Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Human Health Risk Assessment of Heavy metals in Cow Milks from Selected Local Government Areas of Kano state, Nigeria

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(Received: 27 June 2021 Accepted: 1 February 2022)

KEYWORDS

Environmental pollution;
Industrialization;
Milk;
Heavy metals;
General health;
DIM;
THI

ABSTRACT: Environmental pollution is a problem that has emerged due to increased industrialization with its attendant effects on well-being of people. This study determined the contamination level of cow milk by the heavy metals (Pb, Cr, Cd, Cu, Zn, Mn and Fe) from three local governments (Bichi, Rano and Nassarawa) in Kano state. The results obtained showed that, the concentration of Pb, Cd, Cu, Zn and Mn were below permissible limits in milk. Whereas, moderate contamination by Fe and Cr were also observed. The data obtained were used to calculate some risk indices such as daily intake of metals (DIM), and total hazard index (THI). The DIM in the three local governments was within the range of $3.4 \times 10^{-4} – 6.8 \times 10^{-3}$ µg l^{-1}. b.w/day for Cr and 0.367 – 1.55 µg/l. b.w/day for Fe. Lead (Pb) was only detected in Rano local government within the range of 0.104 – 0.117 µg/l. b.w/day, while Zn was detected in Bichi and Rano within the range of 0.048 – 0.113 µg l^{-1}. b.w/day. All hazard indices calculated for metals were found to be below 1. The result therefore, showed tolerable risk in consumed milk at the aforementioned locations. However, risk assessment should be carried out at intervals as a form of environmental assessment to assess the general health of the public, to prevent buildup of environmental pollutants and consequent consequences.

INTRODUCTION

As a major source of protein, calcium, phosphorus and fat-soluble vitamins (A and D), milk and dairy products make a significant contribution to the dietary intake of biochemically, essential nutrients. It has also been reported to consist of other vitamins (such as vitamin C) and minerals (such as magnesium and iodine). A growing number of populations consume raw milk in Sub-saharan Africa. Health benefits, taste, and enhanced nutritional qualities in raw milk are some of the reasons for such decisions. While many lab studies have clearly revealed that, variety of pathogens being associated with human diseases can be source of contamination to raw milk, it has also been found to be unfortunately contaminated by heavy metals through vertical transmission from plants and feed resources [1].

Environmental factors and livestock characteristics on the other hand, affect the quality of raw milk. Therefore, in order to maintain its competitive place in the market, maintaining quality of raw milk is critical and any change on its composition could pose a serious threat to producers of milk (due to price cuts), dairy industry (increased production costs), and consumers (dietary and health aspects [2].

Environmental pollution is significant global challenge which changes the existence of the ecosystems [3], and also constitutes a threat to human health. This concept emerged in the beginning of the industrial revolution. Several heavy metals contaminates the soil and subsequently taken up by plants through their roots, and then transferred to plant consumers (animals and

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DOI: 10.22034/jchr.2022.1934016.1345

humans) or as run off into water bodies consumed by both animals and humans. Evidences have clearly demonstrated that exposure to ubiquitous environmental pollutants for a long period of time can have adverse effects on humans from their early embryonic stage, extending to their postnatal life [4].These pollutant could be in a form of chemical or other material which are released into the environment and whose effect can have direct or indirect impact on humans and other living organisms [5]. Heavy metals, pesticides, aflatoxins, oils and tars, fertilizers and organic chemicals are the common pollutants in soil, with potential risk on humans. Heavy metals are elements that have relatively high density, they include arsenic (As), lead (Pb), chromium (Cr), mercury (Hg), cadmium (Cd), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe) and their density is more than 6 grams/ cm^3 [6]. They are contaminants which are found in variety of human foods, thereby causing pathological impacts on humans and animals and also constitute major agents of environmental pollution. While some of heavy metals such as Fe, Zn and Cu are very essential in maintaining metabolic activity by serving as enzyme components/activators in the body, others such as Cd and Pb proves to have no any essential physiological and biochemical role [7]. Their wide distribution in the environment can be attributed to numerous anthropogenic activities that have to do with rapid industrialization. As reported by Nasiru et al. [7], heavy metals have a number of ways through which they can enter into the human body and pose a lot of risk to the human; they get accumulated in the environment through industrial emissions, release of industrial effluents, agricultural activities, use of leaded gasoline and paints, incineration of toxic substances and indiscriminate disposal of municipal wastes. These metals when ingested through food or drinking water, have a negative effect on the metabolism of living cells once their concentration exceeded the maximum permissible/tolerance levels [8].

Milk is an important component of human diet that contains lot of essential elements, proteins, fats, sugars and vitamins and also constitute a major diet in infants (as a substitute for breast milk), adults in everyday life and also the elderly people. According to Priyanka et al. [10] milk products are widely consumed around the World by adults and children and most especially elderly people. Due to its wide consumption, lots of studies were carried out to ascertain its safety, as of 2015, Razzagh et al. [11] investigated the adulteration of raw cow milk sampled from east Azerbaijan province of Iran, and found out that raw cow milk is adulterated with water, salt and carbonate. Masoud et al., [1] also carried out a study in Qazvin, Iran, on effect of seasonal variations on microbial and chemical quality of raw milk samples. Nowadays, animals and humans are at a risk of the consequences of heavy metals contamination, because milk from animals is polluted by different sources of contamination, such as environmental and apicultural sources [12]. Environmental contaminants include pesticides, heavy metals, aflatoxins, bacteria, and radioactivity, all of which are introduced into the plants through absorption from the roots [12, 13]. Following the ingestion of these contaminated plants by animals, they bioaccumulate and then excreted through the mammary gland. This vertical transfer of the contaminants constitutes a great risk factor for dairy products and above all, the health of the consumer which includes infants and the elderly. The beneficial role, as well as nutritional adverse effects of heavy metals, in human life has been reported by Razzagh et al. [11]. Cadmium, lead and mercury, were reported to be the major contaminants of food supply. These metals are considered to be the most important environmental contaminants. However, some metal such as Fe, Zn and Cu play significant roles in human body metabolism. Since heavy metals are most often non-biodegradable with a long biological half-lives, their bioaccumulation in body organs can cause detrimental side effects. There exist a definite pattern of high correlation between the abundance of nutrient and the mineral composition in plant, and its subsequent bio-accumulation in animal tissues, as well as in the different tissues of human. The richness of the soil in its content of essential elements and the plant's ability to effectively absorb such elements determines to a great extent, content in plant tissue [11, 14].

Contamination and subsequent poisoning by the heavy metals, can affect the functions of the central nervous system leading to mental disorder, may damage kidneys, the lungs, the liver and other vital organs in the body, thereby promoting several disease conditions. Long term exposure and bio-accumulation of heavy metals in animal’s body may result in prompting the progression of physical, muscular and neurological degenerative processes that mimic certain diseases such as Alzheimer’s disease and Parkinsonism [15].The series of health concerns caused by these metals promoted the worldwide regulation of these metals in foods and other consumables as a mitigation strategy. There is paucity of information and reliable data concerning the level of heavy metals level in raw cow milk in Kano State, despite the fact that, these products are typical food stuff that are largely consumed in almost all the local governments in the state. Therefore, The present study therefore, aims to evaluates and assess the human health risk factors associated with contamination by heavy metals, of cow milks consumed in the local governments of Bichi, Rano and Nassarawa of Kano State, Nigeria.

MATERIALS AND METHODS

Background of study areas

Kano state is located in the northwestern part of Nigeria, it is in the sahelian region, south of the Sahara, and it is the second largest city in Nigeria after Lagos and the most populous state in the country. It has a population of about 9,383,682 people (census, 2006). Its bounded by the latitude 12^o 00^1 0.43^{II} N and longitude 8^o 31^I 0.19^{II} E. Nassarawa is one of the local governments of Kano, situated centrally in Kano and has an area of 34 Km^2 and it is densely populated and consist of various industries and roadside occupational sites such as artisans workshops.

Sample collection

A total of 81 samples of cow milk and samples of feed they consume were collected from the three senatorial districts of Kano State of which Bichi, Rano and Nassarawa forms the headquarters. All the samples were analysed for heavy metals, in each of the study site, samples were collected from “Ruga” (A typical Fulani (Cowboy’s) settlement sites). From each “Ruga” nine (9) samples were collected from three different locations. Making 27 samples from each local government. Alongside the milk samples collected, were also the feed samples that the animals fend on, in each “Ruga”.

Sample analysis

Sample preparation and digestion

Digestion of milk samples

Digestion of sample was done as per the method described by Anastasio et al. [16]. the entire milk sample was poured in a clean and dry conical flask and dried at 70°C on a hot plate. To the dried sample (3gm), 30cm^3 of HNO_3 (65%) and 6 cm^3 H_2O_2 (30%) were added. The conical flask containing acid mixture and the milk sample was kept on a hot plate and digestioin was continued until the content of the flask turned colourless. The flask was then removed from the hot plate and kept at room temperature (25^{o}C). After cooling, 20cm^3 of distilled water was added into each flask and the contents were then filtered through Whatman filter paper no. 42 into a 100 cm^3 volumetric flask. The conical flasks were rinsed and washed 3 times with distilled water and filtered into the respective volumetric flasks until their full volume was attainted (100 cm^3). The concentration of heavy metals (Cu, Fe, Cr, Zn, Mn, Pb and Cd) in digested milk samples were estimated using Atomic Absorption Spectrophotometer (AAS).

Digestion of cow feeds

Feed samples were first digested using wet digestion method, as per the method described by Sobia et al. [17]. 0.2gms of sample were mixed with 4cm^3 of HNO_3 in a 100cm^3 volumetric flask and solution was allowed to stand for few hours, the mixture was then carefully heated over water bath till red fumes coming from the flask completely ceased. The flask together with the mixture was allowed to cool at room temperature and 4 cm^3 of perchloric acid was added, after which the flask was reheated again over water bath to evaporate till a small portion which was than filtered through whattman filter paper NO. 42 and made up to 100 cm^3 volume using distilled water.

Health risk assessment (CDI, HRI)

This was calculated based on the methods of Guo et al. [18]

$$DIM = (C\ metal \times C\ factor \times D\ food\ intake) / (B\ average\ weight) \qquad (1)$$

Where: Cmetal = concentration of metal in samples

C factor = conversion Factor

D food intake = Average milk intake daily

Baverage weight = Average body weight

The values used are Cfactor (0.085) [19], Average daily intake of milk (0.14 kg for each person per day) [20]. Average body weight (15.0 kg for children and 70.0 kg for adult), respectively [21, 22].

The health risk index (HRI) for consumers of cow's milk for the population under study was assessed in accordance with equation 2 [3, 18 and 19]

$$HRI = DIM/RfD \qquad (2)$$

Where: DIM and RfD are daily intake of metal and oral reference dose of metal, respectively. The RfD for Cd, Cu, Zn and Pb were 1.0, 40.0, 330.0 and 3.50μg kg^{-1} day^{-1}, respectively. If HRI < 1, it is assumed that, the exposed populations are safe [18, 23].

The total HRI (THRI) of heavy metals for the consumption of the milk was calculated according to equation 3 [24]:

$$\text{THRI} = \text{HRI (toxicant 1)} + \text{HRI (toxicant 3)} + \ldots + \text{HRI (toxicant } n\text{)} \qquad (3)$$

Toxicants as used in equation are Pd, Cd, Cr, Mn, Zn, Fe and Cu

Statistical analysis

Statistical analysis were performed using SPSS statistical package (version 20; SPSS, Chicago, IL). The one-way analysis of variance (ANOVA) was used to verify significant differences in samples from all locations.

RESULTS AND DISCUSSION

Concentrations of heavy metals from Bichi

The mean concentrations (mgL^{-1}) of heavy metals from the sampling sites (Ruga) in Bichi local government were presented. All samples collected were found to be contaminated with some heavy metals while some were totally below the detection limit. The differences observed in concentrations of heavy metals were not statistically significant ($P \leq 0.05$). Zn was only detected in 50% of samples in this location, with a maximum of concentration of 0.665 mg L^{-1} at BR1 and a minimum of 0.285 mg L^{-1} at BR3, this concentration is below the permissible limit and tends not to pose a risk to the consumers, Mn was detected in feed samples, with a negligible concentration not even enough to facilitate or take part in reactions. Cu, Pb and Cd were totally not detected in the samples, this might be as a result of the "Ruga" being far away from the city and the area has no industrial activities that might cause the contamination of water and soil and subsequently the animal feed, which can then be found in the milk of the grazing animals in the site. Cr was detected in 100% of the samples with a minimum concentration of 0.003mg L^{-1} and a maximum concentration of 0.012 mg L^{-1}, these concentrations detected are below the permissible limits set for Cr. Fe contributed to the highest contamination in the site, it was also detected in all the samples in the range of 5.400 - 2.163 mg L^{-1} (Table 1).

Table 1: Heavy metal concentrations (mg L^{-1}) of the milk samples from Bichi LGA.

Samples	Concentration (ppm)						
	Zn	Mn	Cu	Pb	Cd	Cr	Fe
BR1	0.665±0.262	ND	ND	ND	ND	0.012±0.004	3.990±1.342
BR2	ND	ND	ND	ND	ND	0.003±0.001	4.867±1.359
BR3	0.285±0.403	ND	ND	ND	ND	0.004±0.001	5.400±1.198
BRfeed	ND	0.06±0.00	ND	ND	ND	0.008±0.001	2.163±1.430

BR1, BR2, and BR3: Bichi "ruga" point 1, 2, 3 BRfeed: Bichi ruga animal feed

Table 1 results show that all the metals determined had concentrations below 1 mg L^{-1} but Fe had elevated concentration even up to 5 mg L^{-1}, though Fe is required by the body in some concentrations to mediate metabolic reaction, it also tends to be harmful in concentrations greater than permissible limits, and these concentrations detected greatly exceeds or violates the limit set for Fe and might lead to damaged cells in the liver, heart and other organs which can cause significant adverse effects, such as shock, metabolic acidosis, coagulopathy, liver failure, adult respiratory distress syndrome, coma, long term organ damage, and even death [25].These contamination might be from different sources which includes the feeds consumed by the animals, the water they drink and bioaccumulation through other routes.

Concentrations of heavy metals from Rano

Table 2 presents the mean concentration of heavy metals in the raw milk sample and feed from Rano local government area. Significant difference was observed in RR1 and RR2, RR1 and RR3, RR1 and RRfeed in Cr and Zn concentrations in all samples.

Table 2. Heavy metal concentrations (mg L^{-1}) of the milk samples from Rano LGA.

Samples	Concentration (ppm)						
	Zn	Mn	Cu	Pb	Cd	Cr	Fe
BR1	0.115±0.162	ND	ND	0.610±0.00	ND	0.004±0.014 ^{a,b,c}	9.100±3.111 ^{a,b,c}
BR2	ND	ND	ND	0.690±0.00	ND	0.016±0.002^{a}	3.780±0.645^{a}
BR3	ND	ND	ND	ND	ND	0.015±0.005^{b}	3.640±1.063^{b}
BRfeed	ND	1.14±0.00	ND	ND	ND	0.003±0.001^{c}	0.830±0.142^{c}

Superscripts letters (a,b,c) indicate significant difference along the column
RR1, RR2, and RR3: Rano "ruga" point 1, 2, 3 RRfeed: Rano ruga animal feed

Results from Table 2 is the mean concentration of metals from Rano, the "Ruga" from which these milk samples were taken is far away from the city and had rocks within it, with wide bush and grasses. Zn was detected only at a single location, presenting 25% of contamination, this concentration is far below the concentration needed by the body for metabolic reaction and also very low as compared to the level detected in Bichi local government. Mn was not detected in the milk samples but a low concentration was detected in the feed, which signifies that the concentration wasn't even enough to be carried over in the milk. Cu and Cd were below the detection limits in all the samples. While these two metals were not detected just like in Bichi local government, Pb was an exception, a concentration ranging from 0.610 – 0.690 µg L^{-1} was detected, this contamination might possibly be either from their drinking water or inhaled by the animals from the surrounding, because lead could be found in variety of sources such as exhaust from the leaded gasoline, lead painting which might decompose in water, recycling of lead battery and many other ways. The concentration of lead detected was above the permissible limit and might cause toxicity which will cause renal impairment or neurobehavioural decrements [26]. Cr was detected in all samples with a minimum concentration of 0.003mg L^{-1} detected in the feeds and a maximum concentration of 0.016mg L^{-1} detected in location two, these concentrations are minute and negligible as detected in Bichi local government and would not pose a risk. Fe has the highest concentration detected just as it was detected in Bichi, ranging from 0.830 – 9.10 mg L^{-1}, these concentrations have exceedingly violate the permissible limit and might pose a health risk to consumers.

Concentrations of heavy metals from Nassarawa local govt. area

The mean concentrations of heavy metals from Nassarawa local government was recorded in Table 3, Significant difference were recorded between NR3, NR2 and NR-feed in Cr concentrations, NRfeed,NR3 and NR2 also had significant difference in Zn concentrations in samples analysed.

Table 3. Heavy metal concentrations (mg L^{-1}) of the milk samples from Nassarawa LGA

Samples	Concentrations(ppm)						
	Zn	Mn	Cu	Pb	Cd	Cr	Fe
NR1	ND	ND	ND	ND	ND	0.002±0.000	3.87±0.440
NR2	ND	ND	ND	ND	ND	0.002±0.000 ^{b}	3.64±0.501 ^{a}
NR3	ND	ND	ND	ND	ND	0.020±0.004 ^{a,b,}	4.986±0.997 ^{b}
NRfeed	ND	ND	ND	ND	ND	0.006±0.000 ^{c}	1.620±0.740 ^{a,b}

NR1, NR2, and NR3: Nassarawa "ruga" point 1, 2, 3 NRfeed: Nassarawa ruga animal feed
Superscripts letters (a,b,c) indicate significant difference along the column

Table 3 is the results of heavy metal contaminations from Nassarawa local government. This local government has lower contamination as compared to the other two local governments, only two of the seven heavy metals were detected in all the samples. Zn, Mn, Cu, Pb and Cd were below detectable limits. Cr had a concentration ranging from 0.002 – 0.02 mg L^{-1}, these concentrations were below the concentrations detected from the other two local governments, and these concentrations are also below permissible limit and will have no effect on health. Fe was determined in this local government with values ranging from 1.620 – 4.986 mg L^{-1}; these concentrations are also below those determined from the other two local governments.

Figure 1 shows the distribution of heavy metals in the three local governments. The concentrations of Mn, Pb, Fe, Cr and Zn were compared in the three local government, Three was no significant difference (P ≤ 0.05) in the levels of the heavy metals in the three local governments studied Fe recorded the highest concentration in all samples, and Cr with lowest concentration even though it was present in all analysed samples.

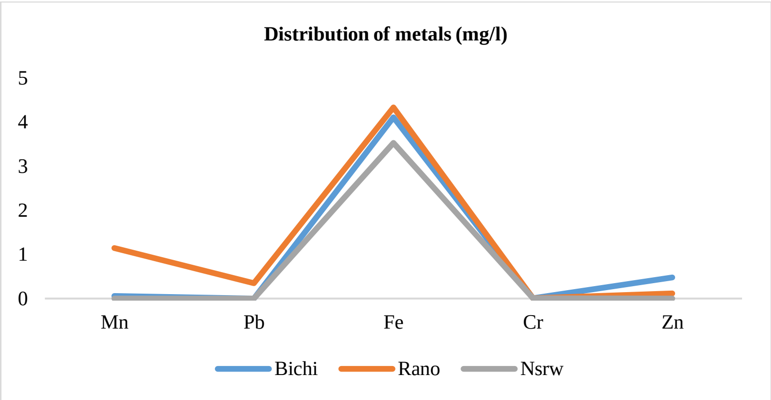

Figure 1. Distribution of metals in the three local governments

Figure 1 compares the average concentrations of metals in the three locations, with Fe having the highest contributing concentration, within the range of 3.529 – 4.33 mg L^{-1}, this concentrations between the three locations were not statistically significant (at P= 0.05), this concentration is slightly lower than the average (5.987412 mg L^{-1}) observed by Ogabiela et al. in [27] in raw milk samples from Challawa, Kano State, and a little bit higher than the average(3.661 µg kg^{-1}) detected by Abdallah [28],and was in agreement with the average detected by Garba et al., [29], at "Kasuwan Shanu" in Maiduguri but very much less than what he detected from University of Maiduguri farm. It has been reported that, Fe can present a problem in dairy products processing due to its catalytic effect on lipids oxidation with development of unpleasant smell,by bounding preferably to proteins and membrane lipoproteins of milk fatty globule [30]. Mn was within the range of 0.06 –

1.14 mg L^{-1}, this concentration was very much low as compared to the work done by Muhib et al., [31], but very much close to the range(0.170 to 1.965) detected by Garba et al., [29] at Auno village of Maiduguri, and also in agreement to the work by Ogabiela et al. [27]. The concentrations presented in this study appears to be higher than what was reported in cow milks from Borena Zone of Ethiopia [32]. However, the element could not be detected in cow's milk samples from Dodoma Urban District, Tanzania [33], just like how it wasn't observed or detected in Nassarawa local government in the present study. Manganese as an element occurrs naturally in soil, rock, water, and food. Manganese plays an essential role in bone mineralization, metabolic regulation, protein and energy metabolism, formation of glycosaminoglycans and cellular protection from damaging free radical species In humans and other animlas [34]. Even though the element is essentially required by both plants and animals, exposure to high levels of the elements via inhalation and ingestion has been suggested to cause some adverse effects especially neurological effects that occurs on a continuum of dose relation [35]. Pb was only detected in Rano local government area with 50% abundance with an average of 0.65mgL^{-1}, this was also the case in the study of Razzagh et al., [36] where he also detected Pb in honey samples from north western region of Iran with average concentration of 0.2 mg L^{-1}. Cr was detected in all locations but having a very low concentration even less than the average concentration detected from Challawa and also the average detected at Zaria by Ogabiella et al. [27] even though the present study has concentrations within the permissible limits. The concentration of Cr detected was very low, ranging from 0.002 – 0.016mg L^{-1}, but difference in Cr concentrations were statistically significant (P= 0.017) between Rano local government area and Nassarawa local government area and also very significant (p= 0.004) between Rano and Bichi local government area, these concentration will cause no harm to the consumers.

Daily intake of metals and health risk index of metals from Bichi, Rano and Nassarawa

Daily intake of metals was calculated for all local governments and presented in Table 4, Results recorded from bichi showed a minimum daily intake of Cr at 0.00051 and a maximum of 0.00204µg l^{-1}, these were slightly lower than DIM of Cr determined from Rano, which were in the range of (0.00068 – 0.0068) but higher than that of Nassarawa which was in the range of (0.00034 – 0.0034), Fe has the highest daily intake irrespective of location, because all the locations had a very high concentration, even though Rano has the highest range (0.619 – 1.55), and Bichi has the lowest range (0.367 – 0.827),the DIM of Zn is very low and was only for the two locations where it was detected. Lead having the lowest occurrence has an approximate DIM of 0.1 but with the highest health risk index.

Table 4. Daily intake of metals and health risk index of metals from Bichi, Rano and Nsrw (µg/l.b.w/ day).

Locations	Metals	DIM	HRI
BR1		2.04×10^{-3}	1.94×10^{-3}
BR2		5.1×10^{-4}	3.4×10^{-4}
BR3		6.8×10^{-4}	4.53×10^{-4}
RR1		6.8×10^{-4}	4.5×10^{-4}
RR2	Cr	5.1×10^{-3}	1.81×10^{-3}
RR3		6.8×10^{-3}	1.70×10^{-6}
NR1		3.40×10^{-4}	2.20×10^{-4}
NR2		3.40×10^{-4}	2.20×10^{-4}
NR3		3.40×10^{-3}	2.20×10^{-3}
BR1		0.678	0.96
BR2		0.827	1.18
BR3	Fe	0.367	1.30
RR1		1.55	2.20
RR2		0.643	0.92

RR3		0.619	0.88
NR1		0.658	0.94
NR2		0.619	0.88
NR3		0.848	1.20
BR1	Zn	0.113	0.437
BR3		0.048	0.162
RR2		0.020	0.065
RR1	Pb	0.104	26.0
RR2		0.117	29.3

DIM = Daily intake of metals, HRI = hazard risk index

Total hazard risk index

Figure 2 compares the total Hazard risk index of Zn, Fe and Cr, and the values obtain were in the sequence of Fe > Zn > Cr, all the values gotten were below one(1), except for Fe, therefore Fe toxicity is highly expected from these regions, but threat due to the other heavy metals in the milk of these areas is very low, but not withstanding, toxicity might still occur, due to the fact that milk is not the only source of heavy metal exposure, other sources include water, foods such as cereals and others and some other times, contamination might occur due to inhalation from the air.

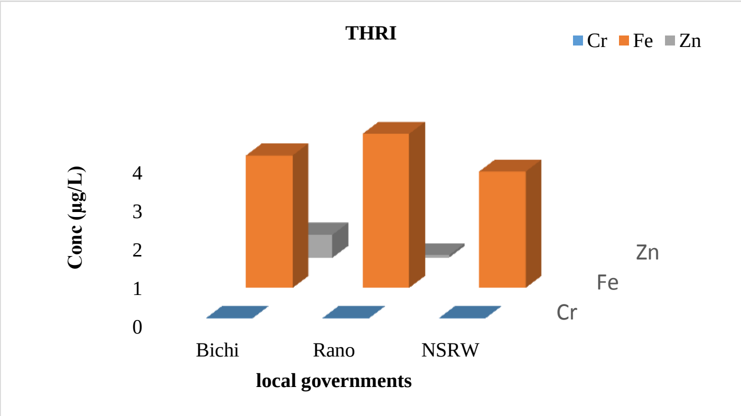

Figure 2. The total hazard risk index of the most prevalent HM (µg/L)

CONCLUSIONS

Heavy metals contamination of milk from the study areas was found to be low, except for Iron. The health risk assessment calculated were within normal range except for Iron, Therefore consumers might suffer Iron toxicity. Consumers are to take caution, and concerned agencies should take proper action in reducing or eliminating these risks.

ACKNOWLEDGEMENTS

The authors wish to acknowledged the support enjoyed from the authourities of the Bayero University, Kano, Nigeria; and Department of Biochemistry, Bayero University, Kano, in particular, for making available the laboratory, reagents and apparatus for the smooth conduct of the research work.

Conflict of interests

The authors declared that, there is no conflict of interest.

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