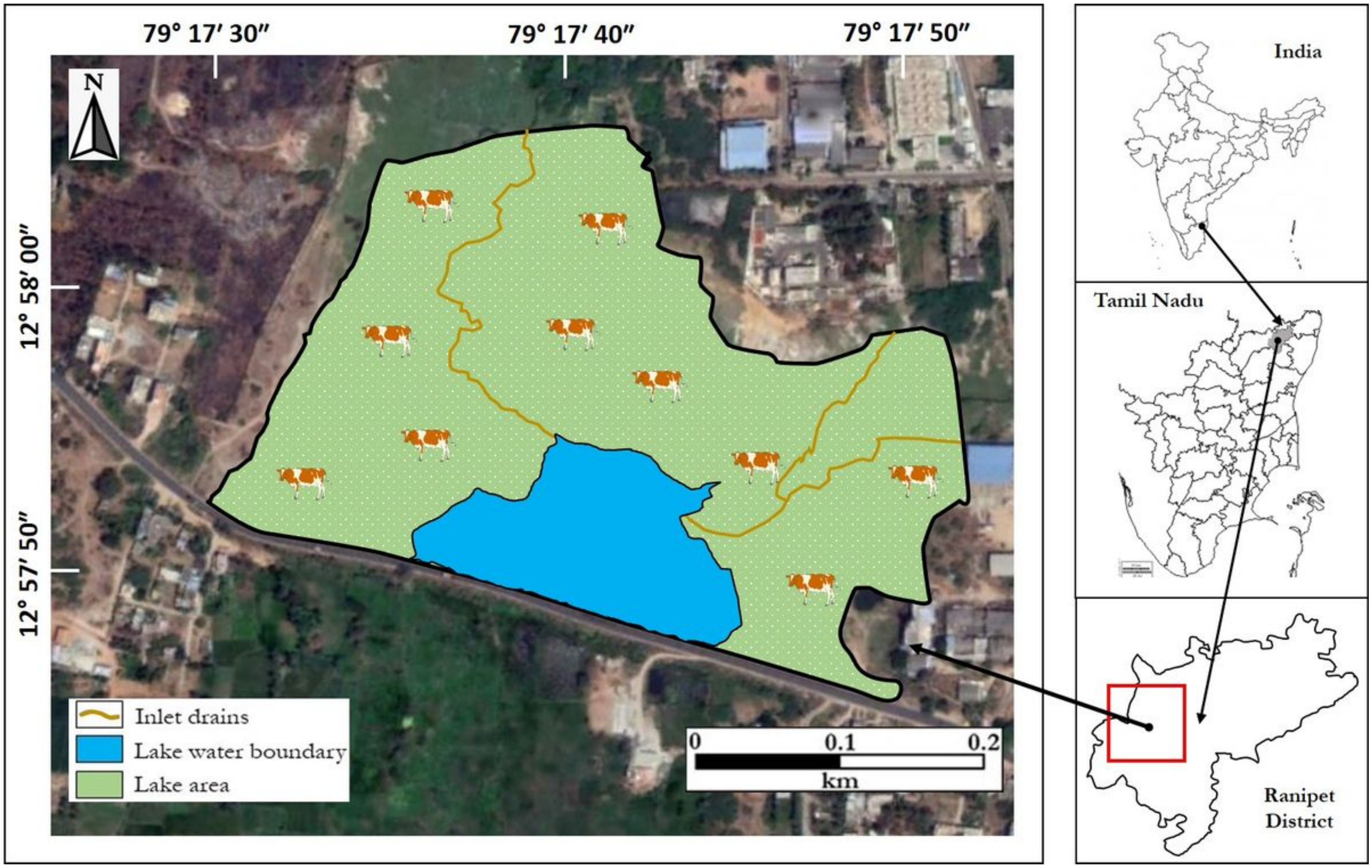

Figure 1

Location of Puliyanthangal lake

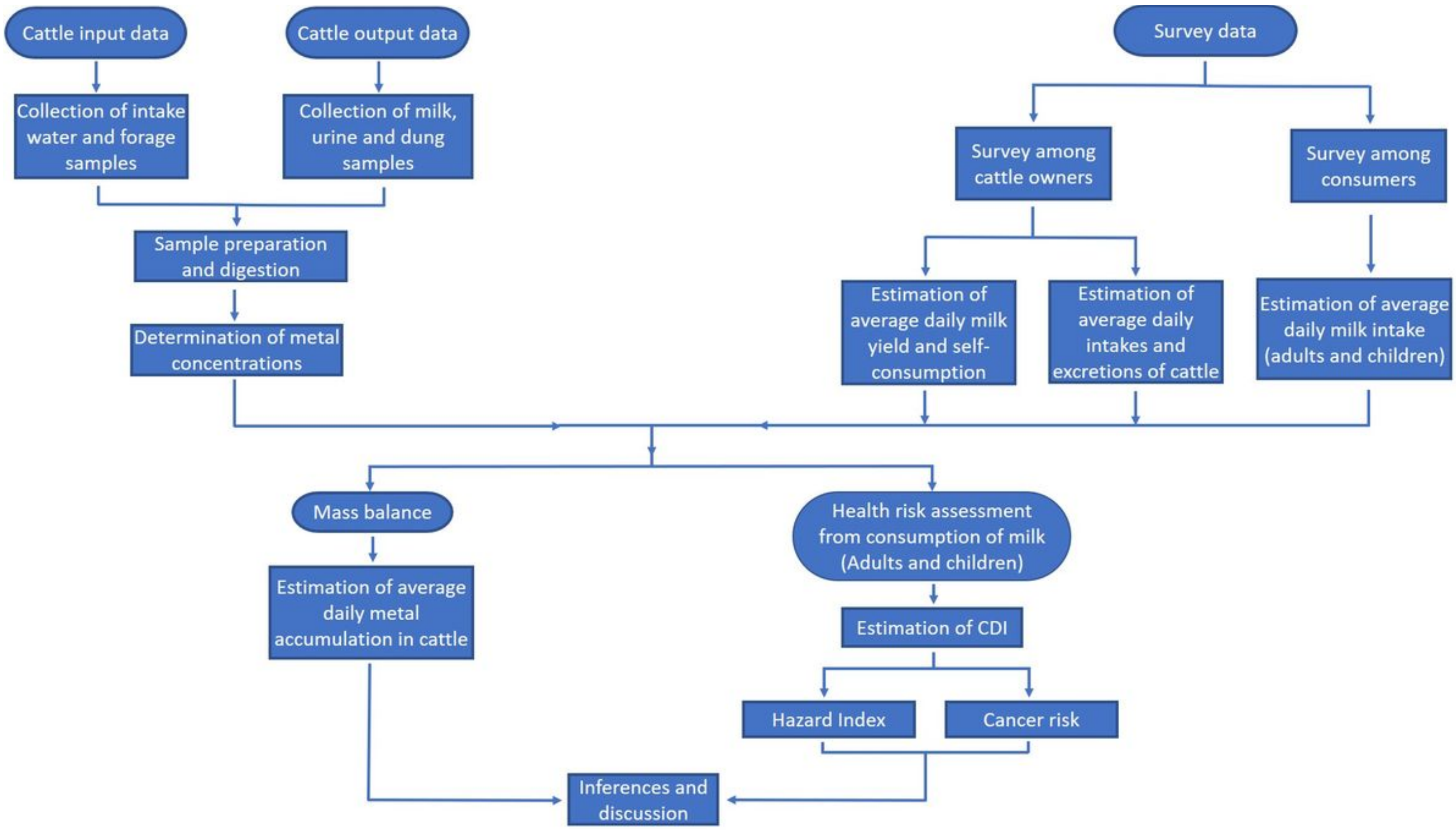

Figure 2

Methodology flow chart

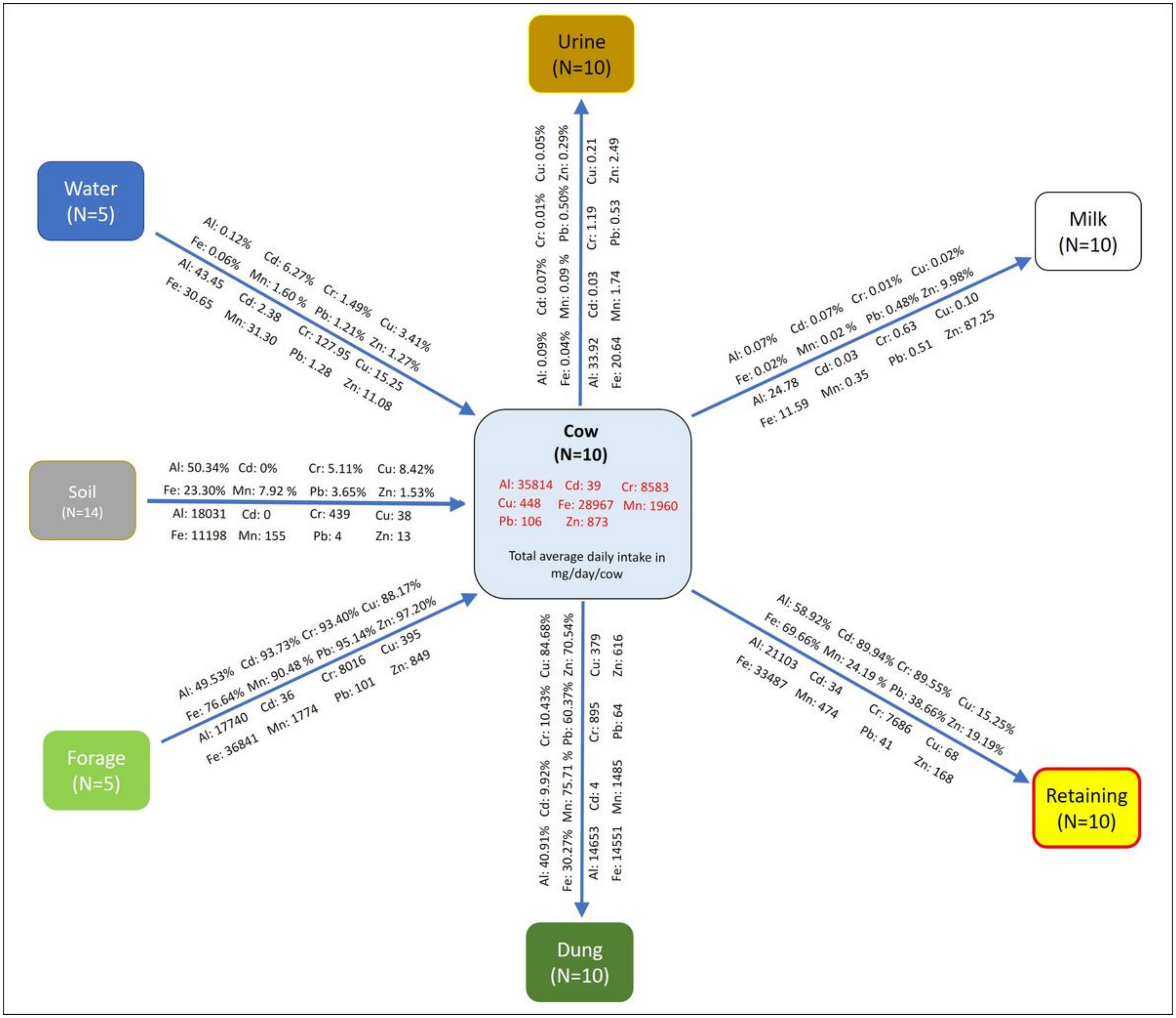

Figure 3

Mass balance chart

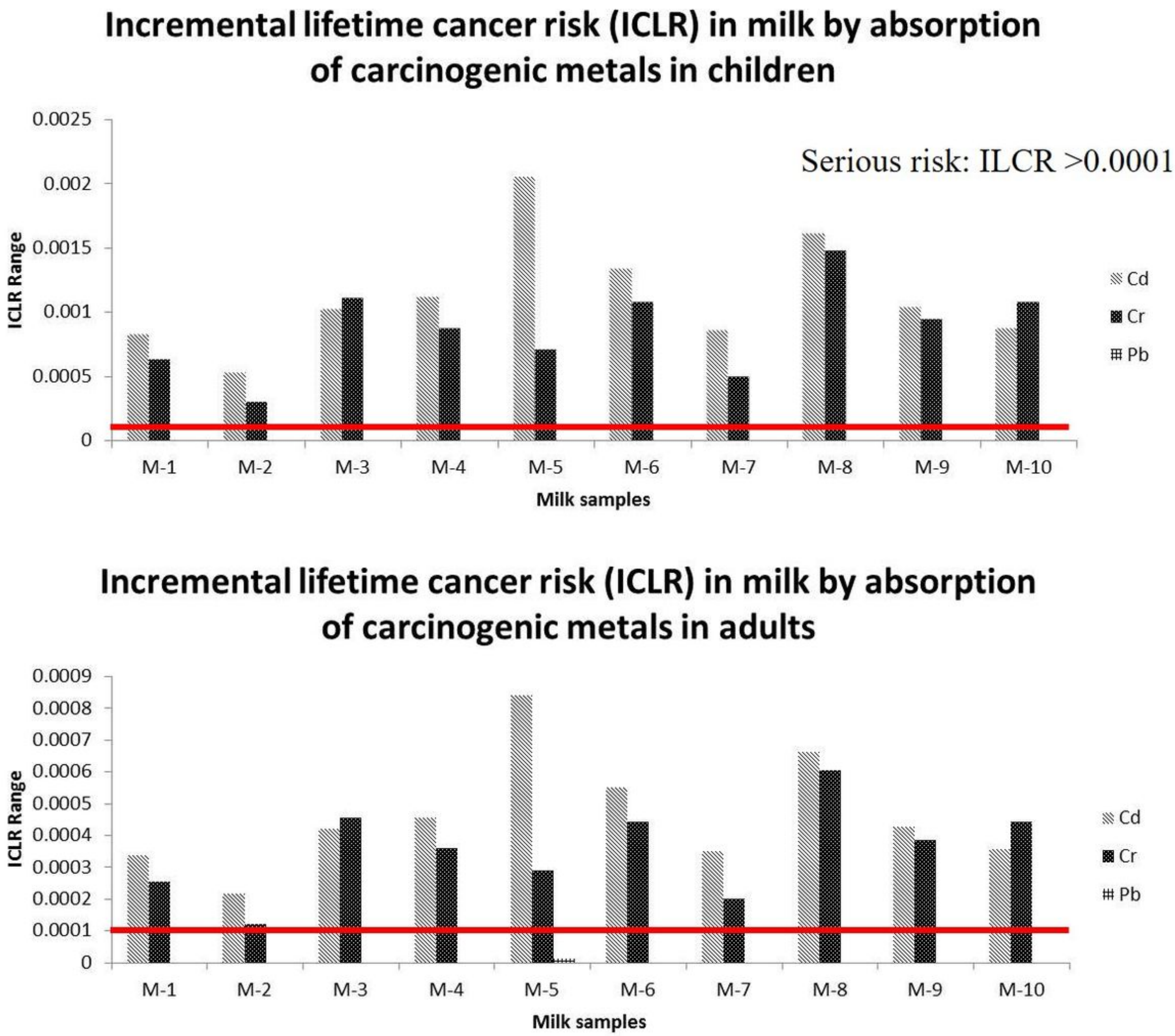

Figure 4

Incremental lifetime Cancer Risk (ILCR) in adults and children

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