PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 26 (8) 1357-1361 August 2022

Assessment of Lead and Cadmium Concentrations in Raw Cow Milk from Farms near Namanve Industrial Park in Kira Division, Wakiso District, Uganda

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ABSTRACT: Lead and Cadmium concentrations were analysed in raw cow milk samples collected from smallholder zero grazing households near Namanve Industrial Park in Kira Division in Wakiso district near Kampala city, Uganda. The concentration of heavy metals in milk was tested with atomic absorption spectrophotometry using the Perkin Elmer AAA Model 2380 after acid digestion. The overall mean concentration of lead and cadmium was 0.01 and 0.0083 mg/kg respectively. Of the three wards, Bweyogerere had the highest lead concentration (0.014mg/kg) and cadmium (0.011mg/kg). Analysis of Variance (ANOVA) test of the mean concentrations of lead between and within wards showed significant differences ($F = 10.85$, $P < 0.05$) and the findings were similar for cadmium ($F=4.77$, $P < 0.05$). Multiple comparison tests (Bonferroni) showed Bweyogerere raw milk had significantly higher lead concentration than milk from Kireka ($P=0.008$) and milk from Kirinya ($P < 0.0001$) and also significantly higher cadmium concentration than milk from Kirinya ($P = 0.0009$). Mean concentration of each metal residue was below the recommended minimum hazardous intake in diet as per Codex/Uganda National Bureau of Standards at 0.02 mg/kg for both lead and cadmium. Therefore, milk from cows reared around Namanve presents no public health-related lead and cadmium hazards.

DOI: **https://dx.doi.org/10.4314/jasem.v26i8.6**

Cite this paper as: BIRYOMUMAISHO, S; ATUKWASE, A; MANIRAKIZA, L (2022). Assessment of Lead and Cadmium concentrations in raw cow milk from farms near Namanve Industrial Park in Kira Division, Wakiso district, Uganda. *J. Appl. Sci. Environ. Manage.* 26 (8) 1357-1361

Dates: Received: 05 August 2022; Revised: 13 August 2022; Accepted: 18 August 2022

Key words: Cow milk; Lead; Cadmium; Industrialisation; Heavy metal milk residues

Milk is the first food for mammals taken immediately after birth to ensure proper growth and postnatal development. Milk is considered a complete food containing almost all nutrients like proteins, carbohydrates and minerals (Meshref *et al.*, 2014). Availability of these nutrients in milk is affected by a variety of factors like animal species, plane of nutrition, genetics, lactation stage and environmental factors (Vahčić *et al.*, 2010). Cow milk is the most consumed livestock milk on Ugandan market although milk from other species like goats and sheep is also available. Milk contains both liposoluble and hydrophilic minerals; hydrophilic vitamins like vitamin C and B-complex are dissolved in the water portion. The liposoluble minerals are found in the fat portion that is vitamin A, D and E. Milk is rich in minerals that include calcium with the biggest proportion, phosphorous, magnesium, zinc, selenium, iodine, copper, sodium, iron and sodium (Akele *et al.*, 2017). Recent studies show increased pollution and contamination of food stuffs consumed by humans worldwide (Sarsembayeva *et al.*, 2020). The sources of these metal contaminants include urbanization, industrialization and chemical agricultural production which have led to massive land and water pollution (Pilarczyk *et al.*, 2013; Ulman and Gezer, 1998). Waste products from these activities contain metals like lead, mercury, cadmium, manganese, nickel, arsenic and cobalt that are disposed of mainly on land and water. Some of these metals end up in the food chain due to erosion of metal surfaces during processing, packaging and transportation of milk and its products (Grimaud *et al.*, 2007). Metal residues in milk also originate from processed animal feeds and

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environment such as paintings, lead alkyl derivatives of combustion, incineration of refuse and manufacturing processes (Meshref *et al.,* 2014). Metal residues are also absorbed by plants from the polluted soil that form the primary link in the food chain and are stored in the plant tissues (Bandani *et al.,* 2016). Plants are later consumed by animals, fish and humans. These metals keep accumulating in different tissues like fat tissue, liver and sometimes excreted in urine, faeces and animal products like milk. Milk fat acts as store for most of these metals (Nyakairu *et al.,* 2011). Monitoring contamination of foodstuffs ensures that safe food is availed to the population and safeguards products of high nutritional value don't contain contaminants beyond recommended maximum levels. The Uganda National Bureau of Standards set standards which must be attained before any food stuff is allowed to be marketed legally, for example vegetables and mineral water and milk (UNBS, 2019). For every dairy product whether processed or raw, it should contain a minimum amount of metal residues beyond which it should not be consumed or be sold to customers. WHO/FAO and CODEX also indicate some metal residues like lead, mercury and cadmium as threats to major foods (Alimentarius Codex, 1999).

Several methods have been employed by these organizations to measure the amount of metal residues in dairy products and include atomic absorption spectrophotometry, Anodic stripping voltammetry, Colorimetry (diethyldiethiocarbamate), Flameless atomic absorption spectrophotometry and Spectrophotometry (1, 5- diphenylthiocarbazone) depending on the nature and components in the food (Alimentarius, Codex, 1999; UNBS, 2019). These metals, when not regulated in milk may be associated with severe healthy complications to both humans and animals like embryonic death, carcinogenic effects, haemorrhagic disturbances, nervous disorders, congenital anomalies and mutations (Enb *et al.,* 2009; Li et al., 2016) hence the need to routinely analyze the foods consumed in the community.

In Uganda, the Dairy Development Authority (DDA), a statutory body under Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) was formed in 1998 under the Act of Parliament to regulate and inspect the standards of milk and its products. The aim was to ensure that consumers are protected, improve public health and produce milk products that can compete internationally (Zirintunda, 2014). On a broader perspective, the East African Dairy Development project (EADD) was formed to oversee the standards of milk and its products in the region working together with Uganda National Bureau of Standards (TechnoServe, 2008; UNBS, 2017). These are the standards that are being used to ascertain whether consumers access healthy milk that does not contain heavy metals beyond the maximum recommended levels. There may be a possibility that milk from farms in Kira is contaminated due to the presence of numerous industries in Namanve that may pollute the water consumed by animals and land where pastures that animals feed on are grown. The metal residues are thereafter excreted in urine, faeces and animal products mainly milk.

The aim of this study, therefore, was to obtain updated information about the status of heavy metals by determining the concentrations of lead and cadmium in raw milk samples from cows in small holder zero grazing households near Namanve Industrial Park in Kira Division in Wakiso District near Kampala city, Uganda.

MATERIALS AND METHODS

Study area: A cross sectional study was conducted in December 2021 in Kira Municipality, Wakiso district. The area is found in the central region of Uganda, approximately 15 km from the capital city Kampala. Milk samples were collected from Kireka, Kirinya and Bweyogerere that comprise three of the six wards of Kira Municipality adjacent to Namanve Industrial park.

Sample collection and preparation: A total of 71 milk samples were collected from different small holder zero grazing households from the three wards; one sample from pooled milk from each farm/household was collected. Samples were taken from households closest to the Namanve Industrial park. Immediately after milking, 250 ml of raw milk was collected into sterile plastic bottles (not metallic) to avoid contamination by metal residues. The samples were transported under ice in a cool box to avoid spoilage during transportation to the central laboratory at College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University until laboratory analysis.

Samples were prepared by wet digestion whereby an organic compound is converted to ash by treatment with nitric or sulfuric acid as described in Analytical Methods for Atomic Absorption Spectroscopy (Elmer, 1996). For each sample, 5ml were placed on a silicate crucible, and evaporated overnight in a hot air oven at 105^oC to dryness as originally described by Murthy *et al.,* (1957). Concentrated sulfuric acid and hydrogen peroxide were added to the dried sample. The mixture was then stirred to make a uniform mixture. Samples were then digested using aluminum block digester (Wagtech International UK) maintained at 360^oC for

3-4 hrs until the samples were clear and digestion was complete as described by Nyakairu *et al.,* 2011). Lanthanum chloride solution was then added to remove any interference from phosphorous and sulfur. Each sample was then made to the mark 500 cm^3 using distilled de-ionized water.

Sample analysis: The milk samples were analyzed at the Government Analytical Laboratory in Wandegeya by a Perkin Elmer Atomic Absorption Spectrophotometer (Model 2380 Perkin Elmer Co. and Northwall CT USA). The concentration of elements was measured from the wave lengths of light specifically absorbed by the element as atoms absorb specific wavelength of light. The heavy metal concentrations expressed as mg/kg were compared with permissible limits for sweetened condensed milk set by the Uganda National Bureau of Standards (UNBS) at 0.02 mg/kg for both lead and cadmium. UNBS is a member of International Organisation for Standardisation (ISO), a contact point for the WHO/FAO Codex Alimentarius Commission on Food Standards, and the National Enquiry Point on Technical Barriers to Trade (TBT) Agreement of the World Trade Organisation (WTO) basing on the Association of Official Agricultural Chemists (AOAC) 999.10 official method for testing selected heavy metals in foods (UNBS, 2019).

Data analysis: Spectrophotometer readings for lead and cadmium in raw milk for individual farms/household were entered in Excel sheet (v2013). Statistical analyses were conducted in Stata v16; mean concentrations of each heavy metal residues were calculated. Analysis of variance (ANOVA) was used to compare concentration of heavy metals of milk from different farms. Pairwise comparison of means of heavy metals from different wards to determine which means were significantly different were compared with multiple comparison (Bonferroni) tests and means were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Kirinya, Bweyogerere and Kireka compose three out of six Kira Municipality wards, Wakiso district and near Kampala city. In the last two decades, the area has seen a surge in human settlement and expansion of the Namanve Industrial park. At the same time, many households in the area keep dairy cattle, mostly under the cut and carry/ zero grazing system. The two metals were selected for investigation because both have various common uses. For almost a century, lead has been used as a gasoline additive to prevent engine knock, in pesticides, batteries and plumbing fixtures (Frank *et al.,* 2019). As an additive lead accelerates drying in paint, makes paint maintain a fresh appearance and also enhances resistance to moisture that causes corrosion (Lokman *et al.,* 2013). Cadmium is among others used in making batteries, lasers, phosphate fertilisers and paint pigment.

Accumulation of these heavy metals in various tissues and organs is associated with severe adverse effects; for lead, these include auditory impairment, severe anaemia, nervous system disorders, kidney and liver damage (Liu *et al.,* 2017). Cadmium on the other hand is associated with damage of various internal organs. Both lead and cadmium have adverse effects on the immune system. In fact, cadmium was classified as a human carcinogen in 1993 (Waalkes, 2003). The extent of heavy metal environmental pollutants from industries around Namanve Industrial Park as well as increased urbanization of the area is not clearly understood. The mean concentration of lead in raw milk from Kira Division was 0.01 mg/kg. Bweyogerere had the highest average lead concentration (0.014mg/kg).

Analysis of Variance (ANOVA) of concentrations of lead in raw milk from different farms showed significant differences (F=10.85, P=0.-0001) between and within different farms. Further, multiple comparison test (Bonferroni test) showed that raw milk from Bweyogerere had significantly higher lead concentration than that from Kireka (P=0.008) and that from Kirinya (P<0.0001) (Table 1).

With increasing urbanization and mechanical and civil engineering works that include plumbing, heavy traffic that involves heavy vehicles that use diesel and numerous petrol-based engines, there is a high chance of lead accumulation in the environment.

Concentration of cadmium in raw milk samples: The mean concentration of cadmium from Kira Division was 0.011 mg/kg; Bweyogerere had the highest average cadmium concentration (0.011mg/kg) (Table 2).

Table 1: Average concentrations of lead (mg/kg) in raw cow milk

Ward	N	Mean	SD	Source	DF	MS	F-statistic	P
Bweyogerere	31	0.014	0.0072	Between farms	2	0.00054	10.85	0.0001*
Kireka	22	0.008	0.007	Within farms	68	0.000051		
Kirinya	18	0.005	0.007					
Overall mean	71	0.01	0.008					

Table 2: Mean concentrations of cadmium (mg/kg) in raw cow milk

Ward	N	Mean	SD	Source	DF	MS	F	P
Bweyogerere	31	0.011	0.0064	Between farms	2	0.000209	4.77	0.0115*
Kireka	22	0.009	0.0067	Within farms	68	0.0000438		
Kirinya	18	0.005	0.0068					
Overall mean	71	0.0083	0.0066					

SD = standard deviation

The concentrations of lead and cadmium obtained from this study differ from results of Nyakairu *et al.,* (2011) who found higher lead and cadmium concentrations in milk in Wakiso above maximum permissible limits whether milk was collected from shops or farms. However, the source of the heavy metals in milk was not investigated further, whether from air, industrial waste, garbage disposal and industrial sludge, poultry waste, soil or polluted water. Likewise, in the present study, lead concentration was high in two farms. This requires that subsequent investigations should dig further to establish the source of the heavy metals when found in higher concentrations in milk. In a study conducted upcountry on heavy metals in milk and beef in Bushenyi district (Kasozi *et al.,* (2019), no cadmium was detected. However, the concentration of lead was higher than the United States Environmental Protection Agency (US EPA) permissible maximum levels. This implied that the lead could be occurring naturally in high amounts in the soil or from man-made activities particularly use of pesticides which are major sources of heavy metal pollution. In another study on heavy metals in drinking water in Kampala city (Bamuwamye *et al.,* 2017), cadmium was not detected. However, lead concentration in tap water was lower than reported in East Africa. The sources could possibly be due to corrosion of older fixtures or from solder that connects pipes including polyvinyl chloride pipes that also contain lead. Although these pipes are in use in the study area, lead levels were below the maximum permissible levels.

The elimination of lead from motor fuels in 2021 was a major step towards reducing effects of lead poisoning in people. This practice began in 1922 when lead in form of tetraethyl lead was first added to motor fuel and over the next 100 years, millions of tons were added to gasoline worldwide and resulted in a global lead pandemic of lead poisoning (Angrand *et al.,* 2022). The adverse effects included impairment of cognitive function, reduction in life expectancy and premature deaths of millions of people. However, lead is still used in fuel for racing cars and aviation fuel for small piston-engine aircrafts in the United States (Heather *et al.,* 2022). Lead exposure of adults living near small aircraft runways in North Carolina showed increased adult cardiovascular mortality suggesting that removing lead from aviation fuel of piston-small engine aircraft could have great health benefits. In a study on heavy metals in vegetables cultivated in a major wetland near Kampala city, lead and cadmium exceeded WHO maximum permissible levels (Mbabazi *et al.,* 2010). However, no recent updated data is available; but with removal of lead in motor fuel, environmental contamination is expected to become lower as has been cited in other parts of the world (Mielke *et al.,* 2019).

Comparison of Maximum Permissible Limits (MPL) of heavy metal concentrations in milk for lead and cadmium residues in the present study was below the recommended minimum hazardous intake in diet as per Codex/UNBS and World Health Organisation standards. This implies that milk from cows in the study area presents no public health risks in relation to lead and cadmium heavy metal residues.

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