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Determination of Concentrations of Selected Heavy metals in Cow's Milk: Dodoma Urban District, Tanzania

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ABSTRACT
Fresh cow's milk samples were collected from five villages Chihanga, Msalato, Mtumba, Ntyuka and Chigongwe of Dodoma district, Tanzania, and preserved in a deep freezer (-20^oC). The milk samples were digested by hot plate using 65% HNO_3 and 30% H_2O_2.The concentrations of Mn, Co, Ni, Cu and Zn were determined by Flame Atomic Absorption Spectrometer. The elements Mn and Ni were not detected in all the milk samples under the study. The concentrations of Co, Cu and Zn were 0.079–0.147, 0.203–0.251 and 10.132–18.419 ppm, respectively in the milk samples of five villages under study. There is significant difference in the mean concentrations of Co and Zn between the milk samples of five villages at P = 0.05. But there is no significant difference in the concentration of Cu among the milk samples of five villages at P = 0.05.The results obtained for detected elements in the present study were also compared with international daily intake guidelines of different international organizations for food and were found below the levels allowed for Cu and Zn, whereas it is more for Co.
***Keywords**:* Heavy metals, Fresh cow's milk, Hot plate digestion, Flame Atomic Absorption Spectrometer.

I. INTRODUCTION

Among foods, milk represents an important intake in a typical diet due to its high nutrient and mineral content. Milk is an excellent source of calcium, vitamin D, riboflavin and phosphorus and a good source of protein, potassium, vitamin A, vitamin B-12 and niacin [1, 2]. Increase in industrial and agricultural activities has resulted in increased concentration of metals in the environment. These metals are taken in by plants through absorption and consequent accumulation in their tissues. Animals that graze on such contaminated plants accumulate such metals in their tissues and milk if lactating [3]. Therefore, milk contaminated with heavy metals such as zinc, lead, cadmium, selenium, sulphur, iodine and possibly even more dangerous arsenic and cyanide [4]. Due to the growing environmental pollution it is necessary to determine and monitor the levels of heavy metals in milk, because they can significantly influence human health [5, 6, 7]. One of the main problems with metals is their ability towards bioaccumulation. Metal residues in milk are of particular concern because milk is largely consumed by infants and children [8].

Heavy metals are persistent contaminants in the environment that can cause serious environmental and health hazards. They are released into the environment from natural as well as anthropogenic activities [9, 10]. Some heavy metals like Mn, Co, Ni, Cu and Zn are essential to maintain proper metabolic activity in living organisms; others like Pb and Cd are non-essential and have no biological role [11, 12, 13]. However, at high concentrations, even essential metals also cause toxicity to living organisms [14].Toxicity of metal is closely related to age, sex, route of exposure, daily intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate and mechanisms/efficiency of excretion [15].

The aim of the present study is to determine the concentrations of heavy metals, namely Mn, Co, Ni, Cu and Zn in fresh cow's milk collected from five villages Chihanga, Msalato, Mtumba, Ntyuka and Chigongwe of Dodoma urban district, Tanzania. The obtained mean elemental concentrations were compared with the corresponding values of different countries in the literature and the daily intake of these elements were also compared with the Recommended Dietary Allowance (RDA) values set by different international organizations.

II. MATERIALS AND METHODS

2.1 Description of Study Area

The study was conducted in Dodoma Urban district, one of the seven districts of the Dodoma Region of Tanzania. It is bordered to the west by Bahi district and to the east by Chamwino district. Dodoma urban district location with in Dodoma region is represented in the Fig. 1. Its coordinates are 6° 10′ 32″ S, 35° 44′ 19″ E. A paved trunk road from Morogoro to Singida and paved trunk road from Iringa to Babati pass through this district [16]. The central railway of Tanzania passes through Dodoma Urban district as well and there is a train station in

Dodoma which is a capital city of Tanzania [17]. Dodoma Airport is located within Dodoma Urban district as well. It is having a population of 410,956.

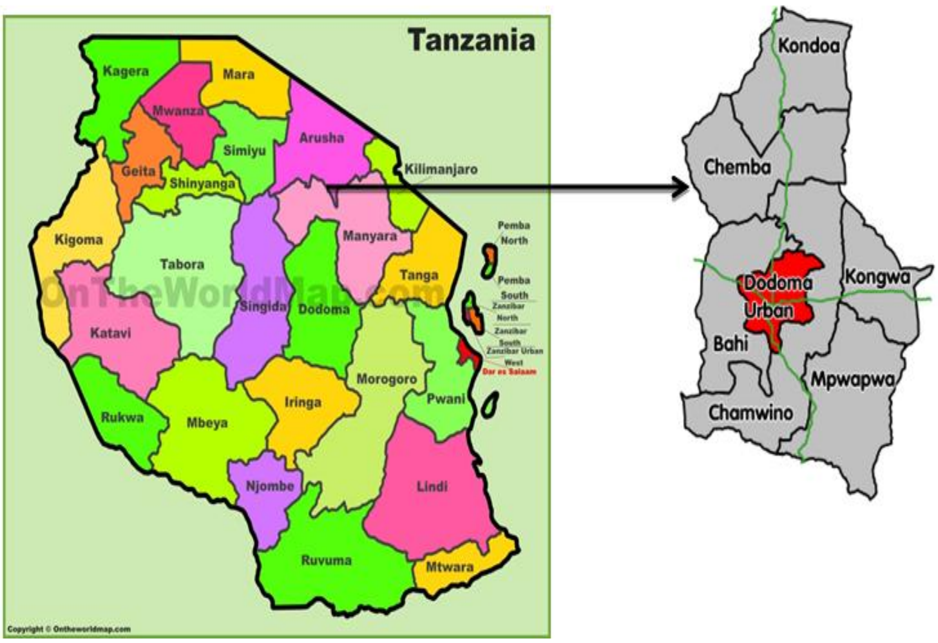

Figure 1: Maps of Tanzania (left) and Dodoma region (right), study area indicated with red colour in the map of Dodoma Region.

The study area was selected based on the dependence of farmers on livestock production mainly from cattle and goat. Fresh cow's milk samples were collected from five villages Chihanga, Msalato, Mtumba, Ntuka and Chigongwe of Dodoma urban district, Tanzania.

2.2 Chemicals and Reagents

Analytical-reagent grade chemicals and deionized water were used to prepare all the solutions. Nitric acid (65%) (Loba Chemie, India) and hydrogen peroxide (30%) (Techno Pharm chem, India) were used for cleaning glassware and digesting milk samples throughout this work. Stock standard solutions of Mn, Co, Ni, Cu and Zn having concentration 1000 mg/L in 0.5 M HNO_3 were purchased from Romil Limited, Cambridge (Great Britain). Intermediate standard solutions of concentration 10 mg/L were prepared by sequential dilution of stock standards. Working standards were prepared from intermediate standards of each metal.

2.3 Apparatus

All glassware were washed before use with deionised water, soaked in nitric acid (30%), then rinsed in deionised water and air dried. The glassware kept in clean place, to avoid contamination. Hot plate with temperature adjustment is used for the digestion of milk samples. 280 FS AA Flame Atomic Absorption Spectrometer (Agilent, USA) (FAAS) equipped with deuterium background corrector and hollow cathode lamps of Mn, Co, Ni, Cu and Zn with air-acetylene flame was used for the determination of their respective metals in milk samples. Before analysis of the sample, the instrument was optimized to give maximum signal strength by adjusting the parameters such as wavelength, slit width, lamp current, air flow and acetylene flow for each element. These optimized conditions of the instrument are given in Table 1.

Table 1: Operating conditions (FAAS) for the determination of heavy metals in milk samples.

Sl. No.	Metal	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Measurement time (s)	Air flow (L/min.)	Acetylene Flow (L/min.)	Burner height (mm)
1	Mn	279.5	0.2	5.0	5.0	13.5	2.0	13.5
2	Co	240.7	0.2	7.0	5.0	13.5	2.0	13.5
3	Ni	232.0	0.2	4.0	5.0	13.5	2.0	13.5
4	Cu	324.8	0.5	4.0	5.0	13.5	2.0	13.5
5	Zn	213.9	1.0	5.0	5.0	13.5	2.0	13.5

2.4 Sample Collection

The polyethylene sampling bottles were soaked in 20% HNO_3 for 24 hours and rinsed with deionised water before collection of raw milk in order to avoid possible contamination. The udder of each cow was washed with distilled water before milking. Ten cows from different farmers were randomly selected from each of five villages Chihanga, Msalato, Mtumba, Ntyuka and Chigongwe and one composite sample representative of each village was prepared. A milk sample of 100 ml was collected during morning milking time from each cow and homogenized, and were kept in an ice box. The samples were transported to laboratory and immediately kept in a deep freeze (-20^{0}C) until hot plate digestion was carried out [18].

2.5 Sample Digestion and Preparation of Analyte Solution for FAAS

The milk sample needs to be brought into clear solution for analysis by atomic absorption spectroscopy. For this reason the sample was digested to dissolve the milk and to remove the organic components of the milk. 5 g of raw cow's milk is treated with 5 ml (65% nitric acid) and 2 ml (30% hydrogen peroxide) and then digested on electric hot plate at 90ºC and the temperature of this mixture was gradually increased to 120ºC until brown fumes appeared, indicating completion of oxidation of organic matter [19]. The organic matrix of milk was destroyed and leaving behind the elements in clear solution. After cooling, the clear solution was filtered in to 25 mL volumetric flask and diluted up to the mark with double distillated water. A blank digestion solution was made for comparison. Finally the milk samples were directly analyzed for heavy metals by FAAS.

2.6 Calibration Curve and Measurement of Metal Concentrations

The calibration curves were drawn for Mn, Co, Ni, Cu and Zn using linear regression analysis of the concentrations of the standard solutions versus absorbance values. Five series of working standard solutions (Table 2) of metals were prepared by diluting the intermediate standard solution (10 mg/L) with deionized water. A blank and standards were run in FAAS and five points of calibration curve were established. Each standard solution was measured three times and the mean was plotted. As an example, calibration graph for Zn is presented in the Fig. 2. The correlation coefficients of calibration curves are given in Table 2. The correlation coefficient of more than 0.99 showed that there is strong linear relationship between concentrations and absorbance. Each of the sample solutions were aspirated into the flame of the AAS instrument and the absorbance values of the metals was recorded. Concentrations of each metal were determined by interpolation from the calibration curves. Triplicate determinations were carried out on each sample.

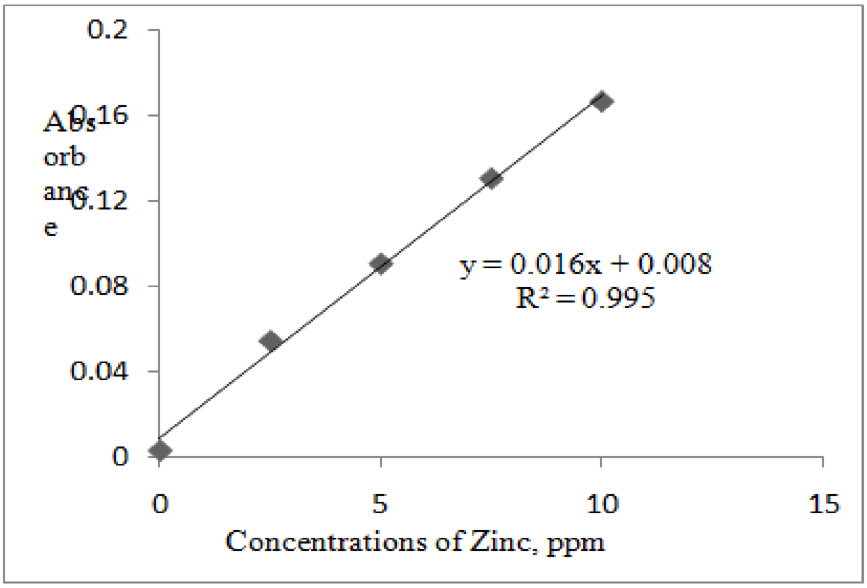

Figure 2: Calibration curve for Zinc.

Table 2: Series of working standards and correlation coefficients of the calibration curves for the determination of metals in milk using FAAS.

Sl. No.	Metal	Concentrations of standards (ppm)	Correlation coefficient, R^2
1	Mn	2.5, 5.0, 7.5, 10.0	0.9998

2	Co	2.5, 5.0, 7.5, 10.0	0.9996
3	Ni	2.5, 5.0, 7.5, 10.0	0.9975
4	Cu	2.5, 5.0, 7.5, 10.0	0.9926
5	Zn	2.5, 5.0, 7.5, 10.0	0.9955

2.7 Method Validation

The recovery of metals was studied by spiking known amount of standard solution to samples. For method validation a milk sample from Msalato village was selected and known amount (5.00 ppm) of standard solution was added to the sample and digested with the same procedure of hot plate digestion for milk samples. After diluting the spiked samples to the required volume with deionized water, they were analyzed with the same procedure followed for the analysis of milk samples. Triplicate samples were prepared and triplicate readings were obtained. The recoveries of the spiked samples were calculated using the following formula.

$$\text{Percentage recovery} = \frac{\text{Concentration in spiked sample} - \text{Concentration in sample}}{\text{Amount spiked}} \text{X } 100$$

2.8 Data Analysis

The data was analysed by t-test using Origin 6.1 software to examine the statistical significance of differences in the mean concentration of the mineral composition of the different milk samples studied.

III. RESULTS AND DISCUSSION

3.1 Evaluation of Analytical Figures of Merit

In this study the precision of the results was evaluated by the standard deviation of the triplicate samples (n = 3), analyzed under the same conditions. Most of the results in both the spiked (Table 3) and real sample (Table 4) showed very less standard deviation indicating high precision. The accuracy and validity of procedure used for digestion was evaluated by analyzing the digests of spiked sample. The recoveries of the detected metals in the spiked milk samples were 95.50%, 94.74% and 100.74% for Co, Cu and Zn, respectively (Table 3). These values show that the method is very accurate and valid for the determination of heavy metals under study.

Table 3: Analytical results obtained for the validation of the optimized procedure.

Sl. No	Metal	Amount spiked, (ppm)	Concentration in sample (ppm), n = 3	Concentration in spiked sample (ppm), n = 3	Recovery (%)
1	Co	5.000	0.147 (0.007)	4.922 (0.023)	95.50
2	Cu	5.000	0.242(0.009)	4.979 (0.054)	94.74
3	Zn	5.000	10.697 (0.125)	15.735 (0.990)	100.74

3.2 Mean Concentration of Heavy Metals in Cow's Milk

The average heavy metal concentrations in the fresh cow's milk from the five villages Chihanga, Msalato, Mtumba, Ntyuka and Chigongwe are presented in Table 4. Among the five elements (Mn, Co, Ni, Cu and Zn) analyzed, three elements (Co, Cu and Zn) were detected whereas the other two (Mn and Ni) were below their method detection limit and hence not detected. From the detected elements, the highest mean concentration obtained was for zinc (13.224 ± 3.281ppm) and lowest for cobalt (0.109 ± 0.027ppm) among all the studied farms of Dodoma urban district. The mean concentrations of heavy metals detected in the milk samples in the present study can be arranged in the order of Zn (13.224 ± 3.281) > Cu (0.232 ± 0.020) > Co (0.109 ± 0.027) in ppm.

Table 4: Average concentration, ppm (SD, n = 3) of metals in milk samples collected from different villages of Dodoma urban district.

Sl. No.	Heavy metal	Average concentrations, ppm (SD, n= 3)				
		Study sites				
		Chihanga	Msalato	Mtumba	Ntyuka	Chigongwe
1	Mn	<MDL	<MDL	<MDL	<MDL	<MDL
2	Co	0.106 (0.002)	0.147 (0.007)	0.079 (0.001)	0.091 (0.003)	0.122 (0.004)
3	Ni	<MDL	<MDL	<MDL	<MDL	<MDL
4	Cu	0.243 (0.012)	0.242 (0.009)	0.203 (0.006)	0.251 (0.015)	0.218 (0.004)

5	Zn	10.132 (0.235)	10.697 (0.125)	13.302 (0.0925)	13.570 (0.102)	18.419 (0.311)

MDL = Method detection limit

In the present study the concentration of Mn and Ni was found to be less than method detection limit. This might be due to less bioavailability of this element to the animal feed and hence to the cow's milk. Also there are no industries and vehicle emissions which are the basic sources of these heavy metals around the study area. Since it is almost rural area, there is no exposure of these toxic heavy metals. The feeds and the water which the cows use are also free from these toxic metals. This indicates the milk of this district is poor source of manganese and nickel. Manganese and nickel are essential in very low concentration for the survival of all forms of life [20]. Manganese plays an important role in a number of physiologic processes as a constituent of multiple enzymes and an activator of other enzymes. Manganese superoxide dismutase is the principal antioxidant enzyme in the mitochondria. A number of manganese-activated enzymes play important roles in the metabolism of carbohydrates, amino acids, and cholesterol. Pyruvate carboxylase, a manganese containing enzyme, and phosphoenolpyruvate carboxykinase, a manganese-activated enzyme, are critical in gluconeogenesis. Arginase, another manganese-containing enzyme, is required by the liver for the urea cycle, a process that detoxifies ammonia generated during amino acid metabolism [21].

In the brain, the manganese-activated enzyme, glutamine synthetase, converts the amino acid glutamate to glutamine. Glutamate is an excitotoxic neurotransmitter and a precursor to an inhibitory neurotransmitter, γ-aminobutyric acid [22]. Manganese deficiency has been observed in a number of animal species. Signs of manganese deficiency include impaired growth, impaired reproductive function, skeletal abnormalities, impaired glucose tolerance, and altered carbohydrate and lipid metabolism. In humans, demonstration of a manganese deficiency syndrome has been less clear. A child on long-term total parenteral nutrition lacking manganese developed bone demineralization and impaired growth that were corrected by manganese supplementation. Young men who were fed a low-manganese diet developed decreased serum cholesterol levels and a transient skin rash [23]. Blood calcium, phosphorus, and alkaline phosphatase levels were also elevated, which may indicate increased bone remodeling as a consequence of insufficient dietary manganese. Young women fed a manganese-poor diet developed mildly abnormal glucose tolerance in response to an intravenous (IV) infusion of glucose [24]. Infants are exposed to varying amounts of manganese depending on their source of nutrition. Manganese concentrations in breast milk, cow-based formula, and soy-based formula range from 3 to 10 μg/L, 30 to 50 μg/L, and 200 to 300 μg/L, respectively. Nickel will pass into human body through your stomach and intestines if you drink water and milk containing nickel than if you eat food containing the same amount of nickel. A small amount of nickel can enter your bloodstream from skin contact. After nickel gets into your body, it can go to all organs, but it mainly goes to the kidneys. The nickel that gets into your bloodstream leaves in the urine. After nickel is eaten, most of it leaves quickly in the feces, and the small amount that gets into your blood leaves in the urine. The most common harmful health effect of nickel in humans is an allergic reaction. Workers who accidentally drank light-green water containing 250 ppm of nickel from a contaminated drinking fountain had stomach aches and suffered adverse effects in their blood and kidneys. The most serious harmful health effects from exposure to nickel, such as chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus [25].

The concentration of cobalt in the milk samples of five villages was found to follow decreasing order: Msalato > Chigongwe > Chihanga > Ntyuka > Mtumba. The average concentration of cobalt is 0.109 ± 0.027 ppm among five villages. The concentration of cobalt ranges from 0.079-0.147 ppm in the Dodoma urban district. There is significant difference in the concentration of cobalt among five villages at a probability of 0.05. The significant variation in concentration of cobalt in different villages might be due to the difference in the concentration of cobalt in soil generally ranging from about 1 to 40 ppm. Bioavailability of this nutrient varies with the plant species and ultimately its accumulation in the cow's milk through cattle feed varies. Cobalt can enter your body when you drink water and milk that contains cobalt, when you eat food that contains cobalt, or when your skin touches materials that contain cobalt. The most likely way you will be exposed to excess cobalt is by eating contaminated food or drinking contaminated water and milk. The amount of cobalt that is absorbed into one's body from food or water and milk depends on many things includes one's state of health, the amount of consumption and the duration of the consumption of foods or fluids containing cobalt. If the human body does not have enough iron then, the body may absorb more cobalt from the consumed foods. Once cobalt enters human body, it is distributed into all tissues, but mainly into the liver, kidney, and bones. Cobalt has both beneficial and harmful effects on human health. Cobalt is beneficial for humans because it is part of vitamin B_{12}, which is essential to maintain human health. When too much cobalt gets into human body, however, harmful health effects can occur. Serious effects on the lungs, including asthma, pneumonia, wheezing, asthma and skin rashes [26]. The recommended daily intake of Co is 5-8 μg/day. In the present study calculated daily intake of Co is 13.4 μg/day, which is greater than the recommended value. Therefore, it is recommended to reduce the cobalt intake from the other sources.

The concentration of cobalt in the milk samples of five villages was found to follow decreasing order: Ntyuka > Chihanga < Msalato < Chigongwe > Mtumba. There is no significant difference in the concentration of copper among the five villages at a probability of 0.05. The mean concentrations in the present study were found in the range of 0.203 to 0.251 ppm in Dodoma urban district. The average concentration of copper is 0.231 ± 0.020 ppm among five villages. This indicates that the cow's milk of this zone is poor source of copper. Copper is an essential trace element that plays a vital role in the physiology of animals for fetal growth and early post-natal development. Excess copper in the body leads to Wilson's disease which is characterized by deficiency of ceruloplasmin [27]. The toxic limit of copper in cow's milk is 0.4 ppm [28]. The concentration of copper in the present study is below this toxic limit. Per capita consumption of milk in Tanzania is 45 litres per annum. That means 123 mL per day per person. Therefore, the daily intake of copper, as found in our study, is 28.4 μg/day is very low compared to 2000-3000 μg/day as per recommended daily intake of copper [14].

In this study, zinc is present in all the milk samples with concentrations ranging from 10.132 to 18.419 ppm. The average concentration of zinc from all the sites is 13.224 ± 3.281 mg/kg among five villages under study. The concentration of cobalt in the milk samples of five villages was found to follow decreasing order: Chigongwe > Ntyuka > Mtumba > Msalato > Chihanga. There is significant difference in the concentration of zinc between five villages at probability 0.05. Taking too much of zinc into the body through food, water, or dietary supplements can affect health. The Recommended Dietary Allowances (RDAs) for zinc is 11 mg/day for men and 8 mg/day for women. If large doses of zinc (10–15 times higher than the RDA) are taken by mouth even for a short time, stomach cramps, nausea, and vomiting may occur. Ingesting high levels of zinc for several months may cause anemia, damage the pancreas, and decrease levels of high-density lipoprotein (HDL) cholesterol. The daily intake was determined to be 1626.55 μg/day while the recommended daily intake is 12000 – 15000 μg/day [14]. Therefore, the concentration of zinc in the milk, as found in our study, is less than the internationally recommended level.

3.3 Comparison of the result of the present Study with that of past studies

There are wide variations in the published data for the elemental concentrations of cow's milk of different countries as shown in Table 5.The observed wide variation in the concentrations of metals in milk is possibly due to nature and composition of the soil of the specific region. This results in different level of accumulation in the grass, which in turn results in the varying concentration of metals in the cow's milk. The concentrations of Mn and Ni were found to be less than method detection limit in present study, where as in some of the literature these concentrations are present in the detectable levels. The concentration of cobalt in the present study is 0.109 ± 0.027 ppm. There are limited reports on the concentration of cobalt in milk samples. According to a report of milk composition and synthesis resource library, the concentration of cobalt is 0.6 ppm. The concentration obtained in the present study is very less compared to the reported value.

The result of Cu (0.231 ± 0.020 ppm) is also closer to the report made by Ogabielain Nigeria (0.214 ± 0.230 ppm) and comparable with the value reported for the milk samples of Egypt, Crotia and Ethiopia. Zinc concentration (13.224 ± 3.281 ppm) in the present study is greater than that of the reported values in different countries in the literature except for china (Table 5).

In general, the concentrations of metals detected in the present study were more or less comparable with the reported literature values. However, relatively comparable concentration of Cu and higher concentration of Zn were observed in this study in comparison to the reported values.

Table 5: Comparison of the elemental concentrations of fresh cow's milk of present study with the literature values of the other countries

Country	Mn	Cu	Zn	Reference
Egypt (mg/kg)	0.056 (0.038)	0.140 (0.116)	3.146 (1.081)	[18]
Saudi Arabia (mg/kg)	NR	0.005 (0.600)	0.944(2.400)	[14]
Ethiopia (mg/kg)	NR	NR	4.923 (0.277)	[29]
Nigeria (mg/L)	0.219 (0.090)	0.214 (0.230)	5.521 (13.900)	[30]

Poland (mg/L)	0.102 (25.63)	0.089 (125.14)	3.163 (710.61)	[31]
China (mg/kg)	0.600 (0.150)	0.420 (0.130)	30.400 (2.600)	[12]
Croatia (mg/kg)	NR	0.380 (0.120)	0.510 (0.160)	[32]
Ethiopia (mg/Kg)	0.427 (0.018)	0.109 (0.006)	5.592 (0.092)	[33]
Tanzania (mg/L)	<MDL	0.231 (0.020)	13.224 (3.281)	Present study

The values in the parenthesis are SD's, NR= Not reported, MDL = Method detection limit

3.4 Recommended daily allowance of heavy metals

The daily intake of the metals depends on both the concentration and the amount of food consumed. The daily dietary intake of milk for an average Mumbai (India) population is 113g. The reported values of daily milk consumption in USA and Spain are 224g and 124g, respectively [14]. In Tanzania, per capita consumption of milk is 45 litres per annum. The value given per year is changed to per day to know the approximate daily intake in Tanzania. That means 123 mL per day per person. Since density of whole milk is very close to the density of water, that is 1.0002 g/mL compared to water 1.0 g/mL. Thus it is possible to assume that 123 mL is equal to 123 g of whole milk. Assuming a value of 123 mL of milk consumption per day, the daily intake of the detected heavy metals from samples of the present study are determined and depicted in Table 6. The 4th column shows the Recommended Dietary Allowance (RDA) as set by different international organizations [14].

From the Table 6, it is observed that the daily intakes of detected elements in the study area were less than the recommended/permissible levels set by different international organizations except for Co. The daily intake of Co is greater than the recommended value whereas Cu and Zn daily intakes are far less than the recommended daily intake values. Therefore, it is suggested that some other alternative sources of food rich in Cu and Zn might be taken to compensate the deficiency in milk.

Table 6: Comparison of daily intakes of metals from 123 mL milk by Tanzanian population with recommended/ permissible values

Element in fresh milk	Concentration (µg/mL)	Daily intake (µg/day)	Recommended/ permissible value (µg/day)	References
Cobalt	0.109	13.4	5-8	[34]
Copper	0.231	28.4	2000 - 3000	[29, 14]
Zinc	13.224	1626.6	12000 - 15000	[29, 14]

IV. CONCLUSIONS

The heavy metals Co, Cu and Zn were detected in milk samples of Dodoma urban district. The concentrations of Co, Cu and Zn were 0.079–0.147, 0.203–0.251 and 10.132–18.419 ppm, respectively in the milk samples of five villages Chihanga, Msalato, Mtumba, Ntyuka and Chigongwe. The other two heavy metals Mn and Co were not detected in the cow's milk of Dodoma urban district even though they were detected in the cow's milk as per past studies. The concentrations of heavy metals observed were comparable with some of the reported values in literature. The results obtained for detected elements in the present study were also compared with international daily intake guidelines of different international organizations for food and were found below the levels allowed for Cu and Zn, whereas it is more for Co.

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