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Determination of the level of pesticide residues, heavy metals and physicochemical compositions of cow's milk in Hawassa city, Ethiopia

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Abstract: *The study was primarily aimed at determining physicochemical composition, heavy metal contents and pesticide residues of the raw cow's milk samples collected from different milk sources of Hawassa city, Ethiopia. All samples were collected using proportional random sampling method. The concentrations of some selected metals (Zn, Cu, Mn, Co, Ni, Cd, Cr and Pb) were determined by using Flame Atomic Absorption Spectrophotometer. Fe was determined by using Ultraviolet-Visible Spectrophotometer. Pesticide residues were determined by using Gas Chromatography coupled with Mass Spectrometer Technique. The mean values of density, pH, titratable acidity, moisture, total solid, ash, solid not fat, fat and protein contents of milk samples collected from different milk sources (peri-urban dairy producers, small scale urban dairy producers and shop milk) were ranged from 1.02-1.029 g/mL, 5.45-6.75, 0.16-0.83%, 86.1-88.7%, 11.3-13.2%, 0.76-0.81%, 6.7-8.8%, 4.33-4.58% and 2.94-3.4%, respectively. The mean concentration of Fe, Zn, Cu and Mn of milk sample collected from milk sources (peri-urban dairy producers, small scale urban dairy producers and shop milk) were ranged from 0.474-0.569 mg/L, 0.748-1.37 mg/L, 0.055-0.129 mg/L and 0.0339-0.037 mg/L, respectively. The percentage recovery for metal analyses was ranged from 89 – 99.1%. The mean values of metals of the raw cow's milk samples in the study area were found to follow the decreasing order; Zn>Fe>Cu>Mn. The contents of metals are below maximum permissible limit as compared with standard levels except Fe. The estimated daily intake values for Fe, Zn, Cu and Mn were lower compared to permissible values. This indicates that the raw cow's milk samples of the study area have deficiency of these metals. Heavy metals (Pb, Co, Ni, Cd and Cr) are below detection limit which indicated that the raw cow milk samples in the study area are safe for consumption. The found physicochemical analysis results discovered that all the collected milk samples satisfied the national and international standards except titratable acidity and pH values of shop milk samples which are beyond the permissible ranges. The limit of detection and limit of quantification of the pesticides were ranged from 0.0008 to 0.004 µg/g and 0.0025 to 0.014 µg/g, respectively while the recovery percentages were ranged from 75-105%. The pesticide residues in all samples were found to be below detection limits which indicated that the raw cow milk samples in the study area are safe to toxicological risk.*

Keywords: *Cow's milk; FAAS; GC-MS; Heavy metals; Milk quality; Physiochemical analysis; Pesticide residue*

1. Background of Study

Farmers extensively used pesticides to increase crops, vegetables and fruits production. Intensive usage of pesticides has resulted in trace contamination of air, water and soil with their residues. The residues of these pesticides can be absorbed by milk producing animals such as cows through contaminated feed, water and inhaled air. Milk is the most versatile organic food product of animal origin. Pesticide residues being highly lipophilic are primarily stored in fatty tissues in cows and later excreted through milk fat. As a result, consumers are at a risk of exposing to these pesticide residues as they are accumulated in fresh milk and fat rich dairy products (Shanmugam et al., 2015; Chizzolini et al., 2005). The people having regular exposure to pesticides are likely to develop skin and eye related problems. These pesticides affect the nervous system, cause cancer, mimic the hormones and lead to death in severe cases (US EPA, 2014). Although milk is an ideal source of micro and macro elements, additional amounts of contaminants like heavy metals might enter the milk and dairy products reaching levels that are harmful to humans. Industrial, mining, agricultural activities (pesticides and fertilizers), use of new technologies, urban emissions and many other activities may contribute towards their significant accumulation in air, water, soil and subsequently, taken by animals or plants and then find their ways to enter the food chain, particularly the dairy products as a result of bioaccumulation (Rahimi, 2013). They cause both acute and chronic effects for organism's especial human beings. Toxic heavy metals cause damages by enhancing the production of free radicals in several organs (brain, liver, kidney and heart) and interfering with cellular mechanisms against oxidation. Their toxicity is largely related to age, sex, routes of exposure, daily intakes, duration of exposure and frequency of intake. These residues in milk are of particular concern even in low concentrations because milk is largely consumed by infants and children (Anadose et al., 2014; Krishnamoorthy et al., 1999). Peri-urban dairy producers (farmers) and a number of small-scale urban dairy producers were found in Hawassa city, Sidama region. Over this area, farmers potentially practice agricultural activities for consumption purpose as well as a major source of income. The major food produce in this area are different crops, vegetables and fruits. Therefore, pesticides are frequently used to control pests. Farmers feed different crop, fruit and vegetable residues for animals to increases milk production. In addition, pesticides applied to crops as well as vegetables and fruits to control pests which transported from agricultural land into water bodies (mainly, surface water and ground water) and graze land through erosion subsequently, animals are ingest grass and water as result of pesticide residues enter animal bodies and causes contamination of milk and the milk products. In addition, urban dairy farm producers produce milk more than small dairy farm producers because they feed their cows not only grass, crop residues, fruit and vegetable residues but also mixed concentrate feed. They used mixed concentrate feed excessively which enhance the production of milk and milk products. These forages are potential source for pesticides and heavy metals. They supply milk and milk products for the consumer that found in the town and currently, more than five hundred thousand of Hawassa city community are dependent on these products. However, it is unquestionable that the milk and its products could be contaminated by pesticide residues and the metallic substances possibly originated from the cows' feed or any one of the sources indicated above. In study area, industrialization is growing very rapidly as well as agricultural activities also spread over. Farmers that found nearby the city frequently used pesticides and fertilizers to enhance the production of agricultural products. Pesticides and fertilizers may contain metals in compound form which found in the soil, water and plants etc. Subsequently, they exist metallic form as result of decomposition due to environmental agent such as temperature and action of microorganisms etc. (Bedaso et al., 2016; Tancin et al., 2016). These are possible sources of contamination for cow's milk and the milk products. As a result, the large population of Hawassa city and the surrounding inhabitants, frequently feeding on these products, could be at risk of pesticide residues and heavy metals contaminations for human health. To detect these pesticide residues and heavy metals meticulously; more sensitive and selective techniques are required such as GC-MS and FAAS, respectively. In this regard, research is important to provide recent information on physicochemical compositions and the level of the contaminants in the cow's milk since there is no recent and enough information and no such research has been conducted on pesticide residues, selected metals and physicochemical compositions in the raw cow's milk in the current study area. Therefore, this study was aimed to determine physicochemical composition, levels of pesticide residues and some selected metals in the raw cow's milk samples collected from peri-urban dairy producers(farmers), small scale urban dairy producers and shop milk which are found in Hawassa city, Sidama region, Ethiopia. Therefore, the study is important and timely to get information on safety to environment and human. The study was conducted with the main objective; to determine the level of pesticide residues, selected metals and physicochemical compositions in the raw cow's milk at Hawassa city.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The study was conducted in and around Hawassa city; Sidama region located 285 km to south of Addis Ababa, Ethiopia. The study area was selected based on the dependence of farmers on livestock production mainly from cattle's and the availability of dairy farm producers. It lies within Latitude 7° 15 0'N, Longitude 38° 45 0'E and Altitude of 1708 m.

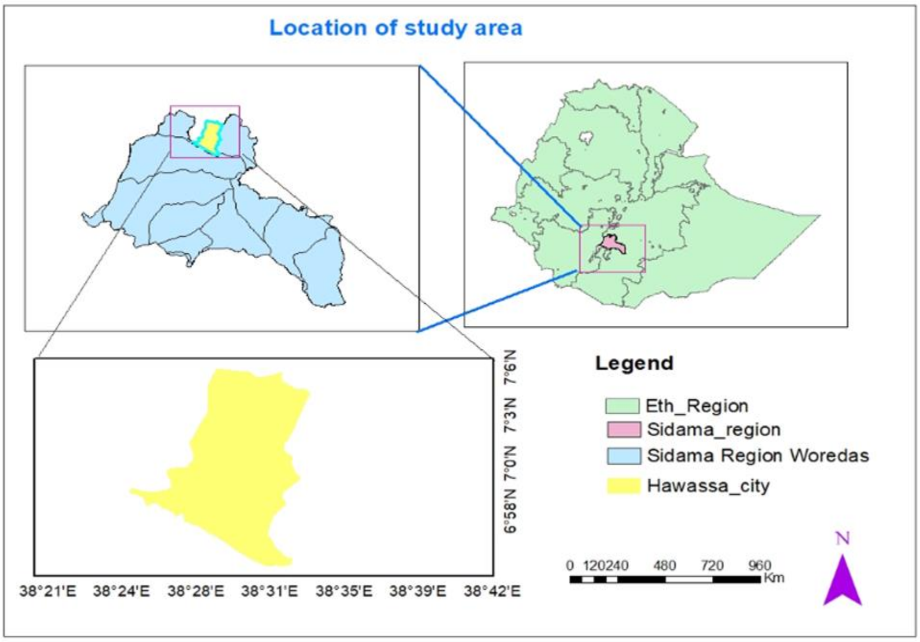

Figure1. *Map of the Study Area*

2.2. Design, Sample Collection, Storages and Pretreatments

From the study area (Hawassa city), five peri-urban dairy producers (farmers) were selected randomly from the location (namely, Chafe). Five lactate cows of peri-urban dairy producers were randomly selected from the location and a composite sample representative were taken for the study.

In addition, one small scale urban dairy producer was selected randomly from the location (namely, Dato). Five lactate cows from small scale urban dairy producer were selected randomly and a composite sample representative were taken for the study. Finally, four milk shops were selected randomly from Hawassa city (Atote, Alamura, Mobeli and Wukiro) and one composite sample representatives were taken for the study.

Milk sample of 100 mL was collected during morning milking time from each cow of peri-urban dairy producers and small-scale urban dairy producer. In addition, 500 mL of raw cow's milk sample was collected from selected shop milk. Then it was mixed and homogenized separately and was kept in an ice box. Finally, 300 mL of five composite fresh milk samples representative were used for the study. A total of five the raw cow's milk samples from peri-urban dairy producers (farmers), small scale urban dairy producer (dairy cooperative milk production center) and shop milk were collected in the study areas. The udder of each cow was washed with distilled water before milking. The fresh cow's milk samples were collected in properly washed and cleaned sample bottles with tight fitting screw caps. The bottles were rinsed with milk samples, filled and sealed, in such a way that no air bubbles remained in the bottles. All milk samples were labeled and transported to laboratory and stored in refrigerator at -20°C until analyses (Enb and Donia, 2009). All analysis was done within 72 hours. Samples were collected in the month of April 2021. To minimize experimental error for analysis of pesticide residues and selected metals, each sample was three times extracted and three times replicated during digestion, respectively. Finally, each composite sample was three times replicated for analysis of pesticide residues, selected metals and physicochemical composition.

Figure2. Show that the Composite the Raw Cow's Milk Sample Collected from Shop Milk, Farmers or Peri-urban Dairy Producers (PUDP) and Small-Scale Urban Dairy Producers (SSUDP) for Physicochemical Compositions, Selected Metals Analysis and Pesticides Analysis

2.3. Chemicals and Reagents

All Reagents and chemicals used in the analysis were of Analytical Grade. Pesticide standards were used from JIJE Laboratory Services in Addis Ababa in Ethiopia with purity between 98.2 and 99.5%. Both acetonitrile and n-hexane (99.99%, Sigma Aldrich, Germany) high performance liquid chromatography grade were used. Anhydrous magnesium sulfate, acetic acid and sodium acetate was obtained from Merck (China). Ultrapure water was prepared from a Milli-Q system (Millipore, Bedford, MA, USA). A concentrated nitric acid (69-72%) HNO_3, UNI-Chem. India) and 30% H_2O_2 (UNI-Chem. Chemical Reagent) were used in digestion of milk samples, blank and spiked solutions. Stock standard solutions (Buck Scientific puro graphics calibration standards, USA) containing 1000 mg/L of the metals Cr, Mn, Ni, Cu, Zn, Pb, Co and Cd from which 10 mg/L of intermediate standard obtained were used for preparation of calibration standards of each metal. 1,10 phenanthroline hydrate (99.8%, Sigma Aldrich, Germany), sodium acetate trihydrate (99%, Sigma Aldrich, Germany), ferrous ammonium sulfate hex hydrate (98%, Sigma Aldrich, Germany) was used for the determination of Iron metal in the milk sample. Throughout the laboratory analysis deionized water was used for sample preparation, dilution and rinsing apparatus before analysis.

2.4. The Raw Cow's Milk Sample Extraction and Clean-Up Procedure for GC-MS Analysis

Extraction of Milk Samples: 10.00 mL amount of homogenized sample was placed into a 50 mL centrifuge tube. A 10 mL aliquot of acetonitrile was then added to each tube, capped and agitated vigorously for one minute. A Bond Elut QuEChERS AOAC extraction salt packet, containing 6 g anhydrous $MgSO_4$ and 1.5 g sodium acetate, was added directly to each tube. Tubes were sealed tightly and agitated vigorously for one minute to ensure that the solvent interacted well with the entire sample and crystalline agglomerates were broken up sufficiently. Finally, sample tubes were centrifuged at 4,500 rpm for 5 minutes.

Dispersive SPE Cleanup: A 2- or 3-mL aliquot of the upper acetonitrile layer was transferred to a Bond Elut QuEChERS AOAC dispersive SPE 15-mL tube. The tube contained 400 mg PSA, 1200 mg of anhydrous $MgSO_4$, 400 mg C18 and 400 mg GCB. A 2 mL of n-hexane was also added to each tube. The tubes were capped tightly and vortexed for 1 minute. The tubes were then centrifuged with a standard centrifuge at 4,500 rpm for 5 minutes. Finally, an aliquot of the upper hexane layer transferred to a 2 mL autosampler vial for injection on GC-MS.

Determination of Pesticide Residues by GC-MS: Pesticide residues were determined by analysis of samples using Agilent Technologies 5977 inert mass selective detector (MSD), Agilent Technologies hyphenated with 6890N GC system.

Analysis conditions were as follows: Chromatographic system was Agilent technologies with 6890N GC; inlet was Split/Splitless mode; detector was Agilent technologies with 5977B MS and column was DB-5 ms, 30 m × 0.25 mm x 0.25 μm.

GC conditions were HP- 5MS capillary column (30 m length X 0.32 mm internal diameter (i.d.), X 0.25 μm film thickness and carrier gas was helium (He) at a flow rate of 1 ml/min. The extract was injected into a single inlet that was split into the dual columns. Instrumental settings were as follows: injector and detector temperatures were 230 ∘C and 300 ∘C, respectively; the gas chromatography oven temperature program was initiated at 100 ∘C for 2 min, raised to 170 ∘C (at a rate of 5 ∘C/min) and held for 10 min, then raised to 220 ∘C (at a rate of 10 ∘C/min) and held for 20 min (with a total run time of 44 min) and the injection volume was 2 μL.

MS experimental conditions were as follows: Ionization mode was electron impact ionization (EI) with 70 eV; EMV mode was gain factor; gain factor was 1; transfer line temperature was 280 °C; ion source temperature was 230 °C; quad temperature was 150 °C; solvent delay time was 3 minutes and acquisition mode were selective ion monitoring mode (SIM). The selective ion monitoring (SIM) mode was used for quantitation purpose and the scan mode was used for identification. Peak area or peak height was used for quantitation. The selective ion monitoring (SIM) mode was designed for targeted pesticides including their retention time windows along with base peak ions. The major monitoring ions, translation and retention time are given in Table 1.

Table1. *The Major Monitoring Ions and Retention Times of Pesticide Residues (OPPs and OCPs)*

No.	Pesticides	RT	Translation	Monitoring ions (m/Z)					
1	Dimethoate	10.701	93	284	249	181	142	93	
2	Profenofos	23.217	339	374	339	318	246	176	
3	Alpha – Lindane	10.190	181	284	249	181	109	93	
4	Hexachlorobenzene	10.314	284	284	249	219	181	142	93
5	Heptachlor	14.905	272	272	237	100			
6	Aldrin	16.813	263	293	263	101			
7	Heptachlor epoxide	19.209	353	353	263	81			
8	Chlordane	20.750	373	373	237	195	170		
9	Endosulfan I	21.543	195	373	237	195	170		
10	4,4'-DDE	23.390	235	339	318	246	176		
11	Endosulfan II	25.572	237	341	237	165			
12	4,4'-DDD	26.114	246	341	235	165			
13	4,4'-DDT	27.710	235	235	165				
14	Methoxychlor	29.671	227	227	212	152			

2.5. Physicochemical Analysis of Milk Samples

All physicochemical analysis of milk samples, such as specific gravity, ash, moisture, total solid, pH, titratable acidity, protein, crude fat and total solid not fat content were determined by to Association of Analytical Chemists (AOAC) methods (AOAC, 2000).

2.6. Sample Digestion and Preparation of Analyte Solution for FAA

Collected milk samples were subjected to optimum wet digestion for mineralization of the milk samples to obtain a clear solution. Optimum condition was obtained by making different trials using various volumes of solvents, times and temperatures of digestion. The 6 ml of freshly prepared 4:2 (2:1, v/v) mixture of concentrated 72%HNO_3 and 30%H_2O_2 was added to each 250 ml round bottomed digestion flask. To Each mixture was then heated on a heating digester for 1.3 h by setting the temperature at 180℃. For each milk sample, triplicate digestions were carried out. Blank digestions, consisting of deionized water and the reagents, were also subjected to a similar digestion procedure (Alem et al., 2015). Six reagent blank samples were prepared for the analysis of the milk samples. All the digested samples were stored in a refrigerator until analysis. All samples were digested in triplicates. The digests were analyzed for the selected metals by using FAAS and UV-VIS in Hawassa University Chemistry Laboratory. Digested samples were aspirated into the fuel-rich air-acetylene flame and the metal concentrations were determined from the calibration curves.

Concentration of iron in the milk samples was determined through the iron- phenanthroline complexing using UV visible spectrophotometer by adjusting the wavelength at 510 nm (ICMR, 1990).

2.7. Method Validation

In present study due to the absence of certified reference materials for milk sample in our laboratory, the validity of the digestion procedure, precision and accuracy of FAAS were assured by spiking the raw cow's milk samples collected from shop milk with standard of known concentration. The spiked and non-spiked milk samples were digested following the same procedure employed in the digestion of the respective samples and analyzed in similar condition.

All the spiked samples were digested in triplicate following the optimized digestion procedure of milk samples. The digested spiked samples were analyzed for their respective metals content using FAAS and UV-VIS. Then the percentage recoveries of the analyte were calculated by (Burns et al., 2002):

$$\%\,Recovery = \frac{C(spiked) - C(non\ spiked)}{C(added)} \times 100\% \qquad \text{Equation (1)}$$

Where; C (spiked) is metal content of the spiked sample, C (non-spiked) metal content of non-spiked sample and C (added) is metal content of metal added.

On the other hand, the precision of the results was evaluated by percentage relative standard deviation of the results of three samples (N=3) and triplicate readings for each sample giving a total of nine measurements for a given bulk sample.

2.8. Statistical Analysis

R software was used for statistical analysis. Statistical evaluations such as mean, standard deviation and relative standard deviation have done for all parameters of milk samples collected from the milk sources. All analyses were carried out in triplicate. Methodological precision was therefore evaluated with standard deviation (SD). Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) to assess significant variation in the mean concentrations of selected metals and physicochemical parameters of the raw cow's milk samples collected from the milk sources (PUDP, SSUDP and shop milk). A Probability level of $p < 0.05$ was considered statistically significant. Pearson's correlation coefficient was used to determine the association between the selected metals as well as physicochemical compositions of the raw cow's milk among the milk sources.

3. Results And Discussion

3.1. Physiochemical Composition of Raw Cow's Milk Samples

Table2. *Physiochemical Analysis of the Raw Cow's Milk Samples (mean ±SD)*

Parameter	Milk source					
	PUDP (Mean ± ^{a}SD)	^{b}RSD (%)	SSUDP (Mean ± ^{a}SD)	^{b}RSD (%)	Shop milk (Mean ± ^{a}SD)	^{b}RSD (%)
Density (g/mL)	1.029 ± 0.001	0.097	1.028 ± 0.0014	0.138	1.02 ± 0.0015	0.15
pH (%)	6.75 ± 0.05	0.74	6.62 ± 0.04	0.66	5.45 ± 0.092	1.7
TA (%)	0.16 ± 0.001	6.25	0.20 ± 0.019	9.28	0.83 ± 0.026	3.19
Moisture (%)	86.1 ± 0.6	0.65	86.8 ± 0.27	0.3	88.7 ± 0.2	0.23
TS (%)	13.1 ± 0.53	4.04	13.2 ± 0.27	2	11.3 ± 0.2	1.8
Ash (%)	0.76 ± 0.017	2.28	0.83 ± 0.015	1.8	0.81 ± 0.02	2.47
SNF (%)	8.8 ± 0.5	5.35	8.62 ± 0.25	2.9	6.7 ± 0.25	3.8
Fat (%)	4.33 ± 0.06	1.4	4.58 ± 0.029	0.63	4.58 ± 0.06	1.26
Protein (%)	3.2 ± 0.38	11.75	3.4 ± 0.13	3.9	2.94 ± 0.29	9.93

*PUDP: the raw cow's milk samples of peri-urban dairy producers (farmers),*UDP: the raw cow's milk samples of small scale urban dairy producers, TA: titratable acidity, TS: total solids, SNF: solid-not-fat, ^{b}RSD: percent relative standard deviation, ^{a}SD: standard deviation, *TS: total solids and, *SNF: solids-not-fat

The physical characteristics such as specific gravity, moisture, pH and TA are important parameters in studying the physicochemical compositions and nutritional aspects of milk. Table 2 shows the various physical parameters of the different milk samples. The specific gravity of milk has previously been shown to be dependent on fat and solids-non-fat (SNF) content in milk, and is normally measured at 25 ℃ (Mohamed, 2004). Milk at normal state has unique physic-chemical properties, which are used as quality indicators. The density/specific gravity of milk among others commonly used for quality test mainly to check for adulteration of water to milk or removal of cream, addition of water to milk reduces milk density, while removal of cream increases it (Mohamed, 2004). From Table 2, mean density values of raw cow's milk in the study area of peri-urban producers, small scale urban producers and milk shop ranges from (1.02 ± 0.0015 to 1.029 ± 0.001) g/mL and is influenced by the relation of its elements, such as fat, lactose, protein, casein, and salts (Mohamed, 2004). The density of milk decreases as the temperature is raised. Increased fat content decreases the specific gravity of milk while increased SNF increases milk specific gravity (Mohamed, 2004).

The density of the raw cow's milk samples collected from shop milk was slightly lower than the raw cow's milk samples collected from both PUDP and UDP. The normal density of milk ranges from (1.028 to 1.033) g/mL (FAO, 1990). The density of shop milk was lower than normal values which are telling of fat skimming off and the addition of water (Rehman and Salariya, 2005). Generally, normally milk has a specific gravity between 1.027 and 1.035 with an average value of 1.032 at 25 ^{o}C mL (FAO, 1990). As observed in the current study the values of the raw cow's milk collected from PUDP and SSUDP were fallen within 1.028-1.032 given to unadulterated milk. However, the values of the raw cow's milk collected from shop milk were below the normal values which indicate that adulteration of the milk (FAO, 1990).

From Table 2, the mean TA and pH values range between 0.16 ± 0.001% to 0.83 ± 0.026% and 5.45 ± 0.092 to 6.75 ± 0.05, respectively. The raw cow's milk samples collected from milk shops are acidic compared with samples collected from peri-urban dairy producers (farmers) and small scale urban dairy producers, which are elsewhere WHO permissible limit for fresh milk (0.17%). This is due to presence of microorganism result in some increment of titratable acidity of the raw cow's milk. Therefore, the possibility that the breakdown of lactose to lactic acid and other acids was responsible for the increase in acidity. A significant decrease in pH and an increase in titratable acidity occurred in shop milk samples (Rehman and Salariya, 2005). However, TA of the raw cow's milk collected from PUDP and SSDP were in WHO limits.

Based on world health organization (WHO) standards and other scientific works, quality milk contains 6.6 pH. The pH values of the raw cow's milk samples collected from milk shops are below allowable limit of WHO and they are somewhat acidic. However, the pH of raw cow's milk collected from SSUDP is comparable with the PUDP (farmers) which are within WHO limit. Physiochemical analysis revealed that the milk samples collected from the milk shops didn't fulfilled the WHO standard in terms of pH and titratable acidity. pH is the parameter that determines the sample acidity and alkalinity and it is one of the parameter which is used to evaluate the quality of milk. At higher pH (up to 7.5) in cases of mastitis than in normal milk of mid–lactation (Rehman and Salariya, 2005).The pH range found in the current study was comparable with the findings in a previous investigation (6.38 ± 0.60 to 6.77 ± 0.88) (Rehman and Salariya, 2005).The results exhibited an average moisture content ranged from 86.1 ± 0 .6% to 88.7 ± 0.2% for milk samples collected from the study area. However, the moisture contents in milk samples collected from shop milk are slightly greater when compared with milk samples collected from both PUDP and UDP. This showed that there is some increment of moisture content of milk samples from shop milk; this is may be due to the addition moisture in the storage material or adulteration and preservation processes (Mohamed, 2004).

The milk samples were also subjected to various chemical analyses and the amount of total protein, ash, fat, were determined as presented in Table 2. Chemical characteristics of samples showed considerable variations and each sample excelled over other in one or other aspect.

From the Table 2, it found that the total solids values are between 11.3 ± 0.2% and 13.2 ± 0.27%. The SNF content of all milk samples are ranged from 6.7 ± 0 .25% to 8.8 ± 0.5%. This value is within the maximum permissible SNF content of WHO standard for quality milk which is 7.71%. The amount of total protein and fat of the raw cow's milk of PUDP, UDP and shop milk were found to be in the range of (2.94 ± 0.29 to 3.4 ± 0.13) % and (4.33 ± 0.06 to 4.58 ± 0.06) %, respectively. Both total protein and fat investigated were within the recommended values. However, the total protein content of the raw cow's milk sample collected from shop milk was slightly less than the raw cow's milk samples collected from both the PUDP and SSUDP. The results demonstrate that, cow's milk is a rich source of protein and casein while the content of total protein and fat were higher in the study area. The milk proteins have the high nutritional value and the principal component of the milk proteins is casein, which constitutes about 75% of all milk proteins (Imran et al., 2008). Beside casein, the other milk proteins are lacalbumin, lactglobulin, etc.

According to current study the protein contents (2.94 ± 0.29% to 3.4 ± 0.13%) are slightly higher as compared with the permissible standards of WHO and other scientific works, which is 2.6%. The study showed that the fat contents of the raw cow's milk samples collected from PUDP (farmers), SSUDP and shop milk, which are good enough and above the minimum standard of WHO which was 3.5%. The white ash is mainly composed of oxides and chlorides of mineral elements, which include lime, magnesia, soda ash, potash, phosphorus oxides, sulfur trioxide, ferric oxide, etc. The ash content values are found between (0.76 ± 0.017% to 0.83 ± 0.015%); these values are slightly higher than that of the maximum permissible limit (0.65%) as reported by (Enb et al., 2009). However, the ash contents in milk samples collected from PUDP are slightly lower when compared with milk samples collected from both UDP and shop milk.

Generally, the results obtained by the physicochemical analysis revealed that all milk samples fulfilled the WHO and other national and international standards except pH and TA content of the shop milk.

3.2. Method Validation

The efficiency of the optimized procedure and analytical method were checked by adding known concentration of each metal in 1 mL of raw cow's milk sample. As shown in Table 3 the results of percentage recoveries for the studied metals in raw cow's milk samples lies within the range of 89 – 99.1%, which is in the acceptable range (80 – 110%), this confirms that, the laboratory performance for each analyte is in control and the optimized wet digestion method is valid (Burns et al., 2002). Therefore, the optimized digestion procedure was valid for the raw cow's milk samples and is believed to remove metal fractions associated with organic matter.

Table3. *Recovery Values of Metals for the Analyzed Milk Samples*

Metals	[a] Conc.in sample (mg/L)	Amount added (mg/L)	[b] Conc.in spiked sample (mg/L)	% [c] Recovery	RSD	[d]Method used
Cu	0.0927 ± 0.0065	0.10	0.190 ± 0.008	97.33 ± 7.095	7.29	FAAS
Mn	0.0383 ± 0.00096	0.10	0.1374 ± 0.001	99.1 ± 0.173	0.175	FAAS
Zn	0.863 ± 0.00436	0.10	0.959 ± 0.0036	98 ± 6.6	6.69	FAAS
Fe	0.485 ± 0.028	1.70	2.003 ± 0.138	89 ± 6.99	7.83	UV - VIS

[a] **values** are mean ± SD of triplicate readings of triplicate samples, [b] **Values** are mean ± SD of triplicate readings of triplicate samples, [c] mean recovery ± SD of percentage recoveries of triplicate reading of triplicate samples, [d] Method is AAS and UV are Atomic Absorption Spectrophotometer and UV-visible spectrophotometer.

3.3. The Level of Some Selected Metals in the Raw Cow's Milk Samples

Among the nine metals analyzed, four metals (Fe, Mn, Cu and Zn) were detected whereas the other five metals (Pb, Cd, Co, Cr and Ni) were below their corresponding method detection limit in all the milk samples in the study area. Probably this was because there are no industries and vehicle emissions which are the basic sources of these toxic heavy metals around the study area. This indicates that the feeds and the water which the cows use are also free from these toxic metals.

The average metal concentrations in the raw cow's milk from the three milk sources (PUDP, SSUDP and shop milk) of Hawassa city were presented in table 4. From the detected elements, the highest mean concentration obtained was range for Zn (0.748±0.029 to 1.37±0.12 mg/L) and lowest range for manganese (0.0339±0.0019 to 0.037±0.0009 mg/L) among all the milk sources in the study area. The mean concentrations of metals detected in the milk samples were collected from different sources (PUDP, SSUDP and shop milk) in the present study can be arranged in the order of Zn (0.748-1.37) > Fe(0.474-0.569) > Cu(0.055-0.129) > Mn(0.0339-0.037) mg/L.

As per recommendations of (WHO, 1996) the presence of zinc in raw cow's milk should be at the level of 3-5 mg/L. Some of the authors (Pechova et al., 2008) also endorse that zinc concentrations should range between 2-6 mg/l. From table 4, we can see that in the entire raw cow's milk samples which were collected from three areas (PUDP, SSUDP and shop milk) the average concentration is below the limited level. However, the concentration of Zn in the raw cow's milk collected from PUDP was less than both SSUDP and shop milk and milk can be considered as deficient regarding this essential element, but the problem lies when this milk is used for infants, to who represents the only source of Zn ions. These low levels are directly associated with animal feeding rations have been poor with nutrition elements. Generally, the concentration of zinc, copper and manganese in this study was below this WHO limit.

Table4. *The Levels of Metals in Raw Cow's Milk Sample (mean ± SD, n = 3)*

Metals	Mean Concentration (mg/L)					
	PUDP (Mean ± [a] SD)	[b]RSD (%)	SSUDP (Mean ± [a] SD)	[b]RSD (%)	Shop Milk (Mean ± [a] SD)	[b]RSD (%)

Cd	[c] BDL	-	[c] BDL	-	[c] BDL	-
Co	[c] BDL	-	[c] BDL	-	[c] BDL	-
Cr	[c] BDL	-	[c] BDL	-	[c] BDL	-
Pb	[c] BDL	-	[c] BDL	-	[c] BDL	-
Ni	[c] BDL	-	[c] BDL	-	[c] BDL	-
Cu	0.129 ± 0.0035	2.7	0.055 ± 0.0056	10.12	0.0637 ± 0.0025	4
Zn	0.748 ± 0.029	3.8	1.37 ± 0.128	9.4	1.28 ± 0.052	4
Mn	0.036 ± 0.001	0.28	0.0339 ± 0.0016	4.7	0.037 ± 0.0009	2.3
Fe	0.569 ± 0.068	12	0.474 ± 0	0	0.480 ± 0.0075	1.6

PUDP: Raw cow's milk samples of peri-urban dairy producers (farmers), **SSUDP:** Raw cow's milk samples of small-scale urban dairy producers, [c] **BDL:** below detection limit**,** [b] **RSD:** Percent relative standard deviation and [a] **SD:** Standard deviation.

Recommended Daily Allowance of Some Selected 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 113 g. The reported values of daily milk consumption in USA and Spain are 224 g and 124 g, respectively (Farid et al., 2004). However, in Ethiopia the daily consumption of milk is very low. It is considered to be 53 mL/day which is calculated from 19 Kg per capita per year (Belete et al., 2014). The value given per year is changed to per day to know the approximate daily intake in Ethiopia. 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 53 g is equal to 53 mL of whole milk.

$$EDI\left(\frac{mg}{day}\right) = Mc \times 53 \text{ ....Equation (2)}$$

Where; Mc = metal concentrations in milk (mg/L), 53 g/day = daily milk consumption rate for Ethiopia (Belete et al., 2014). Assuming a value of 53 mL of milk consumption per day, the daily intake of the detected metals from samples of the present study are determined and depicted in table 5. The 4th column shows the Recommended Dietary Allowance (RDA) as set by different international organizations (Farid et al., 2004).

The daily intakes of detected metals (Mn, Fe, Cu and Fe) in the study area were less than the recommended/permissible levels set by different international organizations. This indicates that the cow's milk of this area is poor source of these metals (Mn, Fe, Cu and Fe). Any way milk is not a significant part of the Ethiopian diet particularly for adults and this deficiency can be compensated by intake of these metals through other staple Ethiopian food items.

Table5. *Comparison of Daily Intakes of Some Selected Metals from 53 mL Milk by Ethiopian Population with Recommended/ Permissible Values*

Metals in the raw cow's milk	**Concentration (mg /L)**	**Daily intake values of metals in present study (mg /day)**	**Recommended/ permissible value (mg/day)**	**Reference**
Copper	0.055-0.129	0.00127-0.00297	2-3	[Farid et al.,2004;Belete et al., 2014]
Zinc	0.748-1.37	0.0397-0.0726	12-15	[Farid et al.,2004;Belete et al., 2014]
Manganese	0.0339-0.037	0.001797-0.00196	2-5	[Ogabiela et al., 2011]
Iron	0.474-0.569	0.0251-0.03016	0.27-27	[Farid et al.,2004;Belete et al., 2014]

3.4. Comparison of the Concentration of Some Selected Metals and Physicochemical Compositions of the Raw Cow's Milk Samples with Literatures Values

There can be dissimilarity in sampling, sample preparation and analysis methods even though various chemical analysis target to a similar objective. Bearing in mind all these, the result of the present study can be compared to the findings of other study findings. Different reports showed that the metal content (Zn, Cu, Mn and Fe) and physicochemical compositions (pH, density, TA, moisture, ash, TS, SNF, fat and protein) of the raw cow's milk samples. The comparisons made for metals content and physicochemical compositions are shown below in table 5 and 6, respectively. As the comparison in the table 5 showed most of the values reported in the literature are higher than the present study**.** There are wide variations in the published data for the elemental concentrations of the raw cow's milk of different countries as shown in table 5. The concentration of Zn in the present study (0.748-1.37 mg/L) was high compared with the corresponding values of the countries such as Saudi Arabia (0.944 ± 2.4 mg/L). The concentration of Zn in the present study was slightly greater compared with the corresponding values of the country such as Croatia (0.510 ± 0.160 mg/L). But, it is much lower compared with the corresponding values of the countries such as Egypt (3.146 ± 1.081 mg/L), Poland (3.163 ± 1.25 mg/L) and Ethiopia (5.592 ± 0.092 mg/L). This showed that the raw cow's milk in the present study area was deficiency in Zinc. The concentration of Mn (0.0339-0.037 mg/L) in the present study was also low compared with the corresponding values of remaining countries in the literature. The result of Cu (0.055-0.129 mg/L) was also closer to the report made by Dobrazanski et al. in Poland (0.089 ±125.14 mg/L), Croatia (0.109 ± 0.006) and Ethiopia (0.109 ± 0.006 mg/L). But, it was less than the values of Egypt (0.140 ± 0.116 mg/L) whereas very much high compared to the value reported for Saudi Arabia (0.005 ± 0.6 mg/L). In general, the concentrations of metals detected in the present study were more or less comparable with the reported literature values. However, relatively lower concentration of Zn, Mn and Cu were observed in this study in comparison to the reported values.

The concentration of Fe in the raw cow's milk collected from three milk sources (PUDP, SSUDP and shop milk) was slightly greater than EU recommended limit and comparable with WHO recommended limits whereas the concentration of Cu and Zn in the raw cow's milk collected from three milk sources (PUDP, SSUDP and shop milk) were less than the EU and WHO recommended limits which were presented in the table 6.

Table6. *Comparison of the Concentration of Metals in the Raw Cow's Milk Samples with Literatures (mg/L)*

Countries	**Metals (mg/L)**				**Reference**
	Zn	**Cu**	**Mn**	**Fe**	
Egypt(raw)	3.146 ± 1.081	0.140 ± 0.116	0.056 ± 0.038	NR	(Enb et al.,2009)
Saudi Arabia (raw)	0.944 ± 2.4	0.005 ± 0.6	NR	NR	(Farid et al., 2004)
Poland (raw)	3.163 ± 0.25	0.089 ± 0.0025	0.102 ± 0.056	NR	(Doberzanski et al., 2005
Croatia (raw)	0.510 ± 0.160	0.109 ± 0.006	NR	NR	(Sikiric et al., 2003)
Ethiopia (raw)	5.592 ± 0.092	0.109 ± 0.006	0.427 ± 0.018	NR	(Tassew et al., 2014)
Ethiopia(raw)	0.748-1.37	0.055-0.129	0.0339-0.037	0.474-0.569	This study

The comparison of the physicochemical composition of the present study with other literature values was presented in table 7. In the present study, the average values of density of the raw cow's milk samples collected from PUD and SSUDP and shop milk was slightly lower than the values of specific gravity reported in Ethiopia (1.030 g/mL) by and Pakistan (1.03 – 1.07) by (Mohammad et al., 2008). But, the density of the present study of the raw cow's milk samples was comparable with other report in Ethiopia (1.02 – 1.03 g/mL) by (Endale et al., 2020). All physical compositions values of the raw cow's milk observed in the present study except density and pH were lower than earlier findings of (Endale et al., 2020). However, the moisture content in the present study was greater than earlier findings of (Matela et al., 2019). The pH content in the present study was greater than earlier findings of (Endale et al., 2020) whereas comparable with the earlier findings of (Mohammad et al., 2008). All the chemical composition values of raw milk observed in the present study except ash content and TS were comparable with the earlier findings of (Tassew et al., 2014) who reported fat, protein and solids-not-fat (SNF) values 4.38 ± 0.06, 2.96 ± 0.01 and 7.79 ± 0.6, respectively in urban dairy farms in Jimma. However, the results of the values of fat obtained in the current study were lower than the findings of (Alganesh et al., 2007), who reported 6.05% fat, 14.31 TS and 8.22 SNF. The ash value of the present study was slightly greater than the findings of (Alganesh et al., 2007), who reported 0.70 ashes. The protein content of present study was almost equal with the findings of Alganesh et al., 2007). The fat and protein contents in the present study were greater than earlier findings of (Matela et al., 2019) whereas comparable with the earlier findings of (Endale et al., 2020). The variation in milk composition values between the current and previous findings may be due to breed, nutritional status and health of cows, particularly that of udder health. The mean values of fat (4.33-4.58%) and protein (2.94-3.4%) in this study were comparable with the Ethiopian Standard value of 4.5% and 3.20%, respectively. However, the mean values of fat (4.33-4.58%) and protein (2.94-3.4%) in this study were greater than EU and WHO standard.

The ash content in the present study was greater than the WHO standards. All physical content in the present study were comparable with ES, EU and WHO values except the raw cow's milk samples collected from the shop milk were not fulfilled these national and international standard values in terms of TA and pH which were presented in the table 9. The SNF and TS content of the entire raw cow's milk samples of present study were fulfilled the national and international standard values except the raw milk samples collected from the shop milk which slightly deviate with these standard values.

Table7. *Comparison of the Physicochemical Compositions in the Raw Cow's Milk Samples with Literatures*

Countries	Physicochemical Parameters									Ref.
	Density (g/mL)	**pH**	**TA (%)**	**Moisture (%)**	**Ash (%)**	**TS (%)**	**SNF (%)**	**Protein (%)**	**Fat (%)**	
Ethiopia (raw)	NR	NR	NR	76.08-80.07	0.28-0.95	19.93-23.56	NR	1.95-2.70	1.49-3.50	Matela et al., 2019
Ethiopia (raw)	1.020-1.030	4.98-5.23	0.85-2.21	87.08-89.57	0.41-0.63	10.89-12.22	NR	3.02-4.21	4.51-5.24	Endale and Aragay, 2020
Pakistan (raw)	1.03-1.07	6.38-6.77	0.81-1.44	76.4-86.9	0.40-0.88	12.9-15.8	NR	2.59-3.28	NR	Mohammad et al., 2008
Ethiopia (raw)	NR	NR	0.30	NR	0.70	14.31	8.22	3.31	6.05	Alganesh et al., 2007
Ethiopia (raw)	NR	NR	NR	NR	NR	NR	7.79	2.96	4.38	Janssen, 2020
Ethiopia (raw)	1.02-1.029	5.45-6.75	0.16-0.83	86.1-88.7	0.76-0.83	11.3-13.2	6.7-8.8	2.94-3.4	4.33-4.58	**This study**

Table8. *National and International Maximum Permissible Limits of Metals in the Cow's Milk*

Metals in mg/L	**Zn**	**Cu**	**Fe**	**Mn**	**Ni**	**Co**	**Cd**	**Cr**	**Pb**
EC, MRL	2-3	0.1	0.37	NA	0.1-1	NA	0.0026	NA	0.02
WHO, MRL	3-5	3.5	0.5	NA	0.05	NA	0.01	NA	0.02
This study	0.748-1.37	0.055-0.129	0.474-0.569	0.0339-0.037	BDL	BDL	BDL	BDL	BDL

Source: (EC, 2006 and FAO, 2012)

Table9. *National and International Standards of Physicochemical Composition in the Cow's Milk*

Quality parameters	**ES**	**EU**	**WHO**	**This Study**	**Ref.**
Density(g/mL)	NA	NA	1.028-1.033	1.02-1.029	[ES, 2009; Raf, 2011; FAO, 2007]
Ph	NA	6.50	6.6	5.45-6.75	[ES, 2009; Raf, 2011; FAO, 2007]
TA (%)	NA	NA	0.17	0.16-0.83	[ES, 2009; Raf, 2011; FAO, 2007]
Moisture (%)	86.75	NA	86.75	86.1-88.7	[ES, 2009; Raf, 2011; FAO, 2007]

TS (%)	12.8	12.5	12.75	11.3-13.2	[ES, 2009; Raf, 2011; FAO, 2007]
SNF (%)	NA	8.25	7.71	6.7-8.8	[ES, 2009; Raf, 2011; FAO, 2007]
Ash (%)	NA	NA	0.65	0.76-0.83	[ES, 2009; Raf, 2011; FAO, 2007]
Fat (%)	4.5	3.25	3.5	4.33-4.58	[ES, 2009; Raf,2011; FAO, 2007]
Protein (%)	3.2	2.73	2.6	2.94-3.4	[ES, 2009; Raf, 2011; FAO, 2007]

Pearson's Correlation between Some Selected Metals and Physicochemical Compositions in the Raw Cow's Milk Samples in the Study Area

The relationships between contents of different metals and physicochemical compositions in the raw cow's milk among the milk sources (PUDP, SSUDP and shop milk) of the study area were analyzed by Pearson's correlation coefficient. The correlation analysis is a bivariant method which is applied to describe the relation between two different metals and physicochemical compositions. The high correlation coefficient (near +1 or −1) means a good relation between two variables, and its correlation around zero means no relationship between them at a significant level of 0.05% level, it is strongly correlated, if $r > 0.7$, whereas r values between 0.5 and 0.7 show moderate correlation between two different parameters (FAO, 2006). Table 10 shows correlation coefficients among the different metals in the among the milk sources (PUDP, SSUDP and shop milk) in the study area. Copper of the raw cow's milk was negatively correlated with zinc and positively correlated with manganese and iron. The Cu and Fe were strong correlated in the three milk sources (PUDP, SSUDP and shop milk) of the raw cow's milk in the study area ($r = 0.999$). Zinc of the raw cow's milk among the milk sources (PUDP, SSUDP and shop milk) was negatively correlated with Cu, Mn and Fe.

Table10 shows correlation coefficients among the different milk quality parameters in the milk sources of the raw cow's milk in the study area. Density of the raw cow's raw milk was negatively correlated with fat content and TA. In addition, density of the raw cow's milk was negatively correlated with moisture content and ash whereas positively correlated with solid non-fat, protein and TS. Protein content was negatively correlated with fat content, moisture content and TA. In addition, protein was correlated positively with ash, TS, pH, SNF and density whereas fat was correlated positively with ash moisture and TA. In addition, fat was negatively correlated with SNF, TS, density and pH. On the other hand, moisture was negatively correlated with protein, SNF, density, TS and pH. In addition, moisture content was positively correlated TA, ash and fat.

Table10. Correlation Coefficient Strength among Different Milk Metals Concentrations in the Study Areas

Variables	Cu	Zn	Mn	Fe
Cu	1	-0.99965	0.304894	0.998683
Zn		1	-0.33003	-0.99697
Mn			1	0.255621
Fe				1

Table11. *Correlation Coefficient Strength among Different Milk Physicochemical Compositions in the Study areas*

Variables	Density	Ph	TA	Moisture	TS	Ash	SNF	Fat	Protein
Density	1	0.999	-0.998	-0.987	0.989	-0.337	0.999	-0.585	0.852
pH		1	-0.999	-0.985	0.9905	-0.327	0.999	-0.576	0.858
TA			1	0.978	-0.995	0.2915	-0.999	0.545	-0.876
Moisture				1	-0.952	0.485	-0.982	0.708	-0.757
TS					1	-0.195	0.992	-0.459	0.9204

Ash						1	-0.315	0.9608	0.205
SNF							1	-0.566	0.865
Fat								1	-0.075
Protein									1

3.5. Statistic Data Analysis

The detected metals Fe, Mn, Cu and Zn, can be found naturally in food. They are essential elements and thus, these metals can be found in cow's milk since it is one of the most important types of food. The significance of variation between samples was analyzed using one-way ANOVA (Kyplot software). The mean concentrations of all metals (Fe, Mn, Zn and Cu) were showed significant difference ($P < 0.05$ at 95% confidence interval) between any two milk sources (PUDP/SSUDP, PUDP/Shop milk and SSUDP/Shop milk). This difference match with the accumulation of metals in soil or water, grass at which the cows grassed in each site may be different especial in PUDP and SSUDP. In addition, this difference may happen as result of variation of feeding sources and types of breeding. These indicate that the concentrations of the metals present in the raw cow's milk depend on cow's feed-milk chain. All physicochemical compositions are significant difference ($P < 0.05$ at 95% confidence interval) between any two sample sites (PUDP/SSUDP, PUDP/Shop milk and SSUDP/Shop milk) except the protein content. These variations were happened due to adulteration (affects the density of the milk), growth of microorganisms (affects TA and pH of the milk), removal of cream and also may be variation in feeding sources of the cows.

3.6. Determination of Pesticide Residues Levels

The level of pesticides in raw cow's milk samples collected from the study area to get information on the residue's levels using GC/MS technique.

3.6.1. Detection Limits and Quantification Limit

The limits of detection (LOD) of the pesticides were calculated as three times the standard deviation of the pesticides level in celebration curve. Similarly, the limits of quantification (LOQ) of the pesticides were calculated as ten times the standard deviation of the pesticides level in celebration curve (Shrivastava and Gupta, 2011).

$$LOD = \frac{3 \times SD}{S} \text{ and } LOQ = \frac{10 \times SD}{S} \text{ ....Equation (3)}$$

Where; *SD* is standard deviations response and *S* is the slope of the calibration curve.
According to this study the residues were found to be lower than the detection limits and quantification limits for the two raw cow's milk samples analyzed.

Table12. *Limit of Detection (LOD) and Limit of Quantification (LOQ) of the Analyzed Pesticides*

Pesticides	**LOD (µg/g)**	**LOQ (µg/g)**
Dimethoate	0.004	0.014
Profenofos	0.001	0.003
Alpha – Lindane	0.001	0.0031
4,4'DDD	0.0025	0.005
4,4'DDE	0.0012	0.0039
4,4'DDT	0.0021	0.007
Endosulfan I	0.0014	0.005
Endosulfan II	0.0024	0.008
Heptachlor	0.00109	0.004
Heptachlor epoxide	0.0013	0.0042
Hexachlorobenzene	0.0019	0.0064
Aldrin	0.0008	0.0025
Methoxychlor	0.0032	0.0107
Chlordane	0.0014	0.0046

The LOD and LOQ were range from 0.0008 to 0.004 µg/g and 0.0025 to 0.014 µg/g, respectively and for the residues analyzed while the recovery percentages were range from 75-105%.

3.6.2. Residual Pesticides Level

The European regulation 149/2008 EC set the limit at 0.01 mg/kg for substances for which no MRL had been established. According to the reported, no detectable residues of the target pesticides (OPPs and OCPs) were found in the analyzed milk. This indicates that the quantified residues in the raw cow's milk samples were below LOD, LOQ and MRL. This finding indicates that the raw cow's milk from the study area is safe for consumption. The European Commission and FAO/WHO have set new MRLs, which mostly are between 4 and 50 ng·g-1 in milk which are given in table 13 (EU, 2008; FAO, 2006). The results of this study revealed that the raw cow's milk samples collected from the study area were free of organochlorines and organophosphorus residues studied. Therefore, the raw cow's milk from the study area is safe for consumption. List of pesticides analyzed and areas of the raw cow's milk sampling with their results presented in the following table 13.

Table13. *Pesticide Residues Findings in Analyzed the Raw Cow's Milk Samples <ND means below the detection limit*

Pesticides	Levels of Pesticide Residues in ppm				
	PUDP (farmers)	**SSUDP**	**Shop milk**	**EU, MRL (µg/g)**	**FAO/WHO, MRL (µg/g)**
Dimethoate	ND	ND	ND	0.01	0.05
Profenofos	ND	ND	ND	0.01	0.01
Alpha – Lindane	ND	ND	ND	0.01	0.001
4, 4' - DDD	ND	ND	ND	0.04	0.02
4, 4' - DDE	ND	ND	ND	0.04	0.02
4, 4' - DDT	ND	ND	ND	0.04	0.02
Endosulfan I	ND	ND	ND	0.05	0.01
Endosulfan II	ND	ND	ND	0.05	0.01
Heptachlor	ND	ND	ND	0.004	0.006
Heptachlor epoxide	ND	ND	ND	0.004	0.006
Hexachlorobenzene	ND	ND	ND	0.01	0.01
Aldrin	ND	ND	ND	0.006	0.006
Methoxychlor	ND	ND	ND	0.01	0.02
Chlordane	ND	ND	ND	0.002	0.002

ND: indicates result less than detection limit, **PUDP:** milk collected from per-urban dairy producers and **SSUDP:** milk collected from small scale urban dairy producers

The estimation of the possible health risks based on EDIs, which is related with exposure to discovered pesticides is not necessary since all pesticides analyzed were less than their individual detection limits and the raw cow's milk from the study area is safe in terms of those pesticides determined according to these findings.

Estimated daily intake (EDI) is determined for the detected pesticide residues in the milk sample following the international guidelines WHO and FAO by using the following equation (FAO/WHO, 2006):

$$\mathrm{EDI} = \frac{\sum \mathrm{C} \times \mathrm{M}}{\mathrm{W}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation(44)}$$

Where, C is the mean of pesticide residues concentration in the milk (µg/kg), M is the milk consumption rate person (53 g per person per day) for Ethiopia, while W is the average body weight of an adult (70 Kg). The hazard index was calculated by dividing the estimated daily intake (EDI) by their corresponding acceptable daily intake (ADI). Since there were no detected residues in the samples, the estimated daily intakes of pesticides in the samples less than detection limit were below the ADIs, which may indicate that the raw cow's milk consumption has a negligible influence to health risk. If the hazard index of the pesticide residue is lower than unity, then the consumer is considered to be adequately safe. The hazard index values showed that all the intakes of pesticide residues remain clearly below the safe limit.

4. Conclusions and Recommendations

4.1. Conclusion

The optimized wet digestion method for the raw cow's milk analysis was found efficient for the metals studied and it was validated through the recovery test and a good percentage recovery was obtained (82.01 – 107.83%). The mean values of metals of the raw cow's milk samples in the study area were found to follow the decreasing order; Zn > Fe > Cu > Mn. From nine analyzed metals, five metals (Cr, Cd, Co, Ni and Pd) were blow detection limit. The residues were below detection limit that indicated safety of the raw cow's milk to consumers. All detected metals are lower than the permissible levels of national and international standards except Fe. Also, EDI of all detected metals are lower than national and international standards. This indicate that the raw cow's milk samples in the study area were showed that the deficiency of these metals.

The outcome of this study showed that, all the milk physicochemical compositions were significantly correlated with national and international guideline standards values except acidity and pH of the raw cow's milk sample collected from shop milk which require more attention to ensure the quality of milk.

The target pesticides (OPPs and OCPs) were not detected in the analyzed milk. This indicates that the quantified residues in the raw cow's milk samples were below LOD, LOQ and MRL. This finding indicates that the raw cow's milk from the study area is safe for consumption. The hazard index values showed that all the intakes of pesticide residues remain clearly below the safe limit. The LOD and LOQ were range from 0.0008 to 0.004 µg/g and 0.0025 to 0.014 µg/g, respectively and for the residues analyzed while the recovery percentages were range from 75-

4.2. Recommendations

Though, the findings of the study shown that there are no significant pesticide residues in the tested the raw cow's milk samples, the fact no pesticides were detected or not exceeded the admitted level does not necessarily mean that farmers are not using pesticides. Even though the results show a negligible risk associated with exposure via the raw cow's milk consumption, a special precaution should be taken with the possible total exposure to these chemicals from various other foods in the future. Additionally, further monitoring studies must be performed using more sensitive analytical instruments not only the studied one but also the other residues in the future.

Additional study should also be undergone on all trace metals by using more sensitive instruments, taking time and increasing economy in the future. The detected metals in the raw cow's milk samples of the study area showed that deficiency of these essential metals which are compensated through feeding of different nutrient rich food and milk producers should feed their cow's different nutrient rich forage which enhances the production of milk as well as nutritional values of the milk.

Physicochemical compositions of the raw cow's milk sample collected from different milk sources were fulfilled national and international standards. However, TA and pH of the raw cow's milk collected from shop milk did not meet national and international standards.

In general, it is not fair to conclude that some of the milk quality parameter sold at Hawassa city, met the minimum legal standards of normal milk. Therefore, it is recommended that introducing different dairy technologies should be supported with a continuous training on how to manage a dairy farm. Stronger milk quality control and quality base payment could help a lot to discourage adulteration.

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