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Authors: Geleta, Girma Selale, Nugussa, Argachew, Faye, Gezahegn, and Ragassa, Girma

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Assessment of Human Health Risks from Aflatoxin M1 in Raw Milk: A Study from North Shewa Zone, Oromia Region, Ethiopia

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Girma Selale Geleta, Argachew Nugussa, Gezahegn Faye and Girma Ragassa

Department of Chemistry, College of Natural Sciences, Salale University, Fiche, Oromia, Ethiopia.

ABSTRACT: This research aimed to assess the levels of AFM1 and evaluate the associated health risks from raw milk samples collected randomly from four selected towns. Ninety (n = 90) raw milk samples were randomly collected from smallholder dairy farmers in the North Shewa Zone, with 23 samples from Fiche, 23 from G/Gurracha, 22 from Dagem, and 22 from Sululta. The concentrations of AFM1 in the raw milk samples were determined using high-performance liquid chromatography with fluorescence detection (HPLC-FLD) after purification via an immunoaffinity column (IAC). AFM1 was detected in 76 (84.4%) raw milk samples, with 53 (58.9%) exceeding the maximum permissible limit established by the European Commission, 0.0500 µg/L. The highest AFM1 content was 2.00 µg/L and the lowest was 0.0100 µg/L. Additionally, risk assessment was performed using the margin of exposure (MOE), estimated daily intake (EDI), hazard index (HI), and cancer risk (CR). The results indicated that based on the average contamination levels of the milk during the study period and typical consumption rates, the average EDI of the adult population to AFM1 ranged from 0.374 to 0.852 ng/kg body weight (bw) per day. The calculated MOE values were less than 10,000, indicating potential health concerns. The mean HI value determined in this study was 2.70, which also suggests adverse health effects. Furthermore, the estimated risk of developing hepatocellular carcinoma (HCC) due to AFM1 exposure from milk consumption among adults was calculated to be 0.00170 cases per 100 000 individuals yearly. This finding indicates a significant risk of HCC, which justifies its continuous monitoring of dairy products throughout the entire supply chain, from production to consumption. Furthermore, our research highlights the need for further investigation into the risks posed by AFM1 in children, given their higher levels of milk consumption relative to adults.

KEYWORDS: AFM1, raw cow milk, Oromia region, North Shewa, Ethiopia

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CORRESPONDING AUTHOR: Girma Selale Geleta, College of Natural Sciences, Department of Chemistry, Salale University, P.O. Box 245, Fiche, Oromia, Ethiopia. Email: ggirma245@gmail.com

Introduction

Milk and milk products are widely recognized as having high nutritional value, as they provide vital nutrients essential for human growth, development, and the maintenance of overall health.[1-3] The majority sources of global milk production is cow's milk, which constitutes around 81% of the total output.[4] In developing countries, such as Ethiopia, milk and its products are important agricultural commodities and present viable investment opportunities for smallholder farmers.[5] However, milk can also be susceptible to food contamination from various chemical contaminants, including pesticides, heavy metals, and antibiotics, as well as microbial contaminants like bacteria, fungi, and molds, which pose public health risks and can lead to economic losses within the dairy sector.[6,7] Therefore, it is imperative that milk and dairy products undergo continuous inspection and monitoring for these contaminants.[8]

Among the various contaminants, fungal contamination is particularly concerning due to the potential production of aflatoxins, including AFB1, aflatoxin B2 (AFB2), aflatoxin G1(AFG1), aflatoxin G2 (AFG2), and AFM1. AFB1, in particular, is recognized as the most potent and toxic natural carcinogen affecting both humans and animals.[9,10] AFB1, in feeds produced due to poor storage and favorable climatic conditions suitable for fungal growth. These contaminants significantly compromise the quality of animal feeds and can be transferred into the milk of lactating cows that consume aflatoxin-contaminated feeds.[11] When lactating animals ingest AFB1 from contaminated feed, a hydroxylation process occurs, resulting in its metabolism in the liver, where it is converted into AFM1, which subsequently enters the milk of dairy cattle. The level of AFM1 excreted in the milk is directly proportional to the quantity of AFB1 consumed through the feed.[9,12] Research has indicated that between 0.3% and 6.2% of AFB1 in animal feed is converted into AFM1 by the cytochrome P450 enzyme system in the liver and then secreted into milk.[3,13-15] The International Agency for Research on Cancer (IARC) has classified AFM1 as a Group 2B carcinogen, indicating that it is possibly carcinogenic to humans, being 10 times less toxic than AFB1. The presence of AFM1 has also been linked to immunosuppression, mutagenicity, and teratogenic effects.[16-22] Therefore, it is essential to implement regular and comprehensive monitoring of AFM1 levels.

To safeguard food safety and protect public health, several countries and international organizations have set a maximum allowable limit for AFM1 in milk and dairy products. These standards differ according to the economic status of the countries and the global guidelines provided by organizations such as the Food and Agricultural Organization/World Health

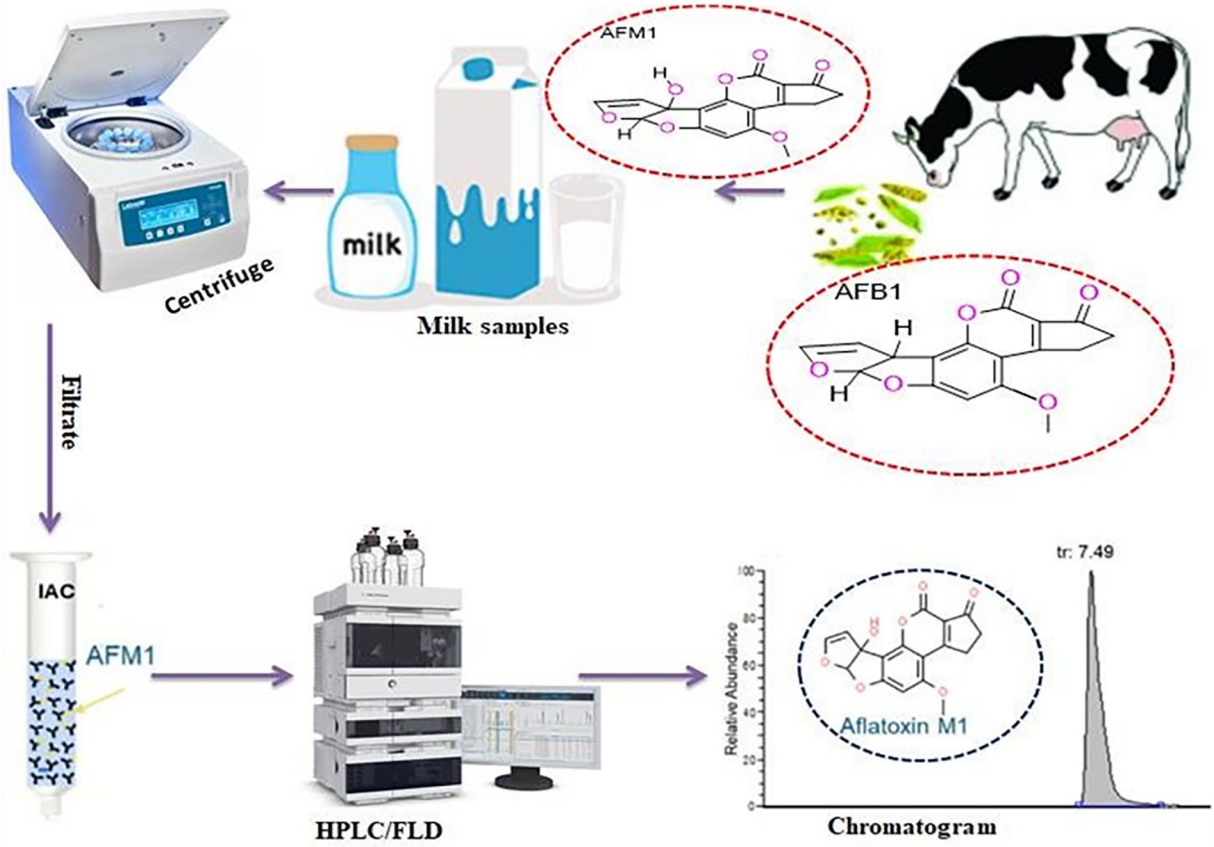

Figure 1. Schematic representation of AFM1 determination in milk samples by UHPLC-FLD.

Organization (FAO/WHO), Codex Alimentarius Commission (CAC),[23] the Food and Drug Administration (FDA). For example, the European Commission has established a maximum residual limit of 0.05 µg/L in milk, whereas the FDA and CAC have set a maximum residual limit of 0.50 µg/L in milk. Ethiopia adheres to the European Commission's regulatory limits of 0.05 µg/kg for raw milk.[24] Moreover, alongside establishing regulatory thresholds for AFM1, it is essential to assess the risk of exposure to AFM1 contaminated milk and dairy products represents a highly effective approach for assessing the severity and likelihood of liver cancer risk. The risk assessment of AFM1 is conducted through the analysis of the EDI, measured in ng/kg body weight per day, along with the MOE and HI. These metrics serve as cancer risk indicators in toxicological research.[25] Therefore, it is essential to conduct regular assessments of AFM1 levels in raw milk to ensure compliance with food safety standards and to mitigate the risks associated with its known toxicity and carcinogenic properties.

Several studies have indicated AFM1 contamination of milk and dairy products from various Ethiopian towns. For example, a recent study conducted in Nekemte city found that AFM1 concentrations in raw cow's milk ranged from 0.01 to 0.35 µg/L, with 58% of samples exceeding the EU's maximum tolerance limit (0.05 µg/L).[24] Similarly, a study by Admasu et al[26] reported that AFM1 was detected in the 99(99%) raw milk samples with values ranging from 0.031 to 5.16 µg/L. Additionally, research by Gizachew et al[27] reported AFM1 levels in milk between 0.028 and 4.98 µg/L, exceeding the maximum permitted level established by the European Union.27 These findings indicate that AFM1 contamination levels in Ethiopian milk vary across different regions, likely influenced by climatic conditions and the feeding habits of dairy cows.[24] Despite the numerous studies conducted in Ethiopia, there remain areas within the country that require thorough investigation. To date, there has been no research assessing AFM1 levels in raw cow's milk in the North Shewa Zone of the Oromia Regional State, Ethiopia. Therefore, the objective of the current study is to evaluate the levels, exposure, and risk characterization of AFM1 in milk samples collected from 4 selected towns from North Shewa, Oromia Region, Ethiopia. This study is expected to provide valuable insights into the current state of AFM1 contamination in raw milk and the potential health risks associated with the consumption of such contaminated raw milk. The findings will be crucial for policymakers, health professionals, and consumers in their efforts to improve food safety and protect public health. The proposed schematic representation for the determination of AFM1 via UHPLC-FLD is shown in Figure 1.

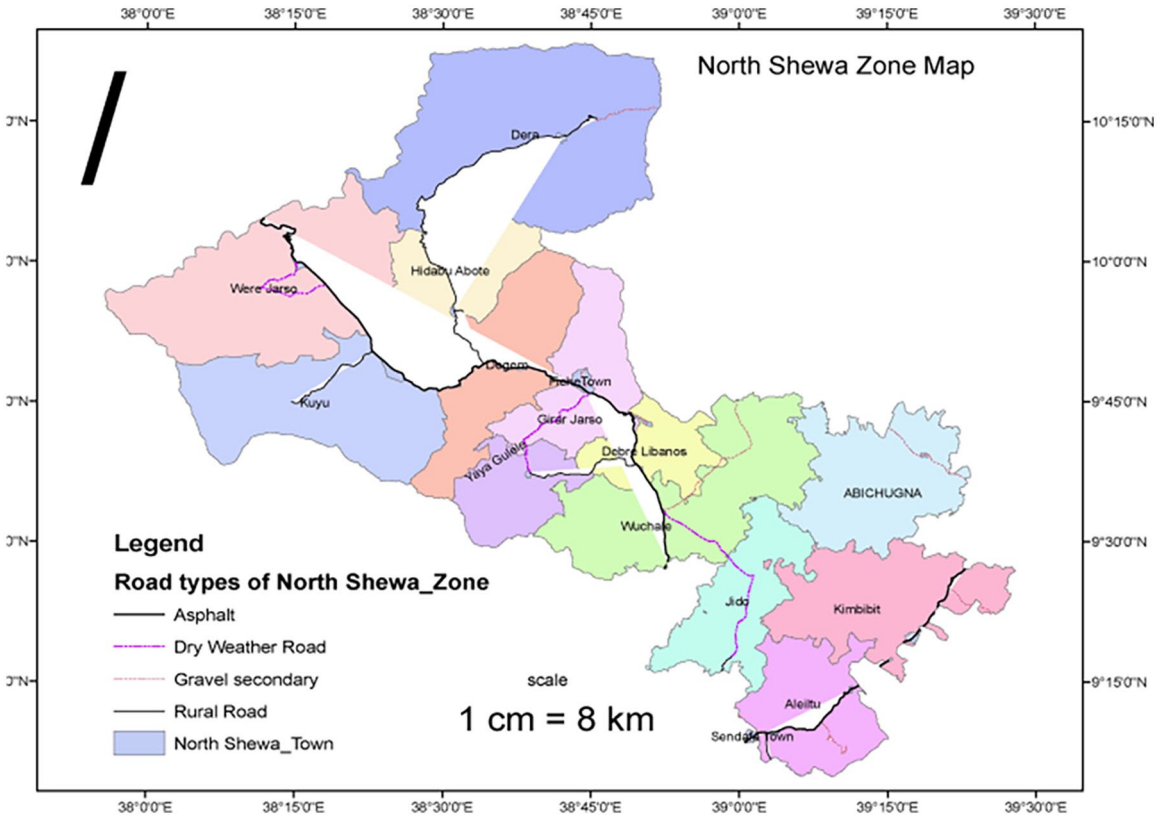

Figure 2. North Shewa Zone map showing the study sites.[28]

Materials and Methods

Study areas and samples collection

This research was conducted at the Department of Chemistry, Salale University, Ethiopia. The research was carried out between February 2024 and May 2024 in the North Shewa Zone, Oromia, Ethiopia. The administrative center of this Zone, Fitche, is located approximately 114 km from Addis Ababa, the capital city of Ethiopia. The North Shewa Zone is located between 2738 and 2782 m above sea level at 90 N, 380E. Since 2008, the zone has been administratively organized into 13 woredas (as shown in Figure 2) and has a density of 3050 people per square kilometer. This makes it the most densely populated zone in the region. It is one of the 20 zones within the Oromia Regional State. According to the national population and housing census conducted in 2007, the Zone has an estimated total population of 1 639 586, comprising 717 552 men and the remainder women. A significant majority of the population, approximately 89.75%, reside in rural areas. A total of 90 (n = 90) raw cow milk samples were randomly collected from smallholder dairy farmers in the North Shewa Zone. The collection comprised 23.0 samples from Fiche, 23.0 from G/Gurracha, 22.0 from Dagem, and 22.0 from Sululta, utilizing plastic bottles for the samples. Each sample consisted of 300 ml of fresh milk, collected directly from the dairy cows during the morning milking sessions at the farmers' houses. Following collection, all milk samples were placed in an insulated cold box for transport to the laboratory, where they were stored in a refrigerator at −20°C until analysis. The 4 study towns were selected purposively based on their high dairy production potential and supply areas for commercial purposes.

Methods of extracting AFM1 from raw milk

The procedure used for aflatoxin extraction and cleanup was based on the instructions of the Ethiopian Confirmative Assessment Enterprise Agency (ECAE). Briefly, raw milk samples were maintained at a temperature of 35°C to 37°C in a water bath. Approximately 300 ml of milk samples were homogenized in a larger beaker. Subsequently, 50.0 ml of the homogenized sample was transferred to a Falcon tube and covered with a cup. This mixture underwent centrifugation at 3000 rpm for 30 minutes to facilitate the separation and removal of the fat layer. The resulting fat-free samples were then filtered using Whatman filter paper, and 25.0 ml of each sample was set aside for the cleanup process. IAC obtained from Libios (Pontcharra-sur-Turdine, France) was prewashed with 10.0 ml of deionized water for the purification of the filtrate. Subsequently, 25.0 ml of the filtrate was added to a conditioned IAC at a rate of 1 drop per second. Afterward, the IAC was rinsed with 10.0 ml of deionized water and allowed to air dry. The AFM1 was then eluted using 3.00 ml of methanol. The resulting eluent was evaporated to dryness under a nitrogen stream at a temperature of 40°C. Finally, and before UHPLC analysis, the final residue

was reconstituted with 1.00 ml of a mobile phase consisting of a deionized water-acetonitrile-methanol mixture (60:25:15).

Analysis method using UHPLC

A UHPLC system (Hitachi High-Tech Corporation, Singapore) was employed for the quantification of AFM1. The chromatographic separation was achieved on a reversed-phase chromatographic C18 column (3.50 mm column; 4.60 × 100 mm) (HS, Bellefonte, USA). The mobile phase consisted of 60% deionized water, 25% acetonitrile, and 15% methanol (v/v/v), which was filtered through a microsyringe filter for use. The following instrument parameters were set: injection volume of 10.00 µl, flow rate of 0.500 mL min^{-1}, run time of 10 minutes, temperature set to 35°C, excitation wavelength of 360 nm, and emission wavelength of 440 nm. AFM1 standard was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and was used to prepare a series of AFM1 standard solutions (0.5, 1, 2, 3, 5, 7, and 10 µg/L) using the deionized water:acetonitrile:methanol mixture (60:25:15 v/v) through dilution. Calibration curves of peak areas versus AFM1 concentrations were then plotted and used for AFM1 determination in samples. The Recommended Daily Intake (RDI) for milk consumption, as outlined in the Ethiopian Food Based Dietary Guideline (EFBDG), is established at 250 g per day for adults aged 19 and older, and this guideline was utilized in the present study.[29,30]

Determination of AFM1 in raw milk samples

The concentrations (µg/L) of AFM1 in raw milk were determined via UHPLC–FLD. The actual amount of AFM1 in µg/L was calculated via the following equation (1).

$$C_m = C_a \times \frac{V_f}{V_i} \times \frac{1}{V_s} \quad (1)$$

where:

C_m = the concentration of AFM1 in the test sample, measured in micrograms per liter (µg/L).

C_a = the mass of AFM1, expressed in nanograms, that is found in the injection volume and corresponds to the area of the AFM1 peak obtained from the calibration graph.

V_f = the final volume of the redissolved eluate, measured in microliters (µL).

V_i = the volume of the injected eluate, in microliters (µL).

V_s = the volume of the test portion, specifically milk, that has passed through the immunoaffinity column, measured in milliliters (mL).

Risk assessment of AFM1

Estimation of exposure via consumption of milk. Exposure assessment is defined as a qualitative and/or quantitative analysis of the potential intake of a chemical agent through food, along with exposure from other relevant sources (FAO/WHO).[31] The EDI (ng/kg bw/day) was calculated by utilizing the average amounts of aflatoxins derived from raw milk samples, the daily consumption of raw milk, and the average body weight (BW). The body weight of an adult in Ethiopia was documented as 65 kg, which was utilized for the calculation of EDI.[7] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) provides guidelines for calculating the EDI of aflatoxins. An EDI value exceeding 1.00 ng/kg body weight per day for AFM1 is associated with an increased risk of HCC.[32,33] The EDI for AFM1 was calculated using a specific formula and is expressed in µg/kg of body weight per day (ng/kg bw/day)[34]:

$$EDI = \frac{Cave\left(\frac{\mu g}{L}\right) \times IR\left(\frac{L}{day}\right)}{bw(kg)} \quad (2)$$

where

The EDI is the average daily dose, Cave is the mean concentration of AFM1 in raw milk (µg/L), IR is the intake rate by the individual consumer of raw milk, and bw is the individual's body weight.

Risk characterization. Risk characterization assesses the likelihood of a negative impact from a contaminant on a human population. The health risk associated with AFM1 was evaluated using methods such as cancer risk assessment, margin of exposure analysis, and hazard index calculations.

Margin of exposure. The European Food Safety Authority (EFSA) has described the risk associated with oral exposure to AFM1 through MOE methodology.[35] The MoE serves as an index for assessing the risk linked to oral exposure to substances that possess carcinogenic and genotoxic characteristics. In the risk assessment of AFM1, the EFSA has approved the application of a potency factor of 0.1 alongside a benchmark dose level confidence limit of 10% (BMDL10) of 0.4 µg/kg bw/day for the induction of HCC due to AFB1. Therefore, MOE was determined as follows using equation (3) by dividing the BMDL10 of 4.00 µg/kg bw/day by the EDI.[35-38]

$$MOE = \frac{BMDL}{EDI} \quad (3)$$

The BMDL10, defined as the benchmark dose level confidence limit of 10%, represents an estimate of the minimum dose that has a 95% probability of resulting in no more than a 10% incidence of cancer. It is advisable to utilize this measure when determining the MoE. The benchmark dose refers to a dose that elicits a low yet quantifiable response. An MoE value of less than 10 000 signifies a significant risk of HCC within the population due to exposure to AFM1, while an MoE value of 10 000 or greater suggests minimal concern regarding public health implications.[34]

HCC in human societies due to the consumption of raw cow milk. The assessment of liver cancer risk associated with aflatoxins was estimated for adults who consume raw milk in the study areas. This assessment involved calculating the cancer

risk per 100 000 individuals, derived from the EDI value and the average potency figure for HCC based on the individual potencies of hepatitis B surface antigen (HBsAg) positive and negative groups. The JECFA has determined that the cancer potency of AFM1 is approximately 10 times lower than that of AFB1.[39,40] For individuals without chronic HBV infection, the cancer potency of AFB1 is quantified as 0.01 cases per 100 000 individuals annually for each nanogram of aflatoxin consumed per kilogram of body weight per day, while for those with chronic HBV infection, it is 0.300 cases. Consequently, the cancer potency of AFM1 is established to be 10 times less than that of AFB1, aligning with JECFA's evaluations of AFM1's carcinogenic potential, which is 0.00100 cases for individuals without chronic HBV infection and 0.0300 cases for those with chronic HBV infection. Additionally, the average prevalence rate of HBsAg+ in Ethiopia, recorded at 7.4%, was utilized, leading to an extrapolated rate of 92.6% for HBsAg-negative individuals.[40,41] Based on the above information, equations (4) and (5) were used to calculate the average potency of AFM1 and CR created by AFM1 in Ethiopia.

$$\begin{aligned}\text{Average potency} &= [0.03 \times \text{HBsAg} + \text{positive individuals in Ethiopia}] + [0.001 \times \text{HBsAg} \\ &\text{- negative individuals/prevalence rate in Ethiopia}] = (0.0300 \times 0.0740) \\ &+ (0.00100 \times 0.926) = 0.003146 \text{ HCC / year per } 100,000 \text{ persons}\end{aligned} \tag{4}$$

Thus, the cancer risk, expressed as the number of cancers per year per 100 000 population per ng of aflatoxin per kg of body weight per day, is determined using the formula

$$\text{Cancer Risk} = \text{Exposure (EDI)} \times \text{Average potency} \tag{5}$$

A carcinogenic risk of 0.0000100 or less than is considered to be of low risk for health concern; however, if the carcinogenic risk associated with AFM1 exceeds 0.0000100, a carcinogenic effect is likely/confirmed.[35,42]

Hazard index. In equation (6), the HI was calculated by taking the estimated intake and dividing it by an acceptable reference value of 0.200 ng/kg bw/day, which represents the tolerable daily intake (TDI) for AFM1. This calculation aims to evaluate public health concerns related to intake.[36,43,44] An HI value of less than 1 typically indicates a minimal risk to consumers of dairy products.

$$\text{HI} = \frac{\text{EDI}}{\text{Reference value}} \tag{6}$$

Statistical analysis. Each reading was performed in duplicate, and the average results are presented as means ± standard deviations. A one-way analysis of variance (ANOVA) was employed to identify significantly different means by comparing the level of aflatoxin contamination of raw milk across the 4 towns ($\alpha = .0500$).

Results and Discussion

Method validation

Calibration curve, limit of detection, and limit of quantitation. The following parameters were tested for validation of the UHPLC-FLD method including limit of detection (LOD), linearity, limit of quantification (LOQ), and accuracy. Linearity was determined by constructing seven-point calibration curves for AFM1 standard solutions across concentration ranges of 0.500, 1.00, 2.00, 3.00, 5.00, 7.00, and 10.0 µg/L. The calibration curves were constructed by plotting the peak area against the concentration of AFM1, with linearity determined through linear regression analysis, indicated by the coefficient of determination (r^2). The limits of detection (LODs) were determined to be 0.0100 µg/L, calculated as 3 times the standard deviation (SD) of the blank sample relative to the analytical curve slope (m) (3SD/m). The limits of quantification (LOQs) were determined to be 0.0400 µg/L, calculated as 10 times the standard deviation of the sample blank relative to the analytical curve slope (10SD/m). The average recovery percentage of AFM1 in spiked milk samples ranged from 80.0% to 85.0%, which is consistent with EU regulation No 401/2006.[45] This regulation specifies that recovery values for spiked concentrations of 0.0100 to 0.0500, 5.00 µg/kg should be between 60% and 120% and 70% and 110%, respectively. The high recovery percentages observed in this study confirm accuracy of the method.

AFM1 contamination in raw milk. A total of 90 milk samples were collected and analyzed for AFM1, comprising 23 samples from Fiche town, 23 from G/Gurracha, 22 from Dagem, and 22 from Sululta. AFM1 was identified in 76 of the analyzed samples, with concentrations varying from 0.0100 to 2.00 µg/L, resulting in an average concentration of 0.234 µg/L. Notably, only 14 samples, representing 15.6% of the total, were found to be below the limit of detection (LOD) of 0.0100 µg/L.

Variation in the level of AFM1 in milk among the four towns. AFM1 was detected in 76 (84.4%) raw milk samples, among these, 53 samples, or 58.9%, exceeded the maximum permissible limit established by the European Commission, 0.0500 µg/L (Table 1). The analysis of raw milk samples from 4 towns revealed varying concentrations of AFM1; however, no statistically significant differences were detected among the samples from these towns at the 95% confidence level, as determined by one-way ANOVA ($P = .102$). As shown in Table 1, the Fiche sample had the highest mean concentration of AFM1 at 0.369 µg/L, followed by G/Guracha at 0.210 µg/L, Sululta at 0.195 µg/L, and Degam at 0.162 µg/L. However, the highest individual measurement of aflatoxin was found in the Degem milk sample at 2.00 µg/L, followed

Table 1. AFM1 (mean ± SD, µg/L, n = 2) in raw milk from different sites determined by UHPLC.

LOCATION OF RAW MILK	NUMBER OF SAMPLES	CONTAMINATED SAMPLES		EXCEEDING EC REGULATIONS > 0.05 (µg/L)				
		NUMBER	%	NUMBER	%	RANGE	AVERAGE	SD
Sululta	23.0	17.0	73.9	13.0	56.5	0.0100-1.42	0.195	0.0139
Fiche	22.0	21.0	95.5	14.0	63.6	0.0100-1.57	0.369	0.000496
G/Guracha	22.0	21.0	95.5	19.0	86.4	0.0170-0.82	0.210	0.00197
Degem	23.0	17.0	73.9	7.00	30.4	0.0100-2.00	0.162	0.0285
Total	90.0	76.0	84.4	53.0	58.9	0.0100-2.00	0.234	0.00794

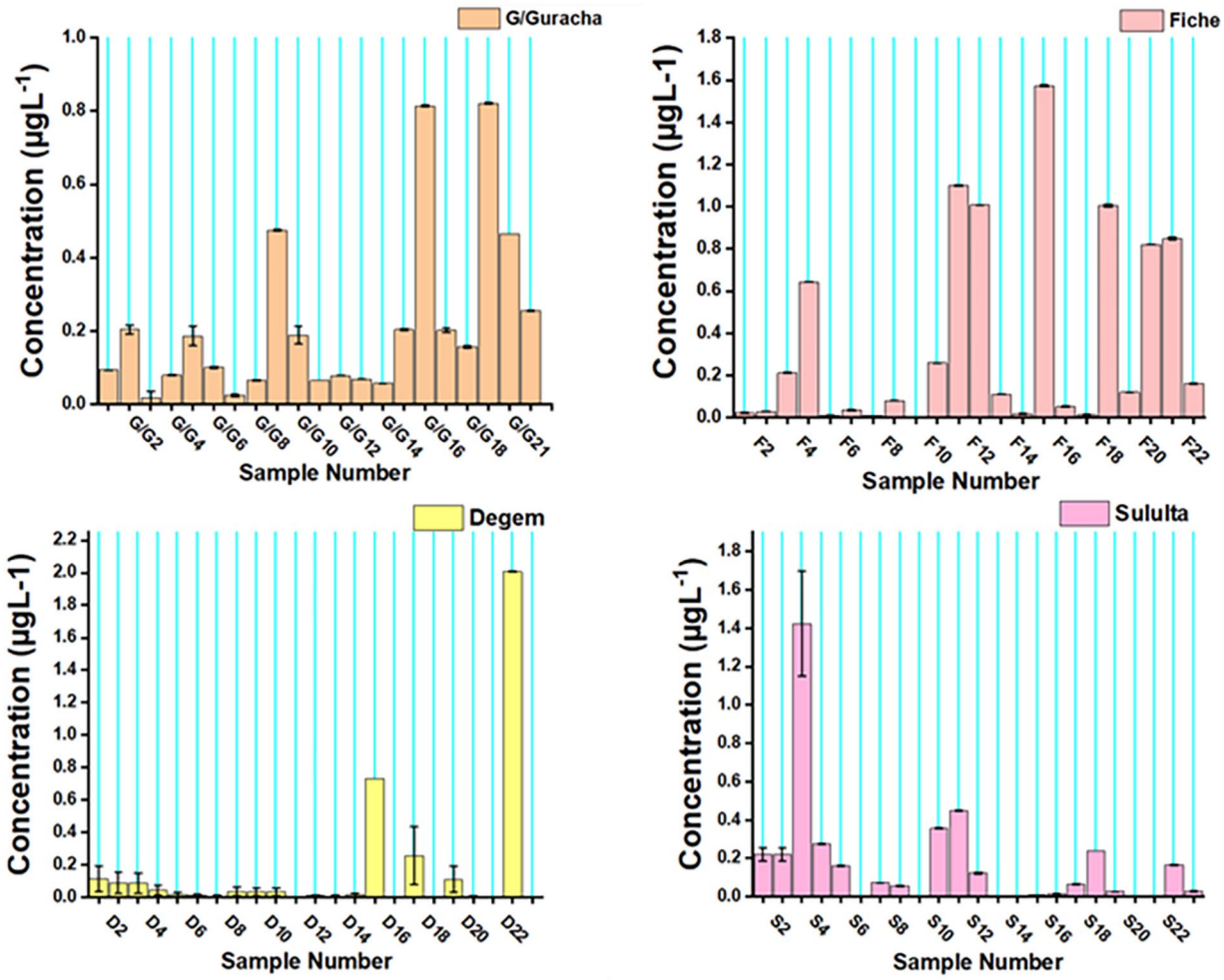

Figure 3. Shows the AFM1 values of individual samples (mean ± SD, µg/L, n = 2) collected from 4 towns.

by Fiche at 1.52 µg/L, Sululta at 1.42 µg/L, and G/Guracha at 0.820 µg/L (Figure 3). The detection of AFM1 in milk samples is likely linked to the presence of AFB1 in animal feed, which is prone to mold contamination. Factors such as local climate, high humidity, and inadequate storage practices may further promote the proliferation of aflatoxigenic fungi in animal feed.[15]

Comparison of the results of this study with those of other studies. The presence of AFM1 contamination in milk samples has been documented in Ethiopia and has also been reported worldwide, as shown in Table 2. The results of this study indicate lower levels of AFM1 in milk samples compared to those reported by Gizachew et al[27] (0.0280 and 4.98 µg/L).27 Similarly, research focused on the reduction of AFM1 levels during the production of traditional Ethiopian fermented milk (Ergo) reported higher concentrations of AFM1 (1.47-5.27 µg/L) in milk samples than those found in the current study.[46] Furthermore, several countries, including Ghana,[15] Iran,[17,36,47] Tunisia,[48] Malawi,[49] and Chile,[50] have reported

Table 2. The concentration of AFM1 in milk samples from various regions in Ethiopia and from reported literature.

TOWNS(LOCATIONS)	SOURCES OF MILK	NUMBER OF SAMPLES	POSITIVE SAMPLES (%)	RANGE (μg/L)	REFERENCES
North Shewa Zone (Fiche, G/Gurracha, Dagem, and Sululta)	Dairy farmers	90.0	84.3	0.0100-2.00	This study
Iran	pasteurized milk			0.00600, 0.0100	Hasninia et al[17]
	Raw milk			0.0490, 0.0140	
South Gondar Zone	Dairy farmers	100.0	99.0	0.0310-5.16	Admasu et al[26]
The greater Addis Ababa Milk Shed,	Dairy farmers	100.0	100.0	0.0280-4.98	Gizachew et al[27]
	Milk traders	10.0	8.00		
Iran	Cheese	2143	72.4	0.160	Massahi et al[44]
Hawassa	Dairy farmers	25.0	20.0	1.47-5.27	Shigute and Washe[46]
Awi Administrative zone (Injibara Town)	Individual farmers	20.0	15.0	0.0460-0.220	Kassa[51]
Gurage Zone,	Dairy farmers	10.0	68.0	0.0200-0.310	Besufekad et al[52]
Central Highlands of Ethiopia (Holetta, Bishoftu, and Hawassa)	Dairy farmers	45.0	71.1	0.00-0.146	Shuib et al[54]
Bishoftu	Milk Pasturized (Industrial)	56.0	100.0	0.555-1.41	Tadesse et al[56]
	Milk Raw (Local)	52.0		0.0290-2.16	

higher AFM1 values in raw milk samples than those observed in this research.

On the other hand, the results of this study exceed those of a previous study that focused on the detection of AFM1 in raw cow's milk in Injbar, Ethiopia, which reported concentrations between 0.0460 and 0.220 μg/L[51]. Furthermore, a study assessing aflatoxin levels in raw milk from various value chain actors across three Ethiopian regions found a concentration of 0.285 μg/L.[7] Another research effort in the Gurage Zone of Ethiopia identified aflatoxin levels in milk and animal feeds at 0.0200 and 0.310 μg/L, respectively.[52] Additionally, Gebrehiowt[53] reported a concentration of AFM1 in milk (0.0880 μg/L) from Bishoftu, followed by Hawassa (0.0570 μg/L) and Holetta (0.0170 μg/L) sites in smallholder urban dairy producers. Mollayusefian et al[3] reported lower level of AFM1 contamination in raw cow milk compared to the finding of this study, with concentrations ranging from 0.0110 to 0.440 μg/L. Additional research conducted in Malaysia,[54] central-southern China (0.00530-0.0362 μg/L),[55] Iran (0.0315 μg/L)[17] and Hungary (30.7 μg/L)[42] also indicated lower levels of AFM1 in raw milk than those observed in the present study. Nevertheless, the results obtained in this study align closely with those from a separate investigation into AFM1 presence in milk and dairy products sold by both local and industrial producers in Bishoftu town, Ethiopia.[56] They reported that 100% of 52 raw milk samples were positive for AFM1. The highest AFM1 content was 2.16 μg/L and the lowest was 0.0290 μg/L; both were obtained from local milk producers, which is comparable to the results of the current study. The levels of AFM1 contamination in milk are affected by various factors, including climatic variations, seasonal variations,[57] geographical conditions, methods of harvesting and storing animal feed, different quantification techniques for AFM1 in milk, as well as transport, storage, processing, and packaging methods, which may explain the variations in aflatoxin contamination across different regions.[3,36]

In Ethiopia, the risk of human exposure to AFM1 contamination in milk is a pressing concern, particularly due to the common practice among dairy farmers of using a variety of mixed concentrate feeds. These feeds often include traditional brewery byproducts (atela), wheat bran, noug (Guizotia abyssinica) cake, maize grains, and silage, all of which are intended to boost milk production. However, these feed components are susceptible to AFB1 contamination. Kaur et al[58] indicate that the primary source of AFM1 in milk is animal feed containing AFB1. To mitigate the toxic levels of AFM1 and the health risks associated with the consumption of raw milk, numerous strategies have been documented worldwide. These strategies encompass biological methods (employing microorganisms such as bacteria and yeast), physical techniques (such as cold plasma and ultraviolet light), and chemical methods (utilizing clays like bentonite, aluminosilicates, and zeolite).[59] Among these approaches, incorporating feed additives into animal diets is recognized as the most effective, practical, and cost-efficient solution, alongside preventive measures that focus on educating dairy producers about appropriate livestock feed management and storage practices.

Table 3. AFM1 exposure among adult populations in 4 towns across Ethiopia.

LOCATIONS	AVERAGE INTAKE (L/DAY)	AVERAGE BODY WEIGHT (KG)	AFM1 (μg/L)	EDI (NG/KG BW/DAY)	MOE	HI	CANCER RISK (CASES/10^5 PERSON/YEAR)
Degem	0.250	65.0	0.162	0.623	6419.5	3.116	0.001960
Fiche	0.250	65.0	0.369	1.42	2818.9	7.095	0.004464
Sululta	0.250	65.0	0.195	0.750	5333.3	3.750	0.002360
G/Guracha	0.250	65.0	0.210	0.808	4952.3	4.0385	0.002541
Average	0.250	65.0	0.234	0.900	4444.4	4.500	0.002831

Estimation of daily consumption of AFM1. The EDI values of AFM1 from raw milk consumption across four locations in Ethiopia are presented in Table 3. In this study, the calculated EDI values of the adults' population consuming raw milk varied between 0.623–1.419 ng/kg bw/day. Although the BW and IR parameters for determining the EDI are alike, the EDI values in Fiche differ from those in other towns due to varying AFM1 concentrations. The EDI value measured in Fiche areas exceeded 1 ng/kg bw/day, indicating a substantial risk of AFM1 exposure through the consumption of raw milk. Meanwhile, Degem, Gebre Guracha, and Suluta have EDI values that fall within the allowed range. The current study reported mean EDI value that is line with previously reported mean EDIs of 0.920 ng/kg bw/day in Iran.[47] However,earlier research in Ethiopia estimated the average EDI for adults as 0.700 ng/kg bw/day, based on an average weight of 57.3 kg, a daily raw milk consumption of 0.130 kg, and AFM1 concentration of 0.320. This value is slightly lower than the current study's finding of 0.900 ng/kg bw/day.[7] Similarly, Ghana's notably low EDI values, ranging from 0.0600 to 0.190, indicate a relatively limited health threat associated with AFM1 in milk, possibly attributed to rigorous monitoring measures.[15] In contrast, EDI values calculated based on Ethiopian food dietary guidelines reported 25.2, 10.5, and 3.80 for children aged 2 to 5, 6 to 9, and adults over 18 years of age by Hiwot et al, which is higher than the estimated value of the current study (0.900 ng/kg bw/day).[34] Similarly, a study by Wu et al,[60] reported the estimated intake amount of AFM1 by Tunisian adults through raw cow milk consumption was 32 ng/kg bw/day. The differences in AFM1 exposure assessments worldwide may be due to differences in EDI-affecting parameters (such as BW, IR, and C-AFM1).[44,61]

Risk characterization of AFM1 exposure. The MOE serves as an indicator of the health risks associated with carcinogenic and genotoxic substances present in food. A MOE value of 10 000 or greater is interpreted as indicating a low risk to public health. The MoE values recorded for raw milk consumers in Degem, Fiche, Sululta, and G/Guracha towns were 6419.5, 2818.9, 5333.3, and 4952.3 respectively. The obtained MoE values are less than 10,000, which indicates a concerning risk level for HCC within the community due to AFM1 exposure. Consistent with our findings, Kortei et al[15] reported MOE values ranging from 197 to 6666.7, which also fell below 10 000, indicating a public health concern associated with the consumption of raw cow milk. Additionally, Zebib et al[7] found that the MOE in the examined Ethiopian regions was below 10 000 among adult populations, highlighting a potential public health risk stemming from elevated AFM1 exposure through raw milk consumption. Similarly, Hassouna et al[48] showed that MOE values obtained were lower than 10,0000 indicating that exposure to AFM1 may increase the risk of developing HCC, which is a serious public health concern. Additionally, Conteçotto et al[33] reported MOE values for AFM1 ranging from 728 to 239, significantly below the safety margin of 10 000. Contrary to our findings, the MOE values for Armenia consumers' exposure to AFM1 were significantly higher than 10 000 among the studied adult population, indicating that there is no health risk associated with AFM1 exposure through consuming milk.[62] Similarly Roila et al[63] and Milićević et al[64] reported higher MOE values for children, adolescents, adults and the elderly that are significantly higher than 10 000, therefore attesting to the absence of health concerns in relation to these age classes.

The average cancer risk estimates for the towns of Degem, Fiche, Sululta, and G/Guracha were found to be 0.00118, 0.00268, 0.00142, and 0.00153 cases per 100 000 individuals per year, respectively. These findings exceed those reported by Omeragic et al[65] in Bosnia and Herzegovina, which indicated an average risk of 0.0000640 to 0.0000740 cases per year per 100 000 individuals. Additionally, a study conducted in Iran indicated that the risk of HCC for milk consumers was 0.000600 in adults, which is lower than the findings of the current study.[36] This finding is also less than the estimates provided by Kortei et al,[15] who assessed the exposure of the adult population in Ghana (ages 18-64) to be between 1.94×10^{-3} and 6.14×10^{-3} ng/kg bw/day. Additionally, a study in Ethiopia focusing on AFM1 risk assessment reported values of 0.180, 0.0400, and 0.0100 per 100 000 population for children aged 2 to 5, 6 to 9, and adults over 18, respectively, which are higher than the findings of the current study.[34] The findings of this study indicate that the carcinogenic risk associated with AFM1 exceeds 0.0000100, suggesting a significant cancer risk at the current levels of contamination and exposure to AFM1.

The calculated HI values for raw milk were found in the range from 1.87 to 4.26, with an average of 2.70. These HI values indicate a significant risk associated with milk

consumption due to AFM1 contamination. Consistent with our findings, Kiani et al[61] reported higher HI values for infants < 6 months, and Branch et al[47] reported higher HI values (4.50) for adults, and Kaur et al[58] reported HI value high for milk (11.5), underscoring a public health issue linked to the intake of raw cow milk. Additionally, higher HI values (18.9) reported in Ethiopia suggest a considerable health risk related to the consumption of raw milk.[34] The HI values observed in this study exceed those reported in Iran (0.535),[66] as well as those documented by Buzás et al[42] in Hungary (0.130-0.450). The evaluation of health risk parameters for AFM1, which encompasses HI, MOE, and CR, reveals significant health concerns for adults in the studied areas. The elevated concentrations of AFM1 found in raw milk samples indicate that lactating cows have likely been subjected to substantial amounts of dietary AFB1. Therefore, it is essential to investigate preventive measures to reduce AFB1 levels in animal feed. Moreover, to mitigate potential health risks for consumers related to AFM1 exposure, there is an urgent need for more comprehensive and frequent monitoring of AFM1 levels in milk, alongside the establishment of regulatory frameworks governing mycotoxins.

Conclusion

In this study, different concentrations of AFM1 was detected in raw cow milk samples collected from 4 study locations within the North Shewa Zone, exhibiting considerable levels of contamination. The overall mean of AFM1 concentration in raw cow milk was 0.234 µg/L. The results of this study indicated that 59.2% of raw milk samples had AFM1 levels exceeding the 0.0500 µg/L limits set by the European Commission. Furthermore, when evaluating health risk assessment indicators such as EDI, MOE, HI, and CR, it became evident that there is a significant health risk to the adult population due to AFM1 exposure through milk consumption in this area. Therefore, it is imperative to implement stringent management and monitoring practices for livestock feeds, along with regular inspections of dairy products, to mitigate AFM1 contamination and safeguard public health and economic interests. Furthermore, it is highly recommended that additional research be conducted on the seasonal variation of AFM1 contamination in milk and the related risks for consumers. This investigation is essential for a comprehensive assessment of the situation, particularly concerning the risks posed to children, who represent a significant portion of milk consumers. This study is, therefore, crucial as it addresses a significant public health concern and offers essential insights for policymakers, health professionals, and consumers aimed at enhancing food safety.

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Author Contributions

The study was carried out by Girma Selale, Argachew Nugussa, Gezahegn Faye, and Girma Ragasa. The manuscript was authored and the figures and tables were prepared by Girma Selale, while Argachew Nugussa, Gezahegn Faye, and Girma Ragasa provided additional insights and discussions regarding the article. All authors have reviewed and consented to the final version of the manuscript for publication.

Ethics Declarations and Ethical Approval

All the authors have read, understood, and complied with the statement on "Ethical responsibilities of Authors," as found in the Instructions for Authors.

ORCID iDs

Girma Selale Geleta https://orcid.org/0000-0002-1726-8869
Gezahegn Faye https://orcid.org/0000-0002-4431-8833

Availability of Data and Materials

All the data generated in the study are included in the manuscript.

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