Atomic Absorption Spectrophotometric Determination of Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb), and Zinc (Zn) in goat's milk

S. T. Garba[1]*, Idi, A. M[1]. and Muhammad, Z. C[1].
[1](Department of Chemistry, P. M. B. 1069. University of Maiduguri, Borno State, Nigeria
stelagarba@yahoo.com; abdullahiim91@gmail.comand zchellube@unimaid.edu.ng
***Corresponding author**
Email Address: stelagarba@yahoo.com
Phone +234 070 3035 2060

ABSTRACT
In this study, the elements; Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb), and Zinc (Zn) in goat's milk samples from three different villages (Fulatami, Musari and Gazabure) in Gubio local government area of Borno State were determined.Milk samples were collected from lactating goats in the morning hours at two different phases; first three and six-month period of lactation. Collection was done from late October 2015 to late March 2016 manually.Samples were digested with HCl, after mineralization. Digested samples were analysed using atomic absorption spectrophotometer and the results obtained indicates that, the elements, Zn, Cu, and Pb were observed in all the samples at the three and six-month period of lactation with the highest concentrations of 0.373; 0.102 and 0.181µg/g for Zn, Cu and Pb from respectively. With the exception of Gazabure village, where Cd was observed at the three and six-month periods, the elements was only observed at the six-month period of lactation in milk from FFulatami and Musari villages with the levels; 0.024 and 0.018 µg/g Cd respectively. Chromium was only observed in milk sample from Musari at the six-month period of lactation with the concentration 0.054 µg/g Cr. The study areas are in remote places where there are no or very less traffication talkless of industries for possible emission. Lead (Pb) for instance, the high level observed may be attributed to atmospheric deposition of suspended particulate matter. The levels are found to vary with time for it decreases as the period of lactation increases. In spite of all the milk from the are safe for consumption since all concentrations observed were below the maximum permissible limits.

Keywords *- Environmental, Feeds, Goat's Milk, Heavy metal, Pollution, Soil, Water.*

1. INTRODUCTION

Worldwide, cow milk is the most commonly consumed milk, dominating the world milk production with about 782 million tons in 2013. Thus, 85% of the world milk production is derived fromcattle, followed by milks from other species such as buffalo (11%), goat (2.3%), sheep (1.4%), and camel (0.2%)[1]. Recently, cow's milk and its derived products have suffered poor public perception. People believe it to be high in fat and energy, with consequent negative health effects. In addition, heightened awareness of intolerant and allergic symptoms arising from cow's milk consumption has led those affected to look for alternatives[2].

Milk is a secretion of the mammalian gland, whose physical characteristics and composition vary between species. It is a complex oil-in-water emulsion containing fat, proteins, lactose, minerals,enzymes, cells, hormones, immunoglobulins, and vitamins. The proteins are majorly categorized as insoluble proteins (caseins) and soluble proteins (whey proteins) found in lactoserum. Caseins include αs1, αs2, β, and κ-caseins, although the whey proteins are α-lactalbumin and β-lactoglobulin. Milk also comprises important minor proteins, such as serum albumin, immunoglobulins, lactoferrin, transferrin, calcium-binding protein, prolactin, folatebinding protein, and proteosepeptone [3]. Some proximate composition of cow, goat and sheep milk is as shown in table 1 below.

Table 1: Proximate composition of cow, goat and sheep milk

Parameter	Cow milk	Goat milk	Sheep milk
Moisture (g/100 g)	87.9 ± 0.5	87.6 ± 0.7	82.9 ± 1.4
Fat (g/100 g)	3.3 ± 0.2	3.8 ± 0.1	5.9 ± 0.3
Ash (g/100 g)	0.7 ± 0.0	0.8 ± 0.1	0.9 ± 0.1
Lactose (g/100 g)	4.7 ± 0.4	4.1 ± 0.4	4.8 ± 0.4
Protein (g/100 g)	3.4 ± 0.1	3.7 ± 0.1	5.5 ± 1.1
Casein (g/100 g)	3.0 ± 0.1	2.4 ± 0.1	4.7 ± 0.5
αs1-casein (%)[b]	39.7	5.6	6.7
αs2-casein (%)[b]	10.3	19.2	22.8
β-Casein (%)[b]	32.7	54.8	61.6
κ-Casein (%)[b]	11.6	20.4	8.9

Source: Balthazar et al. [4]

Milk has been part of our staple diet since the agricultural revolution, so eliminating its consumption will have nutritional consequences. It supplies an economical source of nutrients and confers numerous health benefits.Milk is more of a source of nutrients for any neonate of mammalian species, as well as for growth of children and nourishment of adult humans. Apart from its nutritional values, milk borne biologically active compounds such as casein and whey proteins have been found to be increasingly important for physiological and biochemical functions that have crucial impacts on human metabolism and health [5, 6, 7]. Avoidance of cow's milk may not be the only option for those who experience side effects to it. Goats and sheep milk, with its unique composition, could be a valuable alternative.There are nearly numerous breeds of goats and sheep in the world; however, only someis generally raised for their milk purpose.About 600-700 million of dairy goats and sheep are present in the world [8]. They are living in climates ranging from high altitude mountains to deserts [9]. More than 95% of the goat and sheep population is found in developing countries,

Tropical Africa has about 22% and 17% of the total world sheep and goat population of 1,028 million and 765 million respectively[10, 11]. Nigeria is home to about 22.1 million sheep and about 70% of the small ruminants are found in the semi-arid zones of Nigeria and these belong to the agro-pastoral farmers utilizing extensive and semi-intensive management systems[12]. Three main varieties of goat are recognised in Nigeria, the Sahel, Desert or West African. The Sahelian or Desert goat is found along the northern border of Nigeria, particularly in Borno, where it is often known as 'Balami', although this name has not been adopted as it would lead to confusion with the better-known sheep race, [13] uses 'Sahel', which seems appropriate, as this race is distributed from Senegal to Sudan. In Nigeria, the Sahel goat is generally the variety preferred by pastoralists[14]. Whilst, majority of the goat's population in the country are owned by small-holder rural livestock farmers, a few are still in the urban areas[15].Goat's constitute a good source of family income and livelihood, assets andagricultural resources for smallholder farmers [16, 17].

The chemical composition of fresh goat's milk varies over time and among species depending on several factors, such as the stage of lactation, parity, season, environmental temperature, lactation efficiency, animal age and nutrition (forage composition of range-fed animals), genetic factors (species and breed), and diseases of the udder [18, 19]. With increase in the consumption of products of animal origin especially milk, the risk of food borne diseases of humans also increases. The raw food movement, characterized by eating raw rather than cooked food has increased the awareness of consumption of raw food. One product that is commonly distributed in raw form is milk. Milk can become contaminated in many ways.

Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body [20]. Every form of living matter requires these inorganic elements or minerals for their normal life processes[21]. Minerals may be broadly classified as macro (major) or micro (trace) elements. The third category is the ultra-trace elements.The term "heavy metals" has been used in environmental legislation publications related to chemical hazards and the safe use of chemicals[22]. This term is universally used by scientists [23, 24, 25]. Thomton (1995) [26]defined heavy metals as elements with density exceeding 6.0 g cm^{-3} whereas Berkowittz et al. (2008) [27] defined heavy metal as any metallic chemical element that has specific density of more than 5.0 g cm^{-3} and is toxic or poisonous at low concentrations. Concentrations of selected toxic elements,especially of heavy metals in the environment,is generally used as an early indicatorof contamination phenomena, both in the programsof soil quality control and in air qualitymonitoring [28]. Their ecosystem accumulation (water-soil-plant-animal) makes them very toxic and leads to undesirable consequences for live organisms [29, 30]. Animals get access to toxic heavy metals from plants, soil, fodder and water. Mammary gland is the most physiologically active gland which produces milk and therefore, presence of the input and output of heavy metals is clearly reflected in the milk of animals. Lead (Pb) for instance, readily moves into milk with sustained and increased mobilization of maternal skeletal lead during lactation [31]. It has identified that, blood is one of the major medium of transfer of heavy metals into milk[32].

There is less or no information on the heavy metal status in milks, and/or milk product from the study area. The aim of this research work therefore, is to evaluate the concentrations of Cd, Cr, Cu, Co, Ni, Pb and Zn in milk deriving from free-grazing goatsin some villages of Gubiolocal Government Area of Borno State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Gubio town, the headquarters of Gubio Local Government Area is located some 87 kilometers south of Maiduguri in the northern part of Borno,it covers an area of 2,464 Km^2. Gubio lie at lat12.4951ºN, long. 12.7710ºE. It has the population of 152,778 according 2006 census.Gubio town has a livestock market which is located in an open expanse of land beside the tarred road that leads to Mobar and Abadam Local Government Areas. It is not only a camel markets, but it also has huge potentials for selling and buying cattle, sheep, goats, donkeys and chicken, among other livestock.The inhabitants, most of whom are Kanuri, Shuwa and Fulani and a handful of ethnic groups from Borno and other parts of Nigeria, generally depend on seasonal agricultural subsistence farming,Gubio is one of the areas worst hit by desertification in the state. The short fall in rain naturally makes the climate hot and unfavourable for vegetation. Naturally, one wonders how the livestock population survive such harsh conditions. But according to local sources, most times, the animals feed on water storing plants and tree barks [33]

2.2 Milk Samplecollection

A total of 64 goats milk samples were procured fromsix different ranges fromthree different villages (Fulatami, Musari, Gazabure) all in GubioLocal Government Area of Borno State.Raw and fresh milksamples were collected from lactating animals in the morning hours by milking manually. Approximately, 50 ml of milk samplewere collected from each animal in a hygienic manner without further contamination.This procedure was repeatedtwice at an interval of three (3) months for six (6) months.In all the sampling locations, four healthy lactating goats (0 - 6 months of lactation) were selected for the sample collection.Samples collected (in a label plastic sample bottles) were kept in a cooler containing ice, transported to the laboratory and stored in a deep-freezer (below 4ºC) awaiting analysis [34]. All sample collection was done from late October 2015 to late March 2016.

2.3 Sample Preparation and Analysis

Milk samples were prepared according to the method described by Vidovicet al. [35] with minormodifications. Ten milliliters of milk were used. Five milliliters of concentrated nitric acid were added and the suspension was evaporated to dryness. The dish was then transferred to muffle furnace and heated to white ash at 450°C for 12 h. After mineralization, 5 mL of 10% HCl were added. The mixture was heated and the solution was filtered to 25 mL in a volumetric flask and made up to volume using deionized water.

Analysis of the digested milk samples for the heavy metals; lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), Nickel (Ni), selenium (Se) and zinc (Zn) was carried out by atomic absorption spectrophotometer (Shimatdzu Model AA 6200).

3. STATISTICAL DATA ANALYSIS

All statistical analyses were performed using SPSS 17 package. Differences in heavy metal concentrations among the samples were detected using One-way ANOVA. A significance level of $P < 0.05$ was used throughout the study.

4. RESULTS AND DISCUSSION

In goats, as in sheep, the sexual activity is seasonal. Hence, the stage of lactation is closely linked to the season of production. Lactation usually begins in the first months of the year, that is during the winter feeding period, when goats are fed on roughage. After they are turned out to pasture, daily milk yield increases, and later (in the autumn months) it decreases steadily until the dry period. Heavy metal contamination of this highly consumable productis detrimental to human especially infants and/or children. Figure 1below showed the concentrations ofthe heavy metals determined in the milk samples from the three different villages (Fulatami, Musari and Gazabure) of Gubio local Government Area. No significant difference was observed in the level of the heavy metals determined from the three villages at ($P < 0.05$). The results indicated that, the level of the elements vary with time of lactation. For instance, the level of Cd was not detected at first three-month of lactation in Fulatami and Musari but was detected at second three-month of lactation. This results however, is in agreement with the report by Ritianne and Everaldo, [36], their observation was, cadmium was completely absent in the milk samples analysed. In Gazabure village of this study, the concentration of Cd was found to be 0.008 µg/g Cd at the first three-month of lactation and later decreases to 0.004µg/g Cd at the second three-month of lactation (Figure 1). The high level of Cd was detected in Musari village (0.024 µg/g Cd). This concentration is higher than what was obtained from unguwauku, Kano State [37]. Cd toxicity in humans may lead to kidney failure as well as liver and skeletal disorders [38]. The maximum limit for Cd in milk reported by International Dairy Federation [39] is 0.0026 µg/g. Comparing our results with this limit, 44% milk samples were found to exceed the limit. The mean Cd level of milk samples is also higher than the maximum permissible limit. It has Reports has it that, Cd in milk might have natural or anthropogenic origins - fertilizers and atmospheric deposition in soil [40].

Lead (Pb) is one of the most toxic heavy metals and its level in milk and milk products is increasing day by day due to theuncontrolled urbanization and industrialization [41]. In this study, the highest level of Pb 0.181 µg/g was observed in milk sample from Gazabure village. This level is higher than the mean concentration of Pb reported by Ismail et al.[42]. It is however many-folds lower than what was earlier reported by Antunovic et al. [43]. The level of Pb in Gazabure, is higher than the maximum permissible limits set by WHO/FAO [44]. This could be attributed to atmospheric deposition of suspended particulate matter.The lowest level of Pb of this study, 0.011 µg/g Pb was detected in milk from Fulatami village at the six-month period of lactation. This level is however, higher than what was reported by Yashir et al. [45]**.** It is also, many-folds lower than what was reported by Oana et al. [46]and El-Badry and Raslam,[47].The maximum permissible limit for Pb in milk given by the Codex Alimentarius Commission[48]is 0.02 µg/g. Sayed et al. [49] measured the level of Pb in milk samples from Egypt and found the mean level of 0.327µg/g, which is much higher as compared to the result of this study. The presence of Pb in milk is attributed to many factors such as transhumance along roads and/or motorways, fodder and water contaminations and climatic factors[50]. A provisional tolerable weekly intake (PTWI) have been established by FAO/WHO expert committee for Cd, 7 µg/kg and for Pb, 25 µg/kg bodyweight [51]. From these remarks, it is necessary to control their concentrations in food [50].

Chromium(Cr) is considered as an essential trace element [52] but it can be a poison at higher level [53]. It has been reported that, hexavalent chromium (Cr^{+6}) is toxic and poisonous, particularly because it causes oxidative DNA damage[54]. Chromium which is very toxic metal may cause abdominal pain, hepatotoxicity, dizziness, cardiotoxicity, hyperemia, neurotoxicity, ulceration and vomiting [55]. Chromium (Cr) compounds are mutagenic and carcinogenic in variety of test systems [56]. However, it is essential to maintain the metabolic systems of human body [53] and plays a role in sugar metabolism as a cofactor with insulin [57]. Trivalent chromium (Cr^{+3}) has been reported as an essential and important mineral and is identified as co-factor of chromodulin, which is known as insulin-resistance factor and plays a significant role in some physiological functions of animals and human beings [54]. Chromium intensifies insulin performance and therefore is essential in the metabolism of carbohydrates, fats and proteins; however according to several studies no interaction between Cr and insulin has been yet identified [58, 59]. In this study, Cr was only detected in milk sample from Musari village at the six-month period of lactation. Similar results (of not detectable) was reported by Ritianne and Everaldo,[36]. The values reported by Coni et al.[60] and Caggiano et al. [28] as (0.15 mg/L) are far less than what was reported by Nazir et al.[54]. The recommended dietary intake of Cr during the first half of infancy is 0.01-0.04 mg/ day [61].

Copper (Cu) is an essential element for skin and blood vessel's strength, for the production of myelin and hemoglobin and for the proper functioning of enzyme systems [62, 63]. In this study, high level of Cu (0.102 µg/g Cu) was found in goat's milk from Musari village at the first three-month period of lactation. This level is however, lower than what was reported by Adetutu et al.[37] and much lower than that of El-Badry and Raslam,[47]. The values of Cu obtained were greater than the reported values by [64]in goat milk. Possible contamination of milk with copper can occur from animal feed, high copper content in animals drinking water and also from copper bearing and copper alloys equipment (Mitchell, 1981).The levels reported so far are less than what was reported by Nazir et al.[54]. The maximum limit for Cu in milk proposed by IDF[39]is 0.01 µg/g. However, this limit seems to be outdated as the normal range for Cu in milk proposed by Puls,[65] is 0.1 – 0.9 µg/g. Notably, therefore all the level of Cu determined in this study were lower than the maximum permissible limits set by WHO/FAO[44].

Cobalt (Co) being a part of vitamin B12 is considered an essential element for normal human growth. However, in excess amounts it can disturb the reproductive system and thyroid glands [66] and is also reported as a probable carcinogenic compound by IARC [67]. The highest mean concentration of Co (0.059) detected in this study was from Gazabure village. This level is much lower than what was reported earlier by Patraet al.[68] from India however, it is higher than the observation made from Korea and Spain by Khan et al. [69] and Rey-Crespo et al.[70], respectively. Cobalt was found not detectable milk samples from Fulatami village and was equally not detected in Gazabure village at the six-month period of lactation. Reports has it that, overexposure of Co may cause activation of caspases, DNA fragmentation and production of highly reactive oxygen species such as hydrogen peroxide [71].

Nickei (Ni) being a cofactor for a number of hormones and enzymes is considered as essential element for humans. However, the excessive intake may result in cell damage, impaired reproductive system, altered hormonal and enzymatic activities, oxidative stress and neurotoxicity [66, 72, 73]. There is substantial proof that nickel is essential to animals and perhaps it may have an important function in the human body although the particular and specified biochemical functions of nickel in higher animals have not yet been defined. For this reason, this element has still not been accepted as essential metal [74]. The highest mean Ni concentration found in this study was 0.041µg/g Ni in Musari village at the six-month period of lactation (the second phase of sampling). This concentration is however, lower than what was earlier reported by Dhanalakshmi and Gawdaman,[75] from non-industrialised area.The upper intake level of Ni through dietary sources is 0.1-1µg/g Ni established by Food and Nutrition Board, [76]. None of the milk samples was found to exceed this limit. Therefore, milk only is not able to breach the upper intake level for Ni in the people of the sampling areas and its environs.

Zinc (Zn) is essential part of more than 200 enzymes involved in digestion, metabolism and reproduction [77]. It is an important mineral for the maintenance of healthy skin, wound healing and is directly involved in both innate and adaptive immunity. Zinc also has antioxidant activity and helps eliminate reactive oxygen species through its role as a cofactor for the antioxidant enzyme, superoxide dismutase (SOD)[78]. While Cu and Zn are essential, they can be toxic when taken in excess; both toxicity and necessity vary from elementto-element[79]. Report has it that, higher concentration of Zn can cause impairment of growth and reproduction[80].In this study, zinc was observed in all the milk samples from the three villages at various concentrations. In each village, the level of Zn was found high at the first three-month of lactation and decreases slightly at six-month period of lactation. The highest concentration of 0.373 µg/g Zn was observed in milk sample from Fulatami village whereas the lowest value (0.191) was found in Gazabure village. This levels however, were far below what was reported by Ritianne and Everaldo, [36] and Ahmad et al. [81]. A possible source of contamination of zinc in milk is used metal cans and milk processing equipment[82]. It is also lower than what was reported form unguwauku and KofarMazugal in Kano State by Adetutu et al.[37]. In this part of study area, atmospheric deposition could also contribute to the level of element in soil and water and consequently into milk via the food chain. Coni et al.[83] reported that concentration ranges of certain health-related elements in milk were closely dependent upon animal species and feeding, season of sample collection, environmental condition and industrially manufacturing processes.

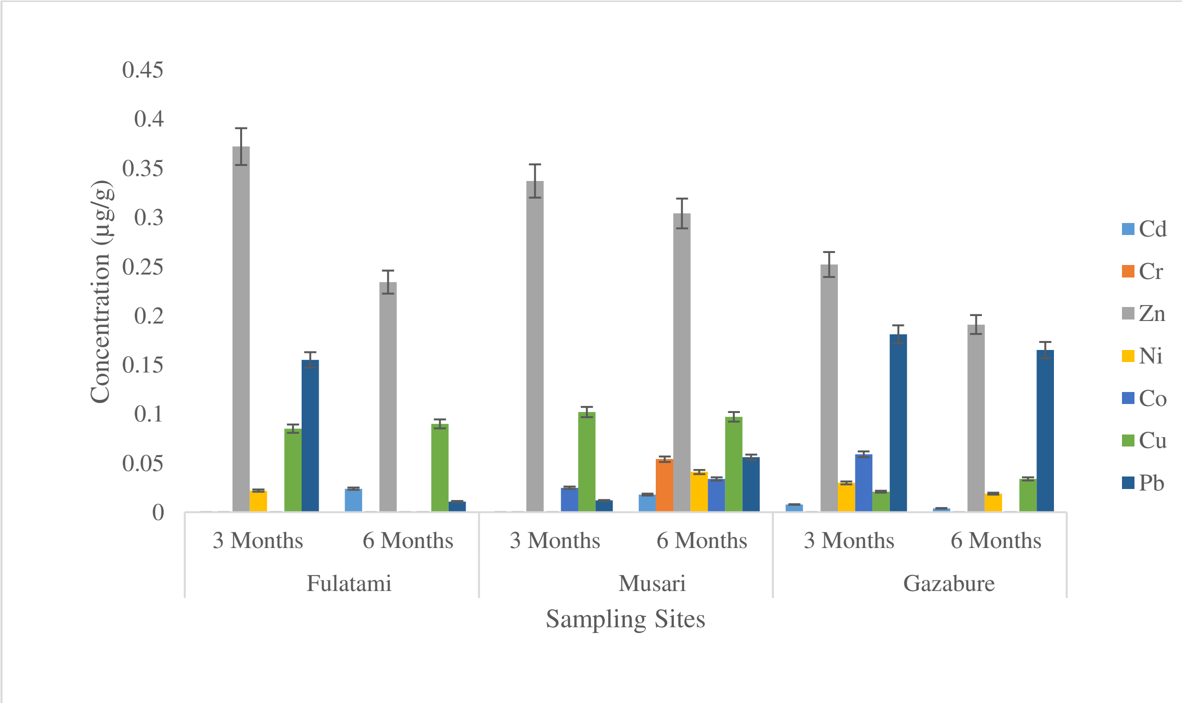

Figure 1: Heavy Metals Concentrations (µg/g) Determined inGoats Milk

5. CONCLUSION

Recently, goat milk consumption and production increases globally as people recognized its advantage especially in the developed nations.Increased urbanization and industrialization has resulted in elevated level of heavy metals in milk and milk products. In this study, the concentration of Pb was found above the permissible limits set by Codex Alimentarius Commission (0.02µg/g) from almost all the villages at different period of lactation and this could be attributed to atmospheric depositions of suspended particulate matter in water and possible the feeds consumed. Although the level decreases as the period of lactation increases. All other elements determined were within the acceptable limits of consumption. This therefore signifies its safety for consumption.

REFERENCE

[1]. FAO. FAOSTAT: Statistics division. food and agriculture organization of the United Nations 2010. Available from: http://faostat.fao.org/. 2015.Accessed 2015 July 31.

[2]. C. Garcia-Ara,T. Boyano-Martinez,J. M. Diaz-Pena,F. Martin-Munoz, M. Reche-Frutos, and M. Martin-Esteban, Specific IgE levels in the diagnosis of immediate hypersensitivity to cow's milk protein in the infant. J Allergy ClinImmunol 2001, 107(1):185-190.

[3]. Selvaggi M, LaudadioV, Dario C, Tufarelli V. Major proteins in goat milk: an updated overview on genetic variability. Mol Biol Rep 2014, 41:1035–48.

[4]. C. F. Balthazar, T. C. Pimentel, L. L. Ferrão, Almada, C. N., Santillo, A., Albenzio, M., N. Mollakhalili, A.M. Mortazavian, J.S. Nascimento, M.C.Silva, M.Q. Freitas, A. S. Sant'Ana, D. Granato, A. G. Cruz, Sheep milk: Physicochemical characteristics and relevance for functional food development. Comprehensive Reviews in Food Science and Food Safety, 2017, 16, 247–262.

[5]. F. L. Schanbacher, R.S. Talhouk, F.A. Murray, L. I. Gherman, and L.B. Willet, Milk–born bioactive peptides. *Int. Dairy J.*1998., 8: 393-403.

[6]. H. Korhonen, and A. Pihlanto - Leppälä, Milk - derived bioactive peptides: Formation and prospects for health promotion. In: *Handbook of Functional Dairy Products* C. Shortt and J. O' Brien (eds). CRC Press, Boca Raton, FL,2004. Pp: 109-124.

[7]. M. Gobbetti, F. Minervini, and C. G. Rizzello, Bioactive peptides in dairy products In: Handbook ofFood Products Manufacturing Y.H. Hui (ed). *John Wiley & Sons, Inc. Hoboken, NJ*. 2007, Pp: 489-517.

[8]. H. K. Kris, The history of domestication of goats. J Archaeolo Sci. 2008, 28: 61-79.

[9]. M. N. Bagley, Meat goat breeds, breeding management, and 4-H Market Goat Management. J Dairy Sci. 2006, 28: 30-5.
[10]. M. Tibbo, Livestock production constraints in a M2-2 Sub - Agro ecological zone with special reference to Goat production. In: Proceedings of Conference at Debub University. Awassa, Ethiopia. Nov. 10-12. 2000, pp 92-106.
[11]. C. Chantalakhana, and P. Skunmum, Sustainable smallholder systems in the tropics. Kasetsart University Press, Bangkok, Thailand. 2002., p. 302.
[12].M. K. Ajala, J. O. Gefu, and P. O. Okaiyeto, Socioeconomic factors influencing small ruminant management practices in Giwa LGA, Kaduna state, Nigeria. In: Nigerian Livestock: A Goldmine for Economic Growth and Food Security. Proceedings of 28th Annual conf. of the Nigerian Society of Animal Production, 2003, vol.28 pp 432- 435.
[13]. I. L. Mason, (3rd ed). *A world dictionary of livestock breeds, types and varieties*. CAB International, Wallingford, UK. 1988.
[14]. R. Blench, Traditional Livestock Breeds: Geographical Distribution and Dynamics in Relation to the Ecology of West Africa. Overseas Development Institute, Portland House Staag Place London, SW1E 5DP. 1999.
[15]. S.A. Sanni, A. O. Ogungbile, and T. K. Atala, Interaction between livestock and crop farming in Northern Nigeria: an integrated farming systems approach. Nigerian Journal Animal Production, 2004, 31(1) 1-2: 94-99.
[16]. E. A. Iyayi, and G. O. Tona, Management practices among small holders of sheep, goats and pigs in derived savannah zone in Oyo state, Nigeria. Nigerian Journal of Animal Production, 2004, 31:1 86-93.
[17]. S. Salem-Ben, and T. Smith, Feeding strategies to increase small ruminant production in dry environments. Small Ruminant Research, 2008, 77 (2-3):174-194.
[18]. A. Y. Tamime,M. Wszolek, R. Bozˇanic´ B. O¨ zer, Popular ovine and caprine fermented milks. Small Ruminant Res, 2011, 101:2–16.
[19]. W. L. Claeys,C. Verraes,S. Cardoen,J. De Block, A. Huyghebaert,K. Raes,K. Dewettinck,L. Herman, Consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits. Food Contr2014, 42:188–201.
[20]. V. K. Malhotra, Biochemistry for Students. Tenth Edition.1998. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India.
[21]. M. Ozcan, Mineral Contents of some Plants used as condiments in Turkey. *Food Chemistry*2003, 84:437-440.
[22]. J. H. Duffus, Heavy metals-a meaningless term?*Pure Appl. Chem.* 2002, 74:793-807.
[23].A. Sherameti, and A. Varma, Soil Heavy Metals. Springer-Verlag Berlin Heidelberg, 492. 2010.
[24.] H. M. Selim, Competitive Sorption and Transport of Trace Elements in Soils and Geological Media.CRC/Taylor and Francis, Boca Raton, FL 425.2012.
[25]. B. Alloway, Heavy Metals in Soils-Trace Metals and Metalloids in Soils and their Bioavailability. *Springer Dordrecht Heidelberg New York London,* Pp:613.2013.
[26]. Thornton, I, Metals in the Global Environment-Facts and Misconceptions, ICME, Ottawa. 1995.
[27]. Berkowitz, B., Dror, I and Yaron, B, Contaminant Geochemistry: Interactions and Transport. *In*: *The Subsurface Environment.* Springer, Heidelberg, Pp: 412. 2008
[28]. Caggiano R, Serena S, Mariagrazia DE, Maria M, Aniello A, Maria R, Salvatore P (2005) Metal levels in fodder, milk, dairy products, and tissues sampled in ovine farms of southern Italy. Environ Res 99:48–57.
[29]. I. Bogut, E. Has-schön, R. Janson, Z. Antunović, and D. Bodakoš, Concentrations of Hg, Pb, Cd and As in meat of fishpond carp (*Cyprinuscarpio*). *Agriculture*, 2000, vol. 6, p. 123–125.
[30]. L. Piskorová, Z. Vasilková, and I. Krupicer, Heavy metal residues in tissues of wild boar (*Susscrofa*) and red fox (*Vulpesvulpes*) in the Central Zemplin region of the Slovak Republic. *Czech Journal of Animal Sciences*, vol. 48, 2003, p. 134–138.
[31]. R. S. Debashis, V. Bharathidhasan, H. K. Mani, and N. Kewalramani, Heavy Metal Contents in Cow and Buffalo Milk Samples from Haryana. Ind. J. Anim. Nutr., 2009, 26(1): 29-33.
[32]. D.O. Nwude, P.A.C.Okoye, and J.O.Babayemi, Blood heavy metal levels in cows at slaughter at Awka abattoir. International Journal of Dairy Science 2010, 5: 264- 270.
[33].https://www.dailytrust.com.ng/sunday/index.php/feature/3238-for-camel-dealers-its-a-hard-time-gubio-market-says-so
[34]. IDF (International Dairy Federation), Trace Element in Milk and Milk products. *Bull. Int. Dairy Fed.*1992, 57**:**278.
[35]. M. A. Vidovic, Sadibasic, S. Cupic and M. Lausevic, Cd and Zn in atmospheric deposit, soil, wheat and milk. Environ. Res., 2005, 97: 26-31.
[36]. S. Ritianne, and A. Everaldo, Determination of Major and Minor Elements in Maltese Sheep, Goat and Cow Milk Using Microwave Plasma-Atomic Emission Spectrophotometry. Journal of Agricultural Science; 2017, Vol. 9, No. 8, 43-50

[37]. O. A. Adetutu, D. F. Muhammad, and H. A. Aminuddeen, Seasonal Evaluation of Mineral Elements, Heavy Metals, Essential Amino Acids, Proximate Compositions and Pesticides in Goat Milk, *World Journal of Analytical Chemistry, 2015, Vol. 3, No. 1, 1-9*

[38]. H. K. Zaidan, M. Al-Terehi, A. M. J. Al–Mamoori,M.B.S.Al-Shuhaib, A. H. Al-Saadi, and K. H. Gathwan, Detection some trace elements in human milk and effect of some factors on its concentrations. *J. biol. med. Sci.,* 2013, **1**: 6-12.

[39]. IDF Standard, *Metal contamination in milk and milk products*. Int. Dairy Fed. Bull., Document no. A. Doe37, 1979.

[40]. S. Maas, E. Lucot, F. Gimbert, Crini and P. M. Badot, Trace metals in raw cow's milk and assessment of transfer to Comté cheese. Food Chemistry 2011, 129: 7-12.

[41]. D. Swarup, R. C. Patra, R. Naresh, P. Kumar, and P. Shekhar, Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. *Sci. Total Environ.,* 2005, 347: 106–110.

[42]. A. Ismail, M. Riaz, S. Akhtar, A. Farooq, A. M. Shakzad, A. Mujtaba, Intake of Heavy Metals through Milk and Toxicity Assessment. Pakistan Journal of Zoology, 2017, Vol. 49(4(, pp. 1413-1419

[43]. Z. Antunovic, T. Klapec, S. Cavar, B. Mioc, J. Novoselec, and Z. Klir, Changes of Heavy Metal Concentrations in Goats Milk During Lactation Stage in Organic Breeding. *Bulgarian Journal of Agricultural Science, 2012, 18 (No 2), 166-170*

[44]. WHO/FAO Expert committee on Food Additives summary and conclusions, 53rd meeting Rome.1999.

[45]. B. Yabrir, A. Chenouf, N. S. Chenouf, A. Bouzidi, F. Gaucheron, and A. Mati, Heavy metals in small ruminant's milk from Algerian area steppe. International Food Research Journal,2016, 23(3): 1012-1016

[46]. C. Oana, C. Tănaselia, M. Miclean, E. Levei, M. Șenilă, and M. Șenilă, Analysis of Minor and Trace Elements in Cow, Goat and Sheep Milk in the NW Part of Romania. ProEnvironment, 2016, 9: 87-90

[47]. S. El-Badry, and A. Raslam, Estimation of lead and copper residues in sheep, goat milks and Karish cheese. Benha Veterinary Medical Journal, 2016, Vol. 30, NO. 2:1-5

[48]. Codex Alimentarius Commission, *Report of the 50th session of the Codex committee on food additives and contaminants.* Hague: Codex Alimentarius Commission, 2011.

[49]. E.M.E. Sayed, A.M. Hamed, S.M.Badran, and A. A. Mostafa, A survey of selected essential and heavy metals in milk from different regions of Egypt using ICP-AES. *Fd. Addit. Contam. Part B,*2011,4: 294-298.4

[50]. S. Birghila, S. Dobrinas, G. Stanciu, and A. Soceanu, Determination of major and minor elements in milk through ICP-AES. Environmental Engineering and Management Journal, 2008, 7: 805-808.

[51]. FAO/WHO. Evaluation of certain food additives and contaminants: Technical report Series 873; World Health Organization: Geneva, Switzerland, 1993.

[52]. H. J. M. Bowen, Environmental chemistry of the elements. Academic Press, London.1979.

[53]. L.Q. Qin, X. P. Wang, W. Li, X. Tong, and W.J.Tong, The minerals and heavy metals in cow's milk from China and Japan. Journal of Health Science 2009, 55: 300–305.

[54]. R. Nazir, M. Khan, H. Ur Rehman, M. Masab, A. Khan, M. Rehman, A. Khaliq, I. Parveen, and F. Shehnaz, Comparative Study of Heavy Metals (Ni, Cu, Fe and Cr) in the Milk of Cattle and Humans Collected from Khyber Pakhtunkhwa, Pakistan*Global Veterinaria,* 2015, *14 (5): 761-767.*

[55]. Z., A.Hussain, U. S. Nazir, and M. Salman, Comparative study for the determination of metals in milk samples using Flame- AAS and EDTA complexometric titration. J. Sci. Res., 2010, 1: 55-76.

[56]. G.V. Zodape, V.L.Dhawan, and R.R.Wagh, Determination of metals in cow milk collected from Numibia City, India. Eco Revolution, 2012, 270-274.

[57]. W.G. Hoekstra, J.W. Suttie, and H.E.Gauther, Trace element metabolism in animals. UK: University Park Press, 1970.

[58]. WHO. *Trace Elements in Human Nutrition and Health*. Geneva, World Health Organisation, 1996.

[59]. D. M.Stearns, Is chromium a trace essential metal? *BioFactors,* 2000, *11*, 149-162. https://doi.org/10.1002/biof.5520110301

[60]. E. Coni, A. Bocca, P. Coppolelli, S. Caroli, C. Cavallucci, and M. T. Marinucci, Minor and trace element content in sheep and goat milk and dairy products. Food Chem., 1996, 57:253–260. doi:10.1016/0308-8146(95)00216-2.

[61]. C.E. Casey, A. Smith, and A. Zhang, A. Microminerals in Human and Animal Milks. P. 622-674. In: G.J. Robert (ed.). Handbook of Milk Composition. Ed. Academic Press, USA: California.1995.

[62]. E.D. Harris, Copper homeostasis: the role of cellular transporters. *Nutr. Rev.,* 2001, **59**: 281-285. https:// doi.org/10.1111/j.1753-4887. 2001.tb07017.x

[63]. J. Osredkar, and N. Susta, Copper and zinc:Biological role and significance of copper/zinc imbalance. *J. clin. Toxicol.,* 2011, 3: 1-18.

[64]. D.O'Connor, L, Folate in Goat Milk Products With Reference To Other Vitamins And Minerals: *A review small Rum.Res,*1994, 4:143-149.
[65]. R. Puls, In*: Mineral levels in animal health: Diagnostic data (2nd ed.)*. Sherpa International Clearbrook, BC.1994.
[66]. G.F. Nordberg, B.A. Fowler, M. Nordberg, and L. T. Friberg, In: *Handbook on the toxicology of metals* (3rd ed.). Academic Press Inc., USA.2007.
[67]. IARC., *Int. Agen. Res. Cancer. Monogr. Ser.,*1991, 52: 263–472.
[68]. Patra, R. C., Swarup, D., Kumar, P., Nandi, D., Naresh, R. and Ali, S. L. 2008. Milk trace elements in lactining cows environmentally exposed
[69]. N. Khan, I. S. Jeong, I. M. Hwang, J. S. Kim, S. H. Choi, E. Y. Nho, J. Y. Choi, K. S. Park, and K. S. Kim, Analysis of minor and trace elements in milk and yogurts by inductively coupled plasma-mass spectrometry (ICP-MS). Food Chemistry, 2014, 147: 220- 224.
[70]. F. Rey-Crespo, M. Miranda, and M. López-Alonso, Essential trace and toxic element concentrations in organic and conventional milk in NW Spain. *Fd. Chem. Toxicol.,* 2013, 55: 513-518. https://doi.org/10.1016/j.fct.2013.01.040
[71]. I. Bushra, A. Saatea, S. Samina and K. Riaz, Assessment of Toxic Metals in Dairy Milk andAnimal Feed in Peshawar, Pakistan. British Biotechnol. J., 2014, 4(8): 883-893.
[72]. K. Doreswamy, B. Shrilatha, T. Rajeshkumar, and Muralidhara, Nickel-induced oxidative stress in testis of mice: evidence of DNA damage and genotoxic effects. *J. Androl.,* 2004, 25: 996–1003.
[73]. K. K. Das, S. N. Das, and Dhundasi, Nickel, its adverse health effects & oxidative stress. *IndianJ. med.. Res.,*2008,128: 412-425.
[74]. A.A. Manuel, J.S. Pedro, M. Rafael and Z. Gonzalo, Nickel content in raw cow's, ewe's and goat's milk. INRA/Elsevier, Paris, 1998, 78: 699-706.
[75]. B. Dhanalakshmi and G. Gawdaman, Determination of Heavy Metals in Goat Milk Through ICP-OES. *Asian J. Dairy & Food Res.,* 2013, 32 (3): 186-190
[76]. Food and Nutrition Board, *Dietary reference intakes (DRIs) recommended intakes for individual elements.*http://iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRIs/New%20Material/5DRI%20Values%20SummaryTables%2014.pdf, 2001.
[77]. WHO/FAO, Environmental Health Criteria 165. Inorganic Lead, WHO, Geneva. 1996.
[78]. P. Licata, D. Trombetta, M. Cristani, F. Giofre, D. Martino, M. Calo, and F. Naccari, Levels of «toxic» and « essential » metals in samples of bovine milk from various dairy farms in Calabria, Italy. Environment International. 2004, 30: 1-6.
[79]. R.M.Tripathi, R. Raghunath, V.N. Sastry and T.M. Krishnamoorthy, Daily intake of heavy metals by infants through milk and milk products. Sci. Total Environ., 1999, 227: 229-235.
[80]. Nolan, K.R., 1983. Copper toxicity syndrome. J. Orthomol. Psychiatry, 12: 270-282
[81]. I. Ahmad, A. Zaman,N. Samad,M. M. Ayaz,S. Rukh et al. Atomic Absorption Spectrophotometery Detection of Heavy Metals in Milk of Camel, Cattle, Buffalo and Goat from Various Areas of Khyber-Pakhtunkhwa (KPK), Pakistan. J Anal Bioanal Tech.,2017, 8: 367.
[82]. W. D. Jarette, A review of the important trace elements in dairy review products. *Aust. J. Dairy Technol,*1979,34:28-34.
[83]. E. Coni, B. Bocca and S. Caroli, Minor and trace element content of two typical Italian sheep dairy Products. J. Dairy Res., 1999, 66: 589-598.