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Human health risk assessment for heavy metals via intake of contaminated milk and milk products

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Abstract: The present study was conducted to assess the risk of human health against heavy metals (Fe, Cu, Mn, Zn, Cr, Ni, Pb, Cd) through the intake of milk and milk products produced by animals which are feeding the fodder produced by polluted irrigation water. The milk samples contained amounts of Fe (10.43 ppm) Cu (1.23 ppm), Mn (0.60 ppm), Zn (2.32),Cr (0.05 ppm), Ni (0.17 ppm) Pb (0.28 ppm) and Cd (0.13 ppm) than MAL in buffaloes milk and Ni (0.16 ppm) and Pb (0.41 ppm) than MAL in cow milk. The milk samples from the small animals (goat) were associated with only Fe (11.24ppm,), Zn (2.47 ppm), Cr (0.07 ppm) and Pb (0.05 ppm) than maximum allowable limit. The hazardous quotient (HQ) indicated that milks from all types of animals were contaminated with metals. From the results the hazardous quotient (HQ) indicated that higher risk for Pb and Cd (>1) metals contamination in buffalo and cow milk. However HI (2 to 14) for all studied milk sample was found to be not safe. Females are at somewhat higher risk than males. This study projected a high multi-metal threat due to food chain contamination in the study area.

Keywords: Animal milk, Daily intake rate of metals, Hazard index, Heavy metals

INTRODUCTION

Milk has been considered as the complete food for human health and plays an important role during childhood. Now a day becomes the prominent source of oral exposure to pollutants. Element content of any milk, including essential trace elements, has thus both nutritional and environmental concerns (Vijaya *et al*., 2015) The concentrations of trace metals in milk depend on the genetic properties of the animals, environmental pollution, lactation and type of feed. Trace metals in dairy products may cause severe health problems. More over an account of metals in the milk and dairy products remains poorly documented; particularly in India. Milk and dairy products are one of the widely consumed foods in the human diet, thereby contribute large fraction of trace and toxic elements. The strict control of trace and toxic elements levels in foods is highly essential and given priority in the recent food legislation (Simsek *et al*., 2000).

Copper (Cu), zinc (Zn), and manganese (Mn) are the key components in a multitude of enzymes and play an important role in many physiological functions of humans and animals. Similarly, the trivalent form of chromium stabilizes protein and nucleic acids and is a cofactor of sugar metabolism, where it enhances insulin activity by its presence in an organo- metallic molecule known as the glucose tolerance factor (Anderson, 1995). Iron is a part of both the oxygen-carrying system and iron–sulphur proteins (Kaneko, 1997). Inadequate intakes of trace elements cause impairment of various biological functions and pathological changes. The essential trace elements may become toxic in high doses (Underwood, 1983).

Due to low Zn and Cu content in some home grown feeds compared to their recommendations and varying bio-availability, supplementation of these metals to dairy rations as mineral supplements (NRC, 1980; European Commission, 2003) is very necessary for most livestock species. However, when these nutrients are added above their requirements, the animal may restrict their undesired accumulation in fatty tissues by adaptation and their excretion thereby leading to an increase in the Zn and Cu content of manure (Nicholson *et al.*, 1999; McBride and Spiers, 2001). Application of such contaminated manure to soils, recycles these contaminants back into the feed and thus exacerbates heavy metal exposure to dairy animals over long-term.

The mammary glands are the most physiologically active part of dairy animals. Thus, the input and output of toxic elements in these organisms are clearly reflected in the milk. Monitoring the route of toxic elements in relation to the soil–fodder–milk pathway is hence important, as the consequences of their activity have a great impact on both the environment and people health (Markert and Friese, 2000). Trace metals such as Pb, Cd, Zn, Cu, Cr and As are potential bio-

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accumulative toxins in the production system of the milk and dairy products (Li *et al*., 2005) as these are usually bound to the lipids together with casein (Coni *et al.,* 1996). At the same time the higher levels may even lead to poisoning.

Cadmium (Cd) and lead (Pb) are non-essential nutrients that are of direct concern to human and livestock health and may accumulate in the body, particularly in the kidney, liver, and to a lesser extent in the muscle. Only a limited number of instances have been reported where levels in cattle tissue exceeded maximum acceptable limits for human consumption (Schwarz *et al.*, 1991; Koh *et al.*, 1998), but recent work has suggested that dairy cattle may be more susceptible to the accumulation of Cd and Pb than beef cattle (Alonso *et al.*, 2003). Although it is unlikely that Cd would accumulate in products intended for human consumption, accumulation has been observed in the ovaries and uteri of dairy cows (Smith, 1986) that may have an impact on reproduction.

The purpose of this study was to determine the extent of heavy metals (viz. Fe, Cu, Mn, Zn Cr, Ni, Pb and Cd) accumulation in the milk samples from cows, buffaloes and goats. About 10.69% to 42.15% of agricultural lands in the Gurgaon and Mewat districts were drain water irrigated (Kaur *et al*., 2008). Geospatial analysis further showed that illegal practices of cutting private channels, mixing of degraded canal water with the nearby drain waters and the seepage of these contaminated surface waters in to all the downstream areas is the sole reason of extensive soil-water degradation in this area. About 59% of Mewat district area, irrigated with poor quality waters, is salt affected. Further, ground waters of about 39.6% of Mewat district are salt affected (EC_{mean} = 7.05 dS/m and SAR_{mean} = 7.71). Besides, sub-surface drinking waters of almost the entire Mewat district were also observed to be contaminated with undesirable concentrations of chromium (Cr: 2.0–3.23 ppm), manganese (Mn: 0.80–1.55 ppm), nickel (Ni: 0.02–0.10 ppm) and lead (Pb: 0.40–0.83 ppm). The ground water of waterlogged or potentially waterlogged areas in the rural areas of Mewat was more contaminated. These contaminated water were using as a irrigation for cultivation of crops like cereals, fodder crops, and vegetable crops and these cultivated crops were directly consuming by animals and the contaminated water were used for drinking purpose for animals. The contaminated cultivated crops and water were the main source of contamination in animal's milk (Rajaganapathy *et al.*, 2011; Vijaya *et al*., 2015). The main objectives of this study is to estimate the health risks against heavy metals through the consumption of buffalo, cow and goat milk to the general public in Ujjina village (Mewat district) of Haryana.

MATERIALS AND METHODS

Site characteristics: The proposed research work was focused on the Ujjina village (2500 ha) in Mewat district of Haryana. The selection of the test village was based on the previous studies conducted in the Mewat district of Haryana by Kaur *et al.* (2008). Mewat district is carved out from erstwhile Gurgaon and Faridabad districts. Gurgaon district bounds it on north. While Rewari district lies to its west and Faridabad district to its east. On south, the district shares its boundary with Rajasthan state. Geographically Mewat district is situated between 26°.30 ° north latitude and 76 °.78 ° east longitudes. The Ujjina village is located in the most downstream part of the Nuh block of Mewat district (Fig. 1), near the point of confluence of the three major (*viz*., Nuh, Chandeni and Kotla) drains of the Mewat district.

Collection of milk samples: In order to conduct the study, milk samples from different milch animals (Buffalo, Cow and Goat) were collected during both winter and summer seasons. These were collected, during evening time, in sterilized labelled plastic bottles after rinsing each plastic bottle with distilled water for at least five to six times and transferred to laboratory analysis. In laboratory these milk sample were stored at -80°C until further use for heavy metal (Fe, Cu, Mn, Zn, Cr, Ni, Pb, Cd) analysis.

Digestion and estimation : Digestion of sample was done as per the method described by Anastasio *et al.,* (2006). For heavy metal analysis, entire milk sample in a clean and dry conical flask was dried at 70°C on hot plate. In dried 3gm-sample, 30ml of HNO_3 (65%) and 6 ml H_2O_2 (30%) were added. The conical flask containing the milk sample and the acid mixture were kept on a hot plate and digested until the content of flask turned colourless. The flask was then removed from the hot plate and kept at room temperature. After cooling, 20-25 ml of double distilled water was added into each flask (to dilute the acid content) and the contents were then filtered into a 100 ml volumetric flask using Whatman filter paper no. 42. The conical flasks were rinsed and washed 3 times with double distilled water and filtered into the respective volumetric flasks until their full volume was attainted (100 ml).

The concentration of heavy metals (Fe, Cu, Mn, Zn, Cr, Ni, Pb and Cd) in digested milk samples were estimated on Atomic Absorption Spectrophotometer (AAS) / Polarograph using standard solution of (Fe, Cu, Mn, Zn, Cr, Ni, Pb and Cd) against unknown samples of milk. In order to measure the concentration of metals in each sample, the concentration range of standard metal solutions were modified. Blank solution (containing everything except milk sample material) was also prepared to set the initial zero reading and to minimize any metal contamination in samples. Recorded values of metals in unknown samples solutions (ppm) were multiplied by their respective dilution factors to obtain actual concentration of metals in dry milk samples (µg/g dry weight of sample).

Health risk calculation: Health risks were calculated by parameter of daily intake rate of metal (FDA, 2001) target hazardous quotients (USEPA, 2013) and Hazard Index was calculated by (USEPA, 1989)

Statistical analysis: The raw data were averaged and compared by statistical software SAS version 9.2 . Further means were compared graphically (bar charts) over all mean values.

RESULTS AND DISCUSSION

Average concentrations of micronutrients and trace metals in the milk samples of buffalo, cow, and goat collected from Ujjina village are illustrated in Figs. 1-7, respectively.

The analysis clearly indicated that the milk samples from buffalo contained more than permissible amounts of Fe (10.43 ppm, 2.09 times more), Cu (1.23 ppm, 3.08 times more), Mn (0.60 ppm, 4.27 times more), Zn (2.32, 2.32 times more) ,Cr (0.05 ppm, 2.05 times more), Ni (0.17ppm, 16.58 times more), Pb (0.28 ppm, 13.83 times more), and Cd (0.13 ppm, 1.33 times more) than MA (Figs. 2 and 3). The nickel and lead concentrations seemed to be the highest. This appeared to be a direct function of the metal translocation from the metal contaminated feed and waters.

This was also observed in the case of the milk samples derived from the cows (Figs. 4 and 5). Even these samples recorded highest Ni (0.16 ppm; 16.22 times more than MAL) and Pb (0.41 ppm; 20.51 times more) than MAL concentrations thereby indicating its direct relationship with the more than permissible metal concentrations in the local area feed and waters. Not much information regarding Ni residual levels in the milk is available in literature. However these results were in complete confirmation with more than permissible concentrations of nickel by Bilal *et al.*, (2010) and lead by Javed *et al*., (2009) and Simsek *et al.*,(2000) in cattle milk, respectively. Sajid and Musa (2012) also reported the higher concentration of Cd,Cr,Ni and Pb metal in cattle milk compared with maximum allowable limit.

In contrast to these, small sized animals such as the goat were observed to be assimilating more than permissible concentrations of only Fe (11.24ppm; 2.24 times more), Zn (2.47 ppm; 2.46 times more), Cr (0.07 ppm; 3.50 times more) and Pb (0.05 ppm, 2.55 times more (Figs. -6) than maximum allowable limit. However these results in small sized animals were in complete confirmation with more than permissible concentrations of Pb and Cr by Bilal *et al* (2010) .Rest of the metals such as Cu (0.11 ppm;), Mn (0.04 ppm; Ni (0.01 ppm;), and Cd (0.01 ppm,) were found to be within permissible limits for the goats. Tuzen and Soylak (2004**)** and Javed *et al*., (2009**)** also reported within permissible nickel concentrations in the goat milk. It was also reported that the Ni concentration in cattle

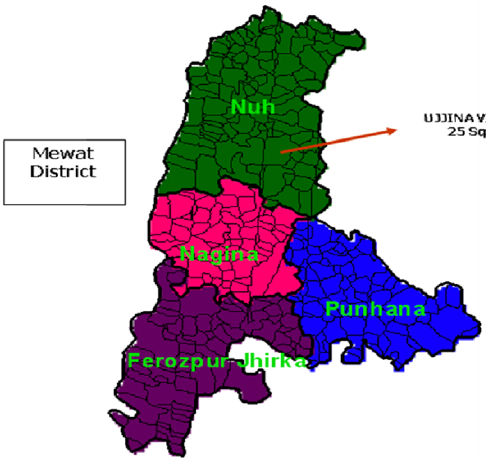

Fig. 1. *Location of study site at Ujjina Viilage (Mewat District).*

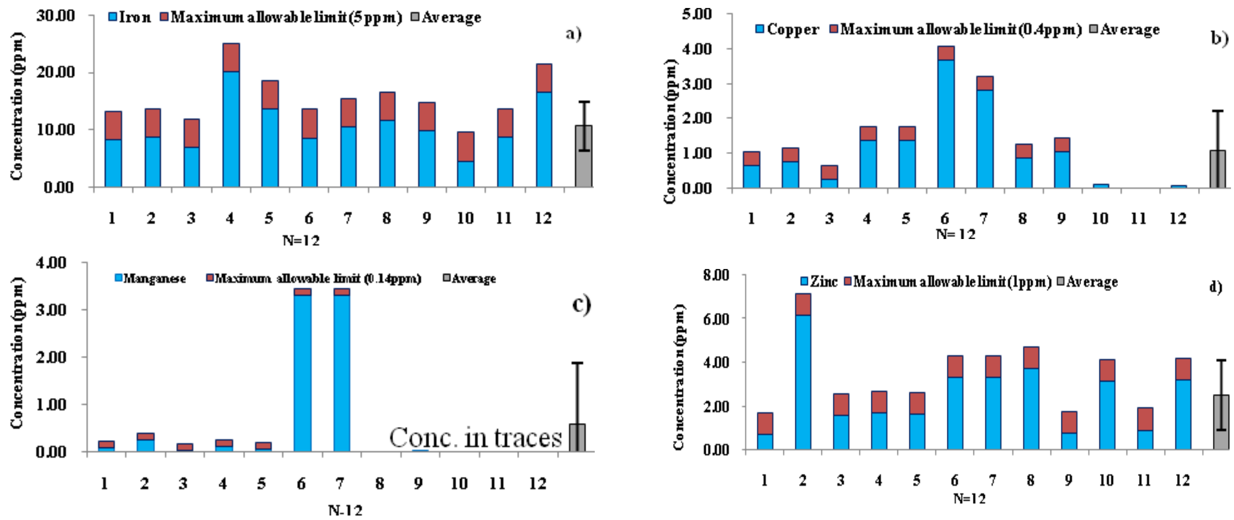

Fig. 2. *Micronutrients a) Iron; b) Copper; c) Manganese and d) Zinc concentration in buffalo milk.*

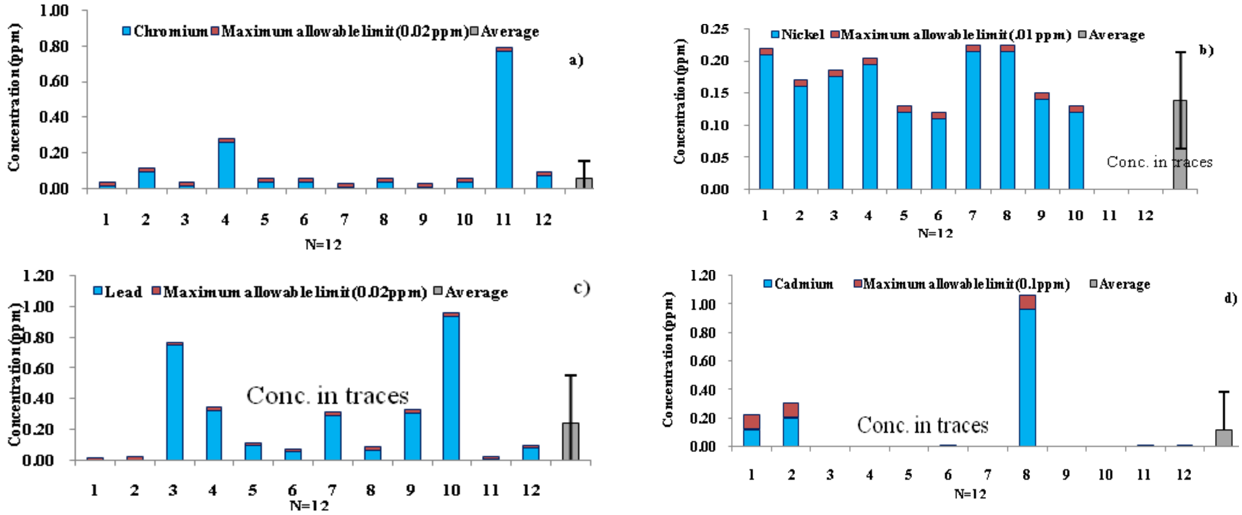

Fig. 3. *Trace metals a) Chromium; b) Nickel; c) Lead and d) Cadmium concentration in buffalo milk.*

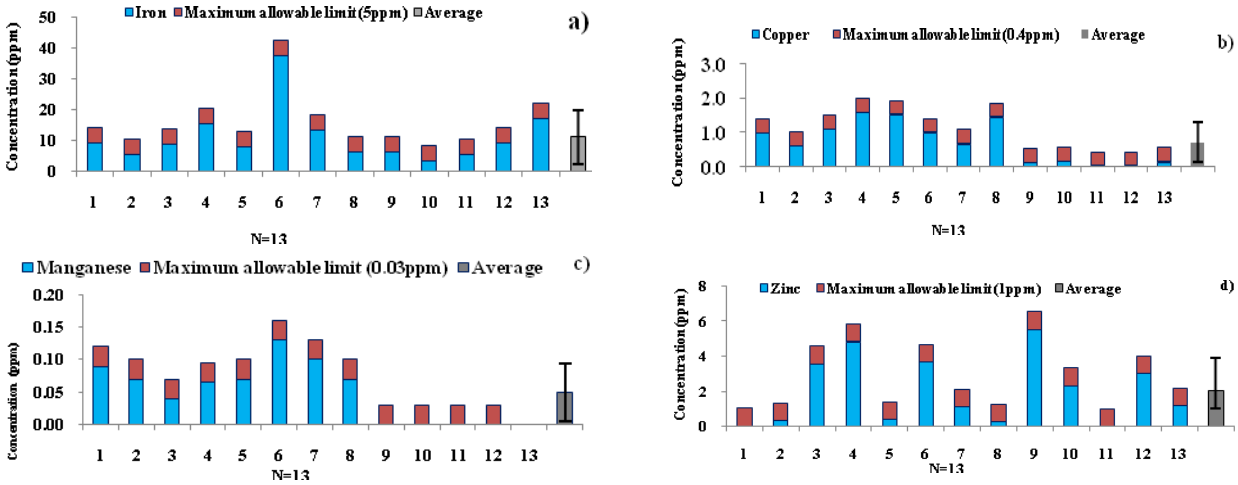

Fig. 4. *Micronutrients a) Iron; b) Copper; c) Manganese and d) Zinc concentration in cow milk.*

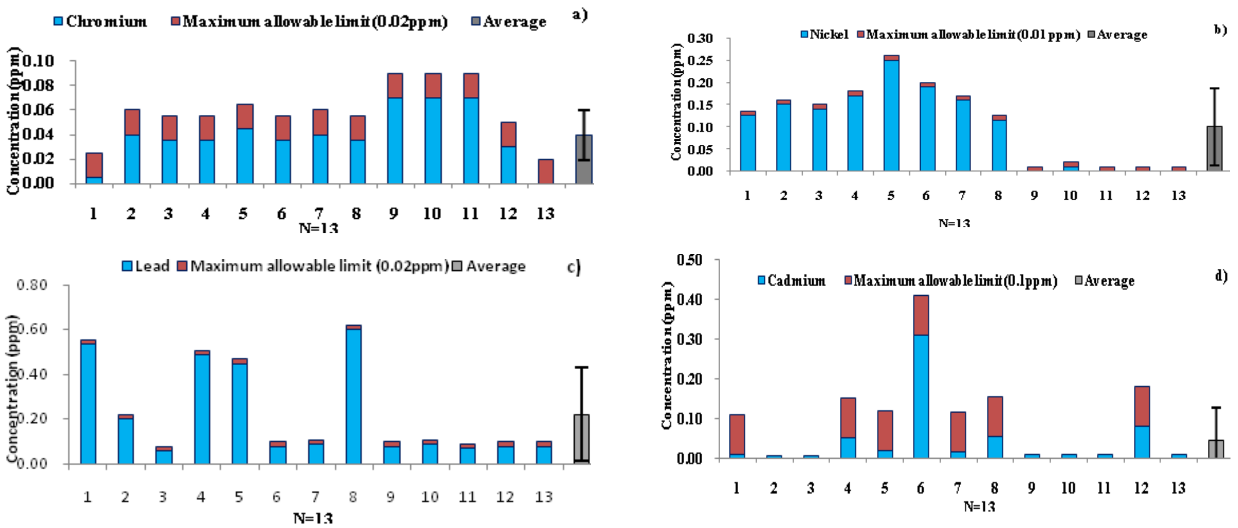

Fig. 5. *Trace metals a) Chromium; b) Nickel; c) Lead and d) Cadmium concentration in cow milk.*

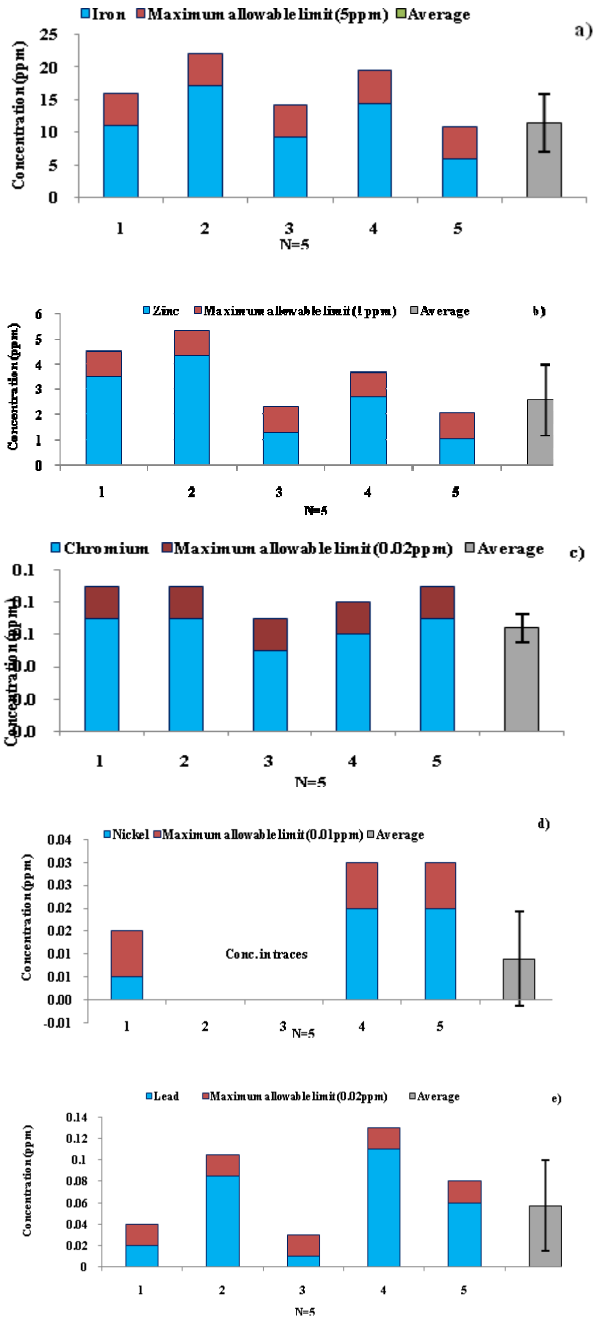

Fig. 6 . *Micronutrients and trace metal a) Iron; b) Zinc c) Chromium; d) Nickel; e) Lead concentration in goat milk.*

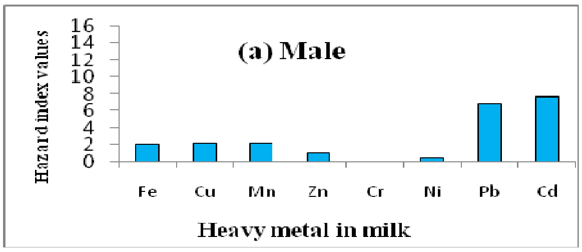

Fig. 7 (a) *Hazard index (HI) for male through consumption of milk collected from Ujjina Village .*

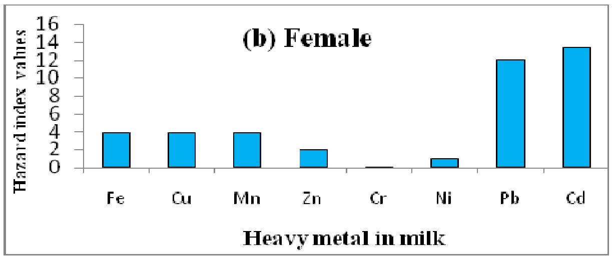

Fig. 8 (b) *Hazard index (HI) for female through consumption of milk collected from Ujjina Village.*

milk were higher than in goat milk and these result were supported by Ibadullah (2012).

Hence, the study projected a heavy and trace metals threat to the human health through the consumption of contaminated milk.

Human health risk assessment

Estimation of daily intake rate of metals: The degree of toxicity of heavy metal to human being depends upon their daily intake rate (Singh, *et al.*, 2010). The DIR estimated for both male and female shown in Table1. Daily milk consumption was according to National Dairy Development Board, per capita milk availability in 2011-12 was 290/gm/day. It was reported that daily metal intake of milk was higher through buffalo and cow milk rather than goat milk. It was also revealed that daily intake of metal rate were higher in female as compared with male (Table 1). Similar study has been found by Monica *et al.* (2011), reported that DIM is higher for female as compared with male through vegetable consumption. This study also reported that the highest intake of Fe, Cu, Mn and Ni with the consumption of buffalo milk and these values exceeded from permissible limits.

Estimation of target hazardous quotients (THQ) : Hazardous Quotients is a complex parameter used for the estimation of potential health risk associated with long term exposure to chemical parameter (Khan *et al.*, 2009). THQ values were calculated on the basis of the oral reference dose. Oral reference doses (R_fDo) for heavy metals are presented in Table 3.

THQ parameter were quantify for both male and female in buffalo, cow and goat milk samples. From the obtained result was revealed that the THQ values for heavy metals Cr, Zn, Ni were >1 (Table 2) in buffalo milk, Mn, Zn, Cr, Ni were >1 (Table 2), in cow milk, Cu, Mn, Zn, Cr, Ni, Cd were >1 (Table 2), in goat milk for male. HQ values for some heavy metal are equal or more than 1. For lead THQ values in buffalo and cow were 3 and goat milk was 1.

Buffalo and cow milk THQ values for Lead is 3 (>1), HQ =1 for goat milk. Cadmium THQ values were observed 5 and 2 in buffalo and cow milk respectively

Table 1. Combined daily intakes of metals (DIM) from 290 gm/litre milk by Indian population with recommended/ permissible value (mg/kg).

Metals	DIM for male				DIM for female			
	Buffalo	Cow	Goat	MAL	Buffalo	Cow	Goat	MAL
Iron	38.67	40.36	41.75	18.13	51.55	53.81	55.67	24.17
Copper	3.93	2.61	0.46	1.45	5.24	3.48	0.61	1.93
Manganese	2.15	0.18	0.13	0.11	2.86	0.24	0.17	0.15
Zinc	9.03	7.31	9.34	3.63	12.04	9.74	12.46	4.83
Chromium	0.42	0.14	0.23	0.07	0.55	0.19	0.31	0.10
Nickel	0.50	0.37	0.03	0.04	0.67	0.49	0.04	0.05
Lead	0.88	0.81	0.21	0.07	1.18	1.08	0.28	0.10
Cadmium	0.41	0.16	0.04	0.36	0.54	0.22	0.05	0.48

***MAL = Maximum allowable limit** (FDA 2001, European Commission 2003a)

Table 2. Combined Target Hazardous Quotients (THQ) for male and female .

Metals	THQ for male			THQ for female		
	Buffalo	Cow	Goat	Buffalo	Cow	Goat
Iron	1	1	1	1	1	1
Copper	1	1	0	2	1	0
Manganese	2	0	0	3	0	0
Zinc	0	0	0	1	1	1
Chromium	0	0	0	0	0	0
Nickel	0	0	0	1	0	0
Lead	3	3	1	6	5	1
Cadmium	5	2	0	9	4	1

Table 3. Oral reference doses (R_fDo) and Upper tolerable daily intake (UL) for investigated metals.

Metals	R_fDo (Mg/kg/day)	References	UL (mg/day)	References
Iron	0.700	USPA(1997)	45	FDA(2001)
Copper	0.040	Khan *et al*, (2008),USPA	10	FDA(2001)
Manganese	0.014	USEPA (1997)	11	FDA(2001)
Zinc	0.300	Khan *et al*, (2008),USPA	40	FDA(2001)
Chromium	1.500	USEPA (1997)	NA	
Nickel	0.020	Khan *et al*, (2008),USPA	1	FDA(2001)
Lead	0.004	Khan et al, (2008)	0.24	Garcia *et al.*,(2007)
Cadmium	0.001	Khan *et al*, (2008),USPA 1997	0.064	Garcia *et al.*,(2007)

(Table 2). The values THQ more than one is cause of concern from health point of view.

THQ values for female were found higher as compared to male. Higher THQ values were more than 1 for Cu, Mn, Zn, Pb and Cd in buffalo milk. (Table 2) and THQ values more than 1 were for Pb and Cd in cow milk (Table 2). In goat milk THQ values were found up to 1.The contribution from all metals brings the combined THQ values to exceeded 1 for Mn, Pb and Cd for male and female. But it was also observed HQ values were higher for female (Cu=2 , Mn=3,Pb=6 and Cd=9 in buffalo milk, Pb=5 and Cd=4 in cow milk). These results were supported by Monica *et al*. (2011) who revealed that THQ values in vegetables were observed higher in female than male. The extremely toxic metals Lead and Cadmium were found in contaminated milk that exceeded normal threshold values. This contaminated milk can raise the intake of Pb and Cd and contribute to great share on the total values of THQ, especially for buffalo and cow milk.

Hazard index (HI): Hazard index is called risk index for residents of ingesting these metals by consuming milk and HI were calculated by summation of THQ of all heavy metals. In present study, it was revealed that the highest HI of heavy metals were for lead and cadmium in both male and female but highest HI value were revealed for female.(Figs.7 and 8).Whereas negligible values of HI were found for chromium. However, the higher values of HI of metals were indicated that there was risk from the intake of milk. The concentrations of different metals are in wide variations in the published data for the elemental concentrations of milk for different countries (Tassew *et al*., 2014).Very high concentration of Fe (10.90 mg/kg) was observed in the present study. Value of Cu (0.903 mg/kg) was higher as compared to the other countries. Although China (0.420 mg/kg) and Nigeria (0.380 mg/kg) had less values as compared with present study (Qin *et al.*,2009, Ogabiela *et al.*,2011) but very much high compared to the values reported for other countries. The concentration of Mn (0.320 mg/kg) is also comparable with Ethopia (0.427 mg/kg) (Tassew *et al*., 2014), but it is higher compared to the respective values of remaining countries are Nigeria (0.219 mg/kg), Poland (0.102 mg/kg) and Egypt (0.056 mg/kg) in the literature. (Ogabiela *et al*, 2011, Doberzanski *et al* ., 2005, Enb *et al.*,2009). Zinc concentration (2.253 mg/kg) is almost same as the reported value of USA (2.235 mg/l). It is higher compared to that of Bangladesh (1.215 mg/kg), Crotia (0.510 mg/kg), Saudi Arabia (0.945 mg/kg), and Spain (1.419 mg/kg).(Lopez *et al.*, 1995, Khan, *et al.*, 1989 Sikiric *et al.*,2003, Farid *et al* ,2004). But presently reported Zn concentration is low with compared to other countries whereas very much lower compared to the China. The concentration of Cr in the present study was (0.077 mg/kg) high compared with the corresponding values of other countries except that of Nigeria (1.568 mg/kg), Ethopia (0.868 mg/kg) and China (0.280 mg/kg) (Ogabiela *et al*, 2011, Tassew *et al*., 2014, Qin *et al* .,2009). The observed concentration of Ni in present study was 0.120 mg/kg). The concentration of Pb (0.234 mg/kg) and Cd (0.234 mg/kg) and these values were not comparable with remaining countries (Table 4). In general, the concentrations of metals detected in the present study were more or less comparable with the reported literature values. However, relatively higher concentration of Cu, Pb, Cd, observed in this study in comparison to the reported values and Fe and Ni concentration were not reported in literature values.

Conclusion

The DIM, THQ and HI indices suggested that metal content in milk is more than permissible limit. The milk samples from buffalo contained more than permissible amounts of Fe,Cu, Mn,Zn,Cr,Ni,Pb,Cd viz 2.09, 3.08, 4.27, 2.32, 2.05, 16.58, 13.83 and 1.33 times more respectively. In the case of the milk samples of cows recorded highest Ni and Pb were 16.22, 20.51 times more than MAL. In contrast to small sized animals such as the goat milk were contained of Fe, Zn,Cr and Pb were 2.24, 2.46, 3.50, 2.55 times more than MAL respectively. The nickel and lead concentrations seemed to be the highest. Therefore, the consumption of such milk in studied areas is not safe for human health. Further researches in this area will be warranted for the interest of public health, mainly to determine the mechanisms of metals absorption in milk

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