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Atomic Absorption Spectrophotometery Detection of Heavy Metals in Milk of Camel, Cattle, Buffalo and Goat from Various Areas of Khyber- Pakhtunkhwa (KPK), Pakistan

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Abstract

The determination of the seven elements was performed by Perkin Elmer Atomic Absorption (AA) spectrophotometer. The present study highlights the importance of seven heavy metals residual concentration including Cd, Cr, Cu, Fe, Mn, Ni and Zn in milk of Camel, Cattle, Buffalo, Sheep and Goat from various areas of Khyber Pakhtunkhwa (KPK), Pakistan. It revealed that milk of camel comprising of high levels of Zn (5.150 ± 0.021 mg/kg), Mn (0.094 ± 0.003 mg/kg) and Fe (1.580 ± 0.530 mg/kg) with a definite correlation. In the milk of buffalo, high concentration of noxious heavy metals including Cu (0.223 ± 0.010 mg/kg) and Cd (0.117 ± 0.086 mg/kg) were found whereas in goat milk, high Ni (1.152 ± 0.045 mg/kg) and Cr (1.152 ± 0.045 mg/kg) was observed and detected. The analysis showed that camel and buffalo have similar high concentration of heavy metals. Overall results showed that milk of cattle shows higher concentration of Zn, Mn and Fe along with Buffalo.

Keywords: Atomic Absorption spectrophotometer; PCA; Cattle's milk; Multi variate analysis

Introduction

Heavy metals are described as those metals which, in their standard states, have density more than five (5) g/cm^3, their atomic weights ranges from 63.546 to 200.590, and their specific gravity must be greater than 4.0 [1]. The unhygienic sources of water and food are of reasons of sickness in human beings. In the environment, among the different contaminants, heavy metals are directly associated to health issues in humans [2]. Heavy metals pollution is a severe risk due to their bioaccumulation, toxic effects, and then continuity in different food chains [3]. These environmental unfriendly pollutants have direct deadly special effects since they are incorporated in body tissues [4]. These metals enter the human body mainly by two routes i.e. inhalation and ingestion [5]. Animals milk contain important elements P, Ca, K, Mg, Na, Cl and trace elements including Cu, Zn, Mn, Fe, Cr, Cd and Ni and proteins [6]. Lactating animals when exposed to high concentration of heavy metals like Cu, Cd, Zn, Ni, Hg, Pb, Fe, As and Cr, then in these animals the metals amass in their milk, which when consumed by consumers cause serious health issues [7]. Regarding the growth and development of infants, there are reported countless studies published showing importance of breast milk. In spite of the ratification of Republic Act No. 7600, the proportion of babies who are entirely fed on breast milk in their starting 6 months dropped from 20% in 1998 to 16% in 2003 [8]. In the environment, the concentration of heavy metals increases due to human activities [9]. Heavy Metals Mn, Zn, Mo, Ni, Cu, Fe and Co is essential as plants require them for their life cycles completion, but they become toxic when they are above the permissible value. Cadmium and chromium have no precise known biological function in plants and are toxic at much low concentration. World Health Organization (WHO) recommends that all the medicinal plants, which are used to prepare herbal product must be checked for heavy metals [10]. Concentration of heavy metals in the environment greater than the permissible value can be destructive to all living species. Intake of above mentioned heavy metals through inhalation, ingestion or by any mean can result in health issues and complications like damage nervous system, cancer and ultimate death [11].

Materials and Methods

Collection of milk samples

Different milk samples were collected from different areas of "Shnwa Gudi Khel" District Karak from different cattle i.e. Cow, Camel, Goat, Sheep and Buffalo in summer season. The samples were collected in plastic bottles. From each cattle eight samples were taken. Then samples were placed in refrigerator to avoid from fermentation. In Figure 1 sample collection, areas are number 19 and 20 in Sample Location [12].

Acid digestion of cattle's milk

All glassware were first cleaned with 10% HNO_3 solution and then further washed with the distilled water. Milk of 10 mL concentration was digested with 1:3 of H_2O_2 and HNO_3 on a hot plate. The samples were heated on hot plate until their volume reduces to 2 mL. This 2 mL sample solution was then diluted with 20 mL distilled water and make a clear solution of it. The contents of the beaker brought to the required volume with distill water and were examined by Flame Atomic Absorption Spectrophotometer.

Preparation of standards of heavy metals

The heavy metals selected for study were Fe, Ni, Cu, Cd, Zn, Mn, Cr and Pb. In each case of the selected metals, three different concentrations

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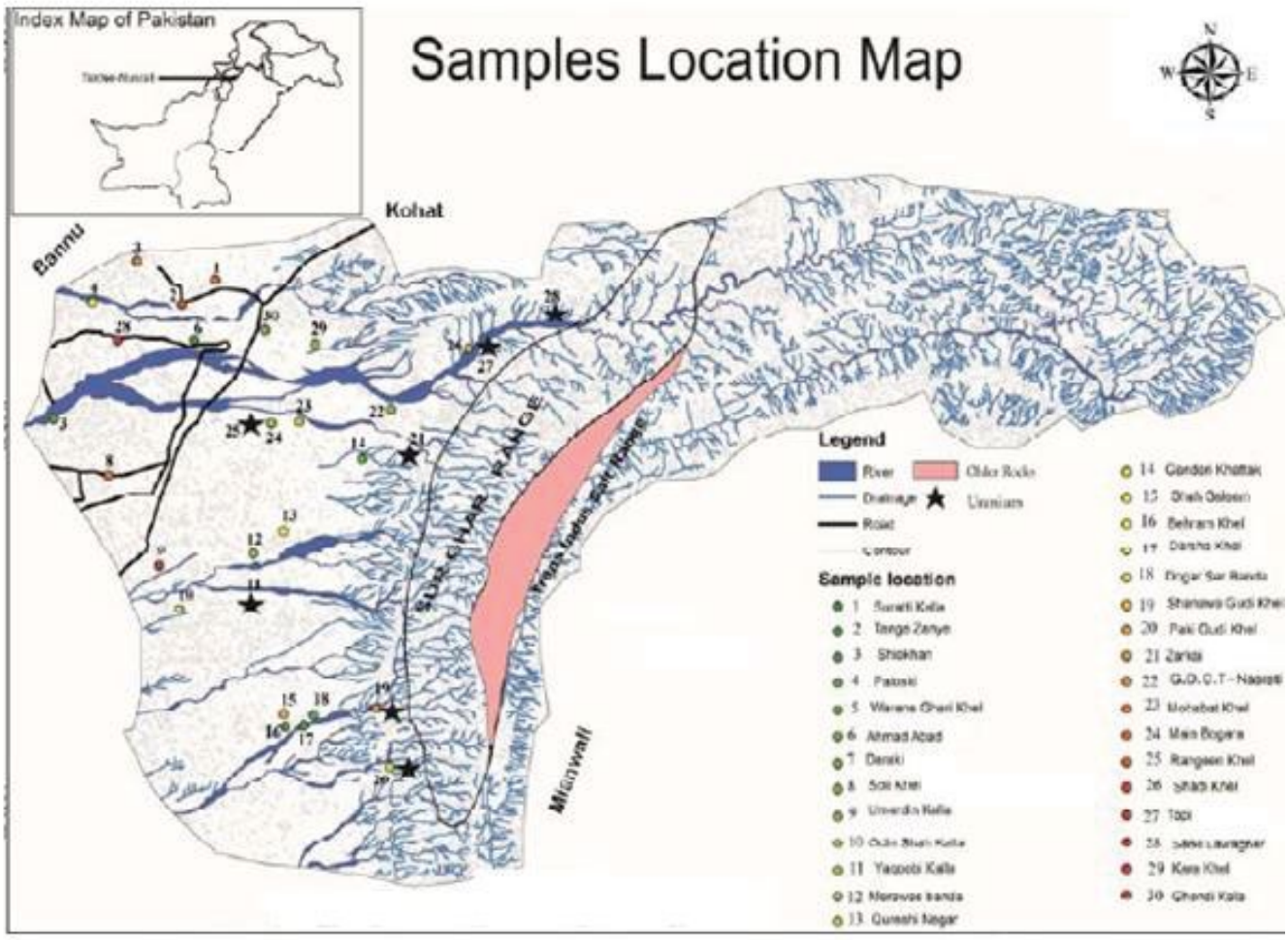

Figure 1: Sample location map of Pakistan.

were made to calibrate the Flame AAS. These concentrations are as follows: 1.0 ppm, 1.5 ppm and 2.0 ppm. The resultant calibration curve of well-prepared standard concentrations gives linear curve by Atomic Absorption Spectrophotometric Analysis by using Perkin Elmer PinAAcle™ 900T atomic absorption (AA) spectrophotometer (Shelton, CT, USA) which is equipped with the sensitive WinLab32™ for AA software running under Microsoft® Windows™ 7 for flame absorption spectrophotometry.

Data assessment

Data was gathered and ordered all data in tables. The concentrations of milk samples in ppm yielding positive results for the occurrence of heavy metals were transformed into concentration in mg/kg.

Results and Discussion

The results for individual heavy metal e.g., Fe, Ni, Cu, Cd, Zn, Mn and Cr are shown in sequel (Tables 1 and 2; Figures 2-4).

Iron concentration

The highest Fe concentration (1.580 ± 0.53 mg/kg) was established in camel milk while the lowest (0.592 ± 0.321 mg/kg) was found in sheep milk. The mean concentration of all the parameters was found within the permissible limits of World Health Organization (WHO). Even the maximum values column of every parameter was also with the allowable limits proposed by WHO (Table 1). The Fe concentration in cattle milk was in the order Camel >Buffalo>Goat>Cow>Sheep. The WHO limit for iron is 0.5 mg/kg [13].

Nickle concentration

The concentration of Ni in the different animal sample (Cow, Buffalo, Sheep, and Goat) has been shown and summarized in Table 1. The highest concentration of Ni (1.152 ± 0.045 mg/kg) was found in Goat milk, while the lowest value of 0.130 ± 0.002 mg/kg was found in Cow milk. The Ni concentration in cattle milk was in the order of Goat >Sheep>Camel>Buffalo>Cow.

Copper concentration

Concentration of copper in all samples of different animals is given in the Table 1. Highest Cu concentration was noticed in milk of Buffalo (0.223 ± 0.010 mg/kg) and lowest was found in Camel milk (0.060 ± 0.040 mg/kg). The Cu concentration in cattle milk was in the order of Buffalo>Goat>Sheep>Cow>Camel. When compared with the permissible limit of 24.2 mg/kg.

Elements	Mean	SD	Minimum	Maximum	WHO limit
Cd	0.076	0.006	0.01	0.117	0.58
Cr	0.034	0.014	0.024	1.152	1.61
Cu	0.141	0.017	0.06	0.223	24.2
Fe	0.692	0.306	0.592	1.58	0.5
Mn	0.056	0.038	0.056	0.094	55.5
Ni	0.130	0.002	0.13	1.152	0.43
Zn	3.136	0.081	3.113	5.15	121

Table 1: Heavy Metals Concentration (mg/kg) in cattle milk, the values are mean ± SD.

Milk Samples	Fe	Ni	Cu	Cd	Zn	Mn	Cr	Pb
Cow	0.692 ± 0.306	0.130 ± 0.002	0.141 ± 0.017	0.076 ± 0.006	3.136 ± 0.081	0.056 ± 0.038	0.034 ± 0.014	BDL
Buffalo	0.960 ± 0.432	0.150 ± 0.010	0.223 ± 0.010	0.117 ± 0.086	4.356 ± 0.081	0.066 ± 0.044	0.032 ± 0.012	BDL
Goat	0.950 ± 0.305	1.152 ± 0.045	0.212 ± 0.010	0.074 ± 0.003	3.345 ± 0.071	0.065 ± 0.032	1.152 ± 0.045	BDL
Sheep	0.592 ± 0.321	0.340 ± 0.001	0.151 ± 0.011	0.01 ± 0.001	3.113 ± 0.072	0.078 ± 0.018	0.044 ± 0.024	BDL
Camel	1.580 ± 0.530	0.220 ± 0.001	0.06 ± 0.040	0.102 ± 0.007	5.150 ± 0.021	0.094 ± 0.003	0.024 ± 0.013	BDL

Table 2: Heavy Metals Concentration (mg/kg) in Livestock milk, the values are mean ± SD.

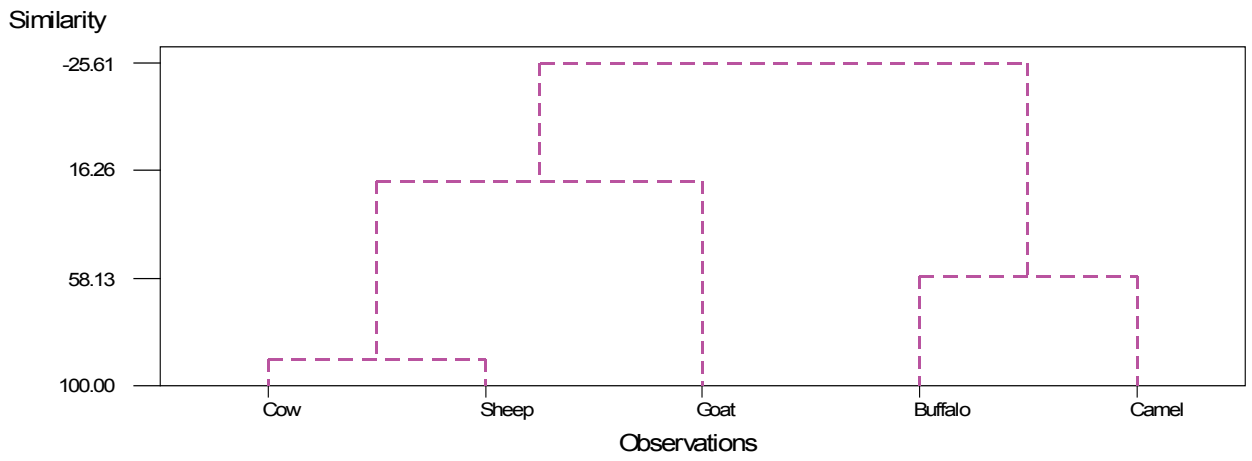

Figure 2: Dendrogram showing cluster analysis of cattle. Two clusters are obvious from the figure.

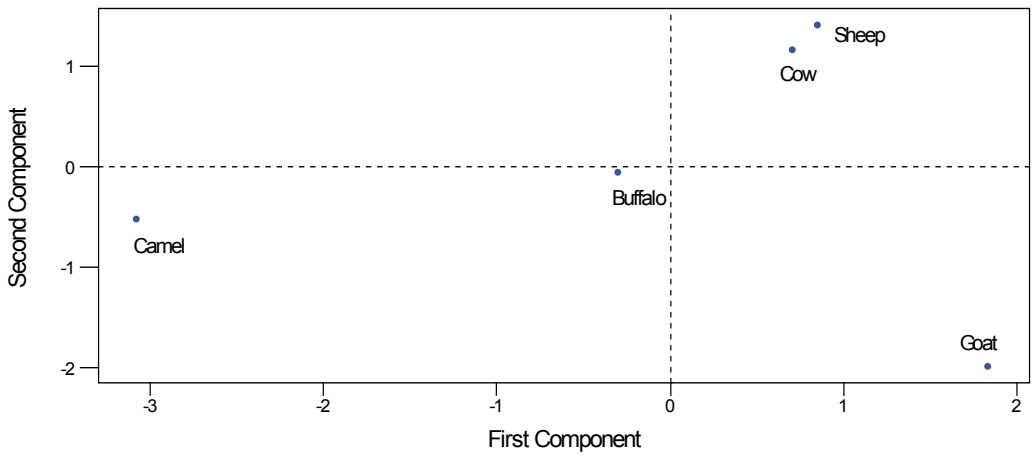

Figure 3: Principal components results of deferent variables from milk sample.

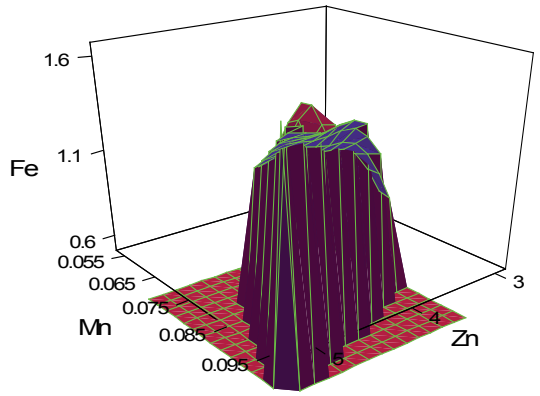

Figure 4: The 3D correlation among Fe-Mn-Zn showing the existence of these elements..

Cadmium concentration

The Cadmium concentration in milk of different animals is shown in Table 1. The highest Cadmium value was found in the milk of buffalo (0.117 ± 0.086 mg/kg) and the lowest value (0.001 mg/kg) was in milk of sheep. The remaining other samples showed very much less variations amongst each other. The Cd concentration in cattle milk was in the order of Buffalo>Camel>Cow>Goat>Sheep. The permissible limit for cadmium has been reported as 0.58 mg/kg [6].

Zinc concentration

The concentration of metal zinc in all the study samples has been given in Table 2. Highest zinc concentration (5.150 ± 0.021 mg/kg) was found in the milk of Camel and lowest concentration (3.113 ± 0.072 mg/kg) in the milk of Sheep. All the values in the study samples were below the permissible limit 121 mg/kg. The Zn concentration in cattle milk was in the order of Camel>Buffalo>Cow>Goat>Sheep.

Chromium concentration

The Chromium concentration in the samples has been shown in Table 2. The highest concentration of Cr was found in milk of Goat (1.152 ± 0.045 mg/kg) and lowest was found in milk of Camel (0.024 ± 0.013 mg/kg). The maximum permissible daily dietary intake of Cr in milk is 1.61 mg/kg [6].

Lead concentration

The Lead concentration in the samples has been shown in Table 2. Lead was present Below Detection Limit in samples of all studied animals milk. The MPL DDA value of Pb in milk is 3.46 mg/kg [6].

Statistical significance

The values of standard deviation and outlier (can be seen in plots) hint that data of variables rotates round the mean values. The Pearson correlation matrix of six elements collected from cattle are exposed in the Table 2. More negative correlations were experimental as compared to positive correlations. It is evident that Cu shows significant correlation with all parameters. The highest relationship was observed between Ni and Cr (0.98) at ($P<0.001$) and between Fe and Zn (0.91 at $P<0.01$). Both relationships are positive explaining the fact that increment in one parameter will cause promotion of the other parameter. Besides this Ni-Cd have the same source as well as Fe and Zn have the same.

Principal Component Results of seven variables collected from milk samples are shown in the Figure 3. First three PCs showed Eigen values >1 hence picked for further description. PC1 represented 50% of the total variance while the rest two PCs contributed 27% and 18% respectively and it explains that not the first factor is the major contributing factor. PC1 articulated elevated loading on Zn (-0.48), Fe (-0.44) and Mn (-0.40) and PC2 expressed high loading on Cr and Ni (-0.58, -0.55) respectively. An elevated value of Cd and Mn was observed in PC3. So here, we have a story that FACTOR1 (PC1) explains the effects of trace elements over milk as shown in the Table 3 and Figures 2 and 3. FACTOR 2 shows that heavy metals including Cr and Ni are the elements that may cause contamination in the milk. This factor also explains that as Cr and Ni have the same source of production and it may enter into the body of these animals through bio augmentation. The authors are of the view that feeding area where these animals consume having plants containing these metals and there are some tannery industries nearby the area creating pollution to these plants. Beside this, samples taken from this mountain areas may contain different heavy metal ore which may contaminate water and plants [10,12].

The results of cluster analysis and principal component analysis of five animals are shown in the sequel. The results showed buffalo and camel formed a separate cluster while rest three are clustered in a separate one. It explains that buffalo and camel have similar response to these heavy metals. The high concentration of heavy metals in soils results increase in concentration of heavy metals in animal [14].

Iron concentration

The highest Fe concentration (1.580 ± 0.53 mg/kg) was established in camel milk while the lowest (0.592 ± 0.321 mg/kg) was found in sheep milk. The mean concentration of all the parameters was found within the permissible limits of World Health Organization (WHO). Even the maximum values column of every parameter was also with the allowable limits proposed by WHO (Table 1). The Fe concentration in cattle milk was in the order Camel> Buffalo>Goat>Cow>Sheep. The WHO limit for iron is 0.5 mg/kg [13]. Our concentration of milk is less than Abdulkhaliq et al. [15]. While Meshref et al. reported very higher values of Fe in milk and milk products as compared to this study [16].

Nickel concentration

The concentration of Ni in the different animal sample (Cow, Buffalo, Sheep, and Goat) has been shown and summarized in Table 1. The highest concentration of Ni (1.152 ± 0.045 mg/kg) was found in Goat milk, while the lowest value of 0.130 ± 0.002 mg/kg was found in Cow milk. The Ni concentration in cattle milk was in the order of Goat>Sheep>Camel>Buffalo>Cow.

Copper concentration

Concentration of copper in all samples of different animals is given in the Table 1. Highest Cu concentration was noticed in milk of Buffalo (0.223 ± 0.010 mg/kg) and lowest was found in Camel milk (0.060 ± 0.040 mg/kg). The Cu concentration in cattle milk was in the order of Buffalo>Goat>Sheep>Cow>Camel. When compared with the permissible limit of 24.2 mg/kg. The Cu value was on higher limits in the study Meshref et al. [16]. The value of copper in this study is in accordance with Shahriar et al. conducted at Bangladesh [17]. Pilarczyk et al. reported lower values of Cu in cow like our study except buffalo [18]. Lutfullah et al. reported lower levels of Cu except buffalo, which are in accordance with present study [19].

Correlation (the Pearson)						
Elements	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**
Cr	-0.0368					
Cu	0.0439	0.4728				
Fe	0.6412	-0.0198	-0.539			
Mn	-0.0234	-0.264	-0.7027*	0.6995		
Ni	-0.1773	0.9829***	0.419	-0.0374	-0.1495	
Zn	0.7051*	-0.3063	-0.4439	0.918**	0.682	-0.3254

Whereas *** = P<0.001, ** = P<0.01 and * = P<0.05

Table 3: The Pearson correlation matrix of six parameters collected from cattle.

Variable	PC1	PC2	PC3
Fe	-0.44	-0.39	0.04
Ni	0.29	-0.55	0.31
Cu	0.39	-0.18	-0.42
Cd	-0.26	-0.33	-0.64
Zn	-0.48	-0.23	-0.15
Mn	-0.40	-0.06	0.50
Cr	0.29	-0.58	0.18
Eigen value	3.53	1.88	1.27
Proportion	0.50	0.27	0.18
Cumulative	0.50	0.77	0.95

Table 4: Factor loading values of seven parameters indicating first three principal factors from cattle.

Cadmium concentration

The Cadmium concentration in milk of different animals is shown in Tables 1 and 4. The highest Cadmium value was found in the milk of buffalo (0.117 ± 0.086 mg/kg) and the lowest value (0.001 mg/kg) was in milk of sheep. The remaining other samples showed very much less variations amongst each other. The Cd concentration in cattle milk was in the order of Buffalo>Camel>Cow>Goat>Sheep. The permissible limit for cadmium has been reported as 0.58 mg/kg [6]. The increased value of Cd in milk has been reported by Doreen in 2014 in Ghana. The similar study has also been conducted by Farid and Baloch in 2012 in Dera Ismail Khan [20]. In this study, the Cd values were on lower limits as reported by Meshref et al. in Egypt [16]. The Cd Value in sheep milk of this study is inconsistent with Poti et al. [21]. Pilarczyk et al. reported lower values of Cd in cow like our study [18]. Abdelkhalek et al. reported the lower values of Cd to this study except buffalo and camel milk where we have high concentration of heavy metals like Cd [22].

Zinc concentration

The concentration of metal zinc in all the study samples has been given in Table 2. Highest zinc concentration (5.150 ± 0.021 mg/kg) was found in the milk of Camel and lowest concentration (3.113 ± 0.072 mg/kg) in the milk of Sheep. All the values in the study samples were below the permissible limit 121 mg/kg. The Zn concentration in cattle milk was in the order of Camel>Buffalo>Cow>Goat>Sheep. Meshref et al. reported upper limits of Zn in milk and milk products as compared to this study [16]. Lutfullah et al. reported greater level of Zn in milk while this study we observed higher value of Zn in camel milk [19].

Chromium concentration

The Chromium concentration in the samples has been shown in Table 2. The highest concentration of Cr was found in milk of Goat (1.152 ± 0.045 mg/kg) and lowest was found in milk of Camel (0.024 ± 0.013 mg/kg). The maximum permissible daily dietary intake of Cr in milk is 1.61 mg/kg [6]. Poti et al. reported higher values of Cr in sheep milk as compared to our study [20]. Muhib et al. reported higher Cr values in cow milk while we have observed high Cr value in goat milk [23]. Abdelkhalek et al. reported the similar values of Cr to this study [22]. Anastasio et al. reported higher values of Cr as compared to our study except in goat [24].

Lead concentration

The Lead concentration in the samples has been shown in Table 2. Lead was present Below Detection Limit in samples of all studied animals milk. The MPL DDA value of Pb in milk is 3.46 mg/kg [6]. The study conducted by Malhat et al. revealed higher concentration of iron, zinc, lead, copper and cadmium in cow milk as compared to this study [25].

Abdulkhaliq et al. reported the higher values of copper and Pb while reported similar value of iron in cow milk to this study [15].

In the previous study conducted by Nazir et al. of the same study, area reported highly polluted cattle milk with heavy metals containing Ni, Cu, Cr, and Fe, which is not in consistent with the study of heavy metals, analysed [26].

Enb et al. showed the lower values of iron and Mn while the similar values of copper and zinc in buffalo milk in comparison to our study [13,27].

Conclusion and Recommendation

The study was done for the concentration of heavy metals and to check whether these heavy metals are within the permissible limits set by the World Health Organization (WHO). The study revealed significant concentrations of iron along with other metals, which were below WHO limits. The highest Fe concentration (1.58 ± 0.53 mg/kg) was found in camel milk while the lowest (0.592 ± 0.321 mg/kg) was found in sheep milk. The Fe concentration in cattle milk was in the order of Camel >Buffalo>Goat>Cow>Sheep. The concentration of Fe was above WHO limits (0.5 mg/kg) in milk of all cattle. The higher values of copper and cadmium were observed in buffalo milk while the higher Cr concentration was found in goat milk in this study. Therefore, it is recommended that a more specific confirmatory test like NIR must be performed by future researchers/studies along with presence of heavy metals in route fodder and under water.

Conflict of Interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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