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Investigation of Levels of Some Selected Heavy Metals in Raw Bovine Milk from Oyam District, Uganda and Estimation of Potential Health Risks

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Abstract: Heavy metal contamination is a serious threat because of their toxicity, bio-magnification and bioaccumulation in food chain. The deficiency of some of these metal elements leads to impairment of vital biological process but when they are present in excess, they become toxic. Raw bovine milk is considered as one of the food sources contaminated with heavy metals, because cows graze on the grass grown in lands which somehow come in contact with the untreated effluent of industries. Milk is a very important component of human diet. The present study was aimed at investigating the levels of selected heavy metals (Cr, Cd, and Pb) in raw bovine milk produced in Oyam District, Uganda and estimation of potential health risks associated with long term exposure to heavy metal contaminated milk from the said area. Experimental results indicated that the mean concentration levels of the metals were 0.17±0.08, 6.84±2.03 and 0.13±0.05 ppm for Lead, Chromium and Cadmium, respectively for all samples taken from Aber Sub County. The mean concentration levels of the metals were 0.15±0.07, 6.55±1.71 and 0.12±0.04 ppm for Lead, Chromium and Cadmium, respectively, for samples taken from Acaba Sub County. The mean concentration levels of the metals were 0.16±0.07, 6.83±1.74 and 0.15±0.11 ppm for Lead, Chromium and Cadmium, respectively, for samples taken from Loro Sub County. The mean concentration levels of the metals were 0.28±0.18, 8.34±2.92 and 0.19±0.13 ppm for Lead, Chromium and Cadmium, respectively, for samples taken from Kamdini Sub County. The Target Hazard Quotient (THQ) of all heavy metals analyzed (Pb, Cr and Cd) in milk samples was found to be less than 1. Hence it would be inferred that it's safe to drink the milk from this area.

Keywords: Lead, Cadmium, Chromium, Bovine Milk, Heavy Metals, Oyam District, Health Risks

1. Introduction

Milk is a nutrient rich, liquid food produced by mammary glands of mammals [1]. As an agricultural product, it is extracted from farm animals during or soon after pregnancy. Milk and its products are very common in our food list due to their high nutrient value, since it is a source of vitamins and lot of mineral constituents which are necessary for proper development and functioning of different tissues and organs. However, chemical hazards and contaminants which are risk factors for dairy products can as well be contained in milk and dairy products [2, 3].

The nutritional components in milk are energy, water, carbohydrate, fat, protein, milk flavor, vitamins, minerals and minor biological proteins and enzymes. Cows are still considered most important among species in milk production with a contribution of 580.5 million kg (83.3%) of 696.6 million kg globally according to FAO 2010 [4]. Milk is also a good source of calcium, phosphorus, potassium, vitamin D, riboflavin, vitamin A, vitamin B-12 and niacin and a good source of protein [5]. Despite the essential benefits of consuming milk, the contamination of milk from moderate agricultural practices, industrial pollutants in the environment, animal feeds and use of sewage sludge in agriculture is increasing and therefore requires urgent attention because of the risk this contamination poses especially to the health of the consumers.

Contamination of milk globally with unwanted substance through animal feeds, heavy metals, mycotoxins, diotoxins and similar pollutants has gained great concern to public health due to their toxic effects on humans and animals [6]. Particular interest has been put on metals because of their ability to bioaccumulate [7]. Many reports have mirrored the presence of heavy metals in milk and other food products [8]. The interest in these elements is increasing due to the available reports of relationships between heavy metals status in food and drinking water and the prevalent oxidative diseases in living beings. Lead, Cadmium, Chromium, Nickel, Arsenic and Mercury are the most common toxic metals of concern according to reports [9].

It is very challenging to determine minute metal elements in milk due to their complex emulsion like matrices and low concentration levels of the metal ions. Many digestion procedures to oxidize organic matrices of samples have been reported in previous studies [10-12]. The most common pre-treatment method for determination of metal element in biological samples is the acid digestion and another well-established method is acid digestion induced by microwave energy [13, 14]. The determination of heavy metals can be performed by several instrumental techniques [15, 16] including indirect photometric chromatography, ion chromatography, flame atomic absorption spectrometry, potentiometric stripping, capillary zone electrophoresis, differential pulse anodic striping voltammetry, mid-infrared spectrometry, particle induced x-ray emission, complex metric titration, and atomic absorption spectrophotometer [17-23]. Atomic absorption spectrophotometer is the method adopted in this study. Target Hazard Quotient (THQ) which is a complex parameter employed for estimating potential health risks associated with long term exposure to heavy metals was employed in this study. The objective of this study was to investigate the levels of some selected heavy metals (Cr, Pb and Cd) in raw bovine milk from Oyam District, Uganda and estimate potential health risks associated with long term exposure to heavy metals contaminated milk from this area.

2. Materials and Methods

2.1. Study Area

Oyam is a district in Northern Uganda. Like most Ugandan districts, it is named after its ‘chief town’ Oyam, where the district headquarters are located. Oyam District was established by Ugandan Parliament in 2006. Prior to that, Oyam District was part of Apac District. Together with Lira District, Alebtong District, Amolatar District, Apac District, Dokolo District, Kole District, Otuke District, Oyam District is part of a larger Lango Sub-Region, a home to an estimated 1.5 million Langi. The district is predominantly rural district.

2.2. Apparatus

The following apparatus were used. Analytical balance, 100 ml round bottomed flasks, Borosilicate volumetric flasks (25, 50 ml, 100 ml & 1000 ml), Measuring cylinders, Pipettes, micropipettes, Muffle furnace, kjeldahy reflux condenser, Desiccators, porcelain crucible, Flame atomic absorption spectrophotometer, Filter paper whatmann № 1.

2.3. Chemicals

All chemicals of high purity analytical grade reagents were used; HNO_3 (69%), $HClO_4$ (70%), Pb $(NO_3)_2$ (99.5%), Cd $(NO_3)_2$ (99.99%), Cr $(NO_3)_2$ (99.99%), H_2SO_4 (98%), Deionized water.

2.4. Sample Collection

Raw milk samples were collected fresh from selected cows during morning milking taking 50 ml per sample. Samples were collected in sterile plastic bottles and kept in iced box at 4°C. The samples were then transported to laboratory and put in deep freezer and kept at - 20°C until time for laboratory analysis.

2.5. Sample Preparation

Exactly 20 ml of fresh milk samples were transferred in to 100ml round bottom flask and mixed with 8 ml of a mixture of HNO_3 (69%) and $HClO_4$ (70%) with a volume ratio of 5:3 (v/v) and digested at 210°C for 2 h and 30 minutes on a kjeldahl digestion apparatus fitted with reflex condenser. The digested samples were allowed to cool at room temperature. 10ml of distilled water was added in to the digested residue and filtered through whatmann filter paper No 1. The volume of the filtrate then made up to 100 ml using distilled water and the solution was further diluted 10 times before determinations of lead (Pb), chromium (Cr) and cadmium (Cd) using AAS.

2.6. Determination of Metal Content

Six standard solutions were prepared by serial dilution of each metal ion stock solution. Calibration curve for each metal ion to be analyzed was prepared by plotting absorbance as a function of metal ion standard concentration.

The concentration of Pb, Cr & Cd were determined using FAAS (Buck scientific model 210 GP) equipped with deuterium arc back ground corrector and standard air acetylene flame system after the parameters (burner and lamp alignment, slit width and wave length) adjustment. Concentration of the metal ions present in the sample were determined by reading their absorbance using FAAS and comparing it on the respective standard calibration curve. Three replicate determinations were carried out on each sample.

2.7. Estimation of Daily Intake

Estimated daily intake (EDI) of the selected heavy metals for people in Oyam were calculated using the equation below [28].

$$EDI = \frac{C \times W}{BW}$$

C (ml/kg) is the level of heavy metals in raw bovine milk, W is the average daily intake of raw milk by people in Oyam by weight (mg/person/day) and BW is average body weight of people (adult) in Oyam. The value obtained for each of the selected heavy metal was used for calculation of Target Hazard Quotient (THQ).

W (average daily intake of raw milk in Oyam by an adult person) was obtained by interviewing adults in Oyam on their daily amount of milk consumption. The average daily milk consumption for these adults were taken as the average daily milk consumption by adults in Oyam.

BW (average body weight of adult person in Oyam) was also determined by weighing the adults who participated in answering the research questionnaire. Each individual's weight was recorded and average body weight for participants computed, representing average body weight for adult people in Oyam.

2.8. Target Hazard Quotient (THQ)

This is a parameter used for estimating potential health risks associated with long term exposure to heavy metals. It is the ratio of EDI to RfD. It was calculated using the formula below [26, 29].

$$THQ = \frac{EDI}{RfD}$$

Where; THQ is Target Hazard Quotient, EDI is the estimated daily intake of each selected heavy metal and RfD (reference oral dose) developed for each heavy metal by US-EPA. According to US-EPA, RfD is an estimate with uncertainty of a daily oral exposure to human population that is likely to be without appreciable risk of deleterious effects during a lifetime. The value of THQ got for each selected heavy metal will be used for assessing health risk of those metals to the people of Oyam due to consumption of contaminated raw milk. THQ less than 1 is assumed to be safe for exposed population implying no risk for that population. THQ values equal to 1 and above indicate potential health risk [29].

3. Results and Discussion

The samples were analyzed using Atomic Absorption Spectrometer (AAS) for the determination of lead, chromium and cadmium at their corresponding wave length 283.3, 228.8 and 357.9 nm, respectively. The concentrations of these metals in raw bovine milk samples collected from different Sub Counties in Oyam District were obtained in mg/kg (ppm) as recorded in Table 1 below.

Table 1. *Levels heavy metals (mg/kg) in milk samples from different sub-counties.*

Metals	Aber	Acaba	Loro	Kamdini
Pb	0.17±0.08	0.15±0.07	0.16±0.07	0.28±0.18
Cr	6.84±2.03	6.55±1.71	6.83±1.74	8.34±2.92
Cd	0.13±0.05	0.12±0.04	0.15±0.11	0.19±0.13

Results for levels of heavy metals (Pb, Cr and Cd) in milk were presented in Table 1. All the three metals were detectable in all samples and their concentrations were in the increasing order of Cr > Pb > Cd. The mean concentration levels of the metals were 0.17±0.08, 6.84±2.03 and 0.13±0.05 ppm for Lead, Chromium and Cadmium, respectively, for samples taken from Aber Sub County. The mean concentration levels of the metals were 0.15±0.07, 6.55±1.71 and 0.12±0.04 ppm for Lead, Chromium and Cadmium, respectively, for samples taken from Acaba Sub County. The mean concentration levels of the metals were 0.16±0.07, 6.83±1.74 and 0.15±0.11 ppm for Lead, Chromium and Cadmium, respectively, for samples taken from Loro Sub County. The mean concentration levels of the metals were 0.28±0.18, 8.34±2.92 and 0.19±0.13 ppm for Lead, Chromium and Cadmium, respectively, for samples taken from Kamdini Sub- County. Lead, Chromium and Cadmium levels showed significant difference (p < 0.05) among samples from different Sub Counties. Kamdini Sub-County reported the highest level for all the metals tested while Acaba Sub-County reported the least level for all the metals tested.

The mean concentration of each heavy metal (Pb, Cr and Cd) was determined for all samples. The average body weight of adult person in Oyam District was found to be 63 kg. The average daily milk consumption per adult person was considered to be 200 ml. Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) of metals were calculated basing on this information and the result is shown in Table 2.

Table 2. *Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) of metals.*

Metals	Mean levels	EDI	RFD0	THQ
Pb	0.19	0.0006	4.0E-03	0.1508
Cr	7.14	0.0227	1.5	0.0151
Cd	0.1475	0.0005	1.0E-03	0.4683

Using the reference oral dose (RFDo) for each metal established by the Food and Nutrition Board of the Institute of Medicine, the THQ of all metals (Pb, Cd and Cr) is less than 1.

4. Discussions

Lead contamination of the environment is primarily due to anthropogenic activities, making it the most ubiquitous toxic metal. Lead readily accumulates in the humus rich surface layer of the soils due to its complexity with organic matter and it was reported to be the least mobile heavy metal in soil under reducing and non-reducing conditions [30]. The Target Hazard Quotient (THQ) of lead in milk samples was found to be less than 1. Hence it could be inferred that it is safe in terms of lead food poisoning. The presence of lead in raw bovine milk samples could be attributed to the exposure of lactating cows to contaminated fodder, climatic factors such as wind, use of Agro-chemicals and contaminated water. Furthermore, the lactating cows graze along the road sides, and lead which is a fuel additive could be emitted from the car exhaust to contaminate the grazing areas along the roadside. High level of lead is particularly of great concern especially due to the fact that milk and dairy products are consumed mostly by infants and children who are uniquely susceptible to the effect of lead.

Chromium is an essential nutrient for plant and animal metabolism, however, the increasing accumulation of chromium in the environment from industrial outputs has caused great concern. Chromium exists in III (+) and VI (+) oxidation states as all other oxidation states are not stable in aqueous solutions. Both valences of chromium are potentially harmful although the latter is more toxic [36].

The difference was statistically significant at $p > 0.05$. The Target Hazard Quotient (THQ) of chromium in milk samples was found to be less than 1. Hence it could be inferred that it's safe in terms of chromium poisoning for human being to drink the milk produced in this area. Chromium is an important mineral the body must have to function properly. The body stores chromium in the blood and in the hair. It's responsible for stimulating the activities of insulin in the body and also help controls blood cholesterol levels. Low level exposure of chromium can cause skin irritation, and ulceration. Long term exposure can cause kidney and liver damage as well circulatory and nerve tissue problems.

Of all the toxic metals resulted in large quantities in to the environment cadmium is generally regarded as the most likely to accumulate in the human food chain. Cadmium is a heavy metal of considerable toxicity with destructive impact on most organ systems [37]. The Target Hazard Quotient (THQ) of Cadmium in milk samples was found to be less than 1. Hence it could be inferred that it is safe in terms of Cadmium food poisoning.

The presence of Pb, Cr and Cd in the raw bovine milk samples are certainly due to addition of some fertilizers and herbicides on the farm and farm sides. Although the study does not aim to point out the source of contamination, there are probable sources. Heavy metals accumulate in the water supply through human activities, such as industrial and consumer waste, commercial processes like mining, agriculture, manufacturing and the discarding of wastes in landfills are common sources of heavy metal contamination [38, 39]. Even rain water, with its acidic pH, can cause these compounds to leach into the surface and underground water supplies from the surrounding soil and rock. Contamination of aquatic and terrestrial bionetworks with toxic heavy metals is an environmental issue of public health concern. Being persistent pollutants, heavy metals accumulate in the environment and consequently contaminate the food chains. Accumulation of potentially toxic heavy metals in biota causes a potential health threat to their consumers including human beings [38]. The primary threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic [40].

5. Conclusion

In this study the levels of Pb, Cr, and Cd in raw bovine milk produced within Oyam District were determined by Atomic Absorption Spectroscopy. The objective of the study was to determine the levels of selected heavy metals (Pb, Cr and Cd) in raw bovine milk produced in Oyam district. All the three metals were detectable in all samples and their concentrations were in the order of Cr > Pb > Cd. Finally, human health risk associated with drinking the milk was identified using Target Health Quotient (THQ) measure and found that Lead, Cadmium and Chromium inferred that it is safe in terms of poisoning for consumption by humans. With regards to presence of heavy metals in raw bovine milk, the necessity of vigorous regular national monitoring of milk contamination as well as quality of safe animal feed as a main source of contamination should be emphasized**.**

Conflicts of Interest

None of the authors have any conflict of interest associate with this study.

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