

# Determination of Heavy Metal Ions (As, Pb, Cd) and Zinc Mineral (Zn) in Human's and Cow's Milk in Bitlis (Turkey)

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*Human's milk and cow's milk fed-infants, long before the mother is exposed heavy metals are potentially dangerous for both mother and baby. Concentrations of toxic heavy metals (Pb, Cd, As) and major nutritional trace element (Zn) which collected center Bitlis, Tatvan and various villages in the 75 volunteer nursing mothers received 6 months after the birth human's milks and 75 cow milks were analyzed. This study was measured by Inductively Coupled Plasma-Mass Spectrometer. Work was measured in accredited laboratory. In the samples As and Cd were under detectable leves although Zn and Pb were measured. Survey conducted, Zn and Pb levels in the mother milk which collected Tatvan borough were determined the highest. The all metal levels in the mother milk and cow milk which collected Bitlis center higher than villages. Zn levels in the cow milks which collected Bitlis center the highest. As a results of analysis, the avarege values of the lead in the human milks respectively (Bitlis center, Tatvan borough and villages) were found  $0.0003 \pm 0.00004$  mg/kg,  $0.00045 \pm 0.000052$  mg/kg,  $0.0002 \pm 0.00003$  mg/kg; the avarege values of the zinc  $3.2 \pm 0.64$  mg/kg,  $3.34 \pm 0.57$  mg/kg,  $2.5 \pm 0.37$  mg/kg. The avarege values of the lead in the cow milks respectively (Bitlis center, Tatvan borough and villages) were found  $0.002 \pm 0.0004$  mg/kg,  $0.003 \pm 0.0004$  mg/kg,  $0.001 \pm 0.0003$  mg/kg; the avarege values of the zinc  $3.7 \pm 0.51$  mg/kg,  $3.6 \pm 0.49$  mg/kg,  $3.0 \pm 0.41$  mg/kg. As a results of analysis between groups weren't found significant relationship ( $P > 0.05$ ).*

**Keywords:** Heavy metals, Mother milk, Cow milk, ICP-MS

One of the problems on the basis of the environmental issues is the pollution of all water, air and soil or at least one of them [1-2-3]. Air pollution has both acute and chronic effects on human health, affecting a number of different systems and organs [4]. The most common toxic heavy metals are lead, mercury, and cadmium. Lead is a commonly found environmental toxic element that causes a deterioration in health [5-6]. Both human and cow milk is the best natural source of nutrition for feeding infants; it contains the optimal balance of fats, carbohydrates, and proteins for developing babies [7]. Breast milk contains properties which help the infant's immune system fight infections. [8] From the birth, milk has a fundamental place in the development of children, thus contamination risk of the milk is an important issue. Heavy metal contamination in milk can through be water, air or food [9]. As located at the top of the food chain, adult and infants have contact with growing levels of heavy metals. As fetus and infants are in the process of rapid growth and development, they are more sensitive to the toxic effects of heavy metals than adults [2-3-10]. The toxic heavy metals accumulating in the body before the pregnancy can be transferred to the fetus via the umbilical cord. Lead can be mobilized together with calcium during bone metabolism, and may be transferred to the fetus either by the transplacental route or by lactation during breastfeeding [7-11].

Lead (Pb) is used for accumulators, cables, enamels, paints and glass in the industry. In addition, it is used as stabilizers in the preparation of polymers and widely used as the compression inhibitors in the composition of gasoline. In recent years, the most important source of lead is exhaust gases of automobiles [12]. Lead passes easily through placenta so infant contact with Pb starting from prenatal period. The amount of Pb in foetus is associated with the load of Pb in mother's body. During pregnancy, Pb in bone, mobilizes and passes foetus through

placenta. In on-going childhood, the most important ways of Pb contact are ingesting paint dust, paint chips, soil, water and air [13]. Level of lead is low in breast milk however lead level increases in the mother's milk due to encounter. Breast milk lead level is about one tenth of blood level and it is associated with blood level. Foetus encounters Pb from mother through the placenta. Breast milk is only responsible for the baby blood level with 12% [14]. Lead level of smoker mothers' milk is two times more than non-smokers. WHO [14] recommends daily highest lead level in breast milk tolerance limit as 3.57 mg/kg while weekly on a diet is 25 µg/kg [15]. Cadmium (Cd) is used in galvanizing technology, paint, lacquer and plastic pigment in the composition as a carrier in yellow-orange in colour and production of nickel-cadmium batteries. Cd mixes in water and atmosphere during smelting and incineration of waste and mixes into the soil with some phosphate fertilizer [16]. Especially, Cd is found in high concentration at leafy greens, root vegetables such as potatoes, carrots and celery, wheat, grains and oilseeds grown in contaminated soil. In addition, Cd is at high levels in shellfish (clams, crabs), molluscs, animal giblets (especially in the liver and kidney of older animals) and wild mushrooms. Cd is also found in cigarette smoke and enters 1-2 mg into smoker's body with a single package [17]. Arsenic (As) is found in conjunction with other metals in earth's crust. Naturally, soil contains 1.00-70.00 mg/kg arsenic [18]. Underground water, flowing water and plants grown in such waters contain arsenic more than normal. By this way, arsenic poisoning and chronic cancer risk are quite high in people consuming water and plants contaminated with arsenic [19]. Zinc is essential for the normal growth, development, and immune system in infants [20]. Zinc in the body, has the highest concentration in the retinal layer and reproductive organs (prostate fluid). A small amount in the liver serves as the repository meets the immediate

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needs. Zinc in the hair and skin never enters into the systemic metabolism [21]. Zinc in the muscle and bone partially available in physiological and pathological conditions that make up the deficiency of zinc. Breast milk is an important source of zinc. Babies fed with breast milk have high level of zinc especially in the first three months and their growth is rapid. The World Health Organization [5] WHO (1993) has indicated that the mean and range of metals detected in breast milk around the world are: Arsenic 0.3 ppb (0.1-0.8), cadmium 0.1 ppb (0.1-3.8), lead 5.0 ppb (0.0-41.1). WHO's [14] daily zinc intake limit is 25.8 mg. In addition, WHO [14] stated 3 mg/L the mean concentration of zinc in 2006 [22]. The World Health Organization has recommended exclusive breastfeeding during the first 6 months of life, and continued breastfeeding with appropriate complementary food until the age of 2 years or later [23]. In the last few years, the contamination of milk has been considered as one of the most dangerous phenomenon. Trace metals is a general collective term applying to the group of metals and metalloids with an atomic density greater than 6 g/cm. This term is widely recognized and usually applied to elements such as lead (Pb), and zinc (Zn), which are commonly associated with pollution and toxicity problems [24]. One of the main problems with metals is their ability to bioaccumulate. The metal residues in milk are of particular concern because milk is largely consumed by infants and children [25]. Non-essential trace elements need to be measured in milk and dairy products for food safety. Aluminium, arsenic, cadmium and lead accumulate especially in the lungs, liver, kidney, thyroid and brain. (Fernandez-Lorenzo et al. 1999). The aim of this work was to evaluate breast milk and cow milk as a source of exposure to heavy metals in infants living in two different regions of Bitlis and among Tatvan. We aimed to analyze the As, Pb, Cd and Zn content in human and cow milk in Bitlis Turkish mothers at the sixth month of lactation, and to study the association of some maternal and infant characteristics with breast milk As, Pb, Cd and Zn concentrations.

## Experimental part

### Materials and methods

In this study, breast milk and cow milk were used as the material. The milk was collected from four different regions of the Bitlis center, with the recent introduction of the policy of absolute breast feeding of infants in Bitlis for the first six months after birth. The samples were obtained by self milking into sterilized polyethylene bottles and were well labeled. The breast milk samples were collected from 18 to 25 lactating mothers below the ages of twenty five in Bitlis. The milk samples were collected in labeled sterilized polyethylene bottles from both breasts after they have been well washed. The milk was collected by self-milking with the nipple well inserted into the bottles. 75 breast milk and 75 cow's milk samples were analyzed by a Inductively Coupled Plasma- Mass Spectrometer. The samples were measured in an accredited laboratory (Accredite No: AB-0566-T). Mother were selected after birth breastfeeding mother for 6 months and in an age range of 18-25. They were living in a suburban area but not a non-industrial area of Bitlis. All the mothers completed a questionnaire to provide details regarding some of the maternal and infant characteristics; including occupation. The mothers were informed about the purpose of the study and written consent was obtained from all participants. The cow's milk samples were determined according to the position of the region based on traffic. No participant characterized as a consumer of cigarette. The samples were drawn into 20

mL tubes and stored at  $-25^{\circ}\text{C}$  in the freezer.

### Preparation of Milk Samples

The samples maintained at  $-25^{\circ}\text{C}$  were dissolved at refrigerator temperature and preparation carried out. Dry combustion method was used as a method of analysis as stated in the literature [26]. 10 g milk sample was weighed into a porcelain crucible and had pre-drying process in a water bath. Actual drying process was made until it was completely solid in the oven at  $105^{\circ}\text{C}$ . Crucibles containing dried samples were placed in a cold muffle furnace and the burning process carried out by increasing  $50^{\circ}\text{C}$  in every hour until  $450^{\circ}\text{C}$ . At  $450^{\circ}\text{C}$ , burning process was continued until there were no black spots in the crucibles. Total burning time was about 10 h in  $450^{\circ}\text{C}$ . Muffle furnace's temperature was dropped to  $150^{\circ}\text{C}$  and crucibles were taken from the desiccator and cooled. 5 mL 6 M HCl was added. The solution was evaporated in water bath until drying. The residue was dissolved in an amount of 0.1 M  $\text{HNO}_3$  and heated in a closed vessel for 2 h. Then, 50 mL flask was transferred to the washing well with 0.1 M  $\text{HNO}_3$  solution. Flask was filled until having 50 mL solution and the samples were transferred into flask and placed in refrigerator. Argon gas has 99.998% spectral purity. External standard calibration method was applied on all determinations. During the analysis at regular intervals, calibration standards were analysed as a sample to monitor the trend of the device. Blind samples were prepared without the samples by completing the analytical sample procedure. Analytical accuracy was controlled with certified reference materials by measuring [27]. Limits of detection (LOD) and quantification (LOQ) of methods were calculated as 3 and 10-fold the standard deviation from concentrations of 6 independent blank replicates. In order to determine Arsenic, Cadmium, Lead, Zinc LOD ve LOQ were 0.358/0.482 mg/kg, 5.63/7.31  $\mu\text{g}/\text{kg}$ , 6.41/10.18  $\mu\text{g}/\text{kg}$ , 2.25/4.12 mg/kg respectively.

### Statistical Evaluation

Analysis of variance technique was used for the evaluation of data. Concerning statistical analysis were utilized by SPSS (version 16) package program. The resulting values considered as  $\pm$  and significance levels were determined as  $p < 0.05$  by using Duncan multiple comparison test.

## Results and discussions

The present study provides data on the levels of Arsenic, Cadmium, Lead, and Zinc in breast milk and cow milk obtained from Bitlis lactating mothers living in two different regions in Bitlis.

Cd, Pb, Zn and As concentrations in the breast and cow milk samples were determined by ICP-MS. In the samples, As and Cd were under detectable levels but Zn and Pb were measurable. Average concentrations of breast milk by region given at the table 1.

In the samples As and Cd were under detectable levels in table 1 Pb levels in the mother milk collected in Tatvan borough were determined as the highest. The reason of higher lead levels in Tatvan was exposure to traffic gas more than other regions.

As shown in the table 2, As and Cd in the mother milk samples collected in the center of Bitlis, Tatvan and villages were under detectable levels so they could not be detected. Pb levels were determined the highest in Tatvan. The reason of high lead level in Tatvan was exposure of the mothers to more traffic gas than the mothers in the other regions.

	Center of Bitlis	Tatvan	Bitlis's villages
Cd	ND	ND	ND
As	ND	ND	ND
Pb	0.3 ±0.04 µ/kg	0.45±0.052 µ/kg	0.2±0.34 µ/kg
Zn	3.2±0.64 mg/kg	3.34±0.569 mg/kg	2.5±0.37mg/kg

Mean±SD; \*ND:Not Determined; ND means <LOD

**Table 1**  
AVERAGE (MEAN±SD)  
CONCENTRATION OF  
BREASTFEEDING BY  
REGION

	Center of Bitlis	Tatvan	Bitlis's villages
Cd	ND	ND	ND
As	ND	ND	ND
Pb	2.0±0.4 µ/kg	3.0±0.4 µ/kg	1.0±0.4 µ/kg
Zn	3.7±0.51 mg/kg	3.6±0.49 mg/kg	3.0±0.41 mg/kg

Mean±SD; \*ND:Not Determined,ND means <LOD

**Table 2**  
AVERAGE (MEAN±SD)  
CONCENTRATIONS OF  
COW MILK BY REGION

**Table 3**  
Zn CONCENTRATION OF BREASTFEEDING AND COW MILK BY REGION

Zn <sub>1</sub>	Center of Bitlis	Bitlis Villages	Tatvan	Zn <sub>2</sub>	Center of Bitlis	Bitlis Villages	Tatvan
Mean	0.153	0.134	0.226	Mean	0.383	0.422	0.450
Median	0.161	0.133	0.147	Median	0.347	0.474	0.424
Std. Dev	0.102	0.005	0.267	Std. Dev.	0.157	0.158	0.091
Min.	0.026	0.130	0.054	Min.	0.18	0.19	0.320
Max	0.272	0.142	0.856	Max	0.634	0.714	0.609

Zn<sub>1</sub>: Zn Concentration of Breastfeeding milk by Region; Zn<sub>2</sub>: Zn Concentration of cow milk by Region

**Table 4**  
Pb CONCENTRATION OF BREASTFEEDING AND COW MILK BY REGION

Zn <sub>1</sub>	Center of Bitlis	Bitlis Villages	Tatvan	Zn <sub>2</sub>	Center of Bitlis	Bitlis Villages	Tatvan
Mean	0.382	0.354	0.393	Mean	0.332	0.270	0.387
Median	0.386	0.333	0.362	Median	0.362	0.295	0.402
Std. Dev	0.202	0.047	0.152	Std. Dev.	0.129	0.052	0.211
Min.	0.090	0.319	0.239	Min.	0.133	0.192	0.242
Max	0.731	0.444	0.657	Max	0.496	0.314	0.790

Pb<sub>1</sub>: Pb Concentration of Breastfeeding milk by Region; Pb<sub>2</sub>: Pb Concentration of cow milk by Region

Table 3 and table 4 were given Pb and Zn Concentration of Breastfeeding and cow milk by Region. Also these table were given that min. and max. numerical values.

### Zinc

In this study, we found that the level of zinc in the mothers' milk in Bitlis were determined as 3.0 mg/kg. Sun et al. [28] determined 3.25 mg/kg zinc levels in the breast milk in China, which were similar to the results in our study. Robberect et al. [29] determined a limit of a daily intake of 1.2±0.2 mg in their work carried out in Africa. In our study, the zinc levels were higher than in this study. Rajalakshmi and Srikantia [30] determined zinc levels that fell from 5.32 to 1.12 microgram/mL in 7 months in Indian women. This shows us that the breastfeeding rates vary according to the environmental pollution in urban areas. We determined that the level of zinc in the cows' milk in Bitlis was 3.4 mg/kg. Özrenk collected [31] cow's milk from Van city, which was determined to have a 3.003 mg/kg zinc ratio. This was similar to our study. Dawg et al. [32] found 4.923 mg/kg zinc ratio in Addis Ababa; the capital of

Ethiopian. This level was higher than our zinc levels in cow's milk. Licata and colleagues [33] determined a 2.016 mg/kg zinc ratio in Italy. This study achieved a lower ratio when compared to our study.

### Lead

In our study, lead concentration in breast milk were found 0.32µ/kg. Ursinyova and Masanova [34] has achieved that the level of lead in breast milk samples from Slovakia were detected 4.7 µ/kg. Sun et al. [28] found 4.7 µ/kg achieving breast milk samples. Tripathi and colleagues [35] found the amount of lead in the human milk 0.7 µ/kg. In 1993, In addition, Sun et al. [28], Tripathi and colleagues [35] and Ursinyova and Masanova (34) in their study; the average lead levels founding in breast milk have been seen higher than average lead levels in our studies. The estimated weekly intakes of lead of breast-fed infants in this study were in some cases higher than the Provisional Tolerance Weekly Intake (PTWI) recommended by FAO/WHO [36] and this study were higher than our lead levels. Leotsidinis

et al. [37] were found lead values in mother milk  $0.48 \pm 0.60$   $\mu\text{g}/\text{kg}$  at Greek mothers. This study is similar to ours. Because two of the small nature of environmental pollution from industry.

In our study, the average amount of lead found in cow milk were detected below, the value of lead in cow's milk is the same as with lead levels in Bitlis center. Ayar et al. [38] determined in their study of lead level in milk, 103  $\mu\text{g}/\text{kg}$ . Dawg et al. [32] found lead levels in cow milk obtained from the Ethiopia's capital of Addis Ababa 9.98  $\mu\text{g}/\text{kg}$ . Also Murthy and Rhea [39] Temiz and Soylu [40], Ayar et al. [38] in their studies in cow's milk lead levels were higher than our study in cow milk lead values. Although the majority of the studies support the fact that women living in urban areas with heavy traffic and industrial activities have higher breast lead levels than those living in rural areas [41,42]

### Cadmium

Cadmium levels in breast milk samples were not be able to calculated because the average concentrations were below quantifiable levels. Ursinyova and Masanova [34] found cadmium level as 0.43  $\mu\text{g}/\text{kg}$  in samples of breast milk collected from mothers in Slovakia. Larsson et al. [43] found cadmium levels 0.1  $\mu\text{g}/\text{kg}$  in milk collected from Swedish mothers. In 1993, WHO's [44] average cadmium level for breastfeeding was calculated as 0.1  $\mu\text{g}/\text{kg}$ . Cadmium was not detected in cow milk samples as in the case of breast milk at research region.

### Arsenic

Arsenic present in milk could have a natural origin, because it has been demonstrated that it is secreted in breast milk, or provided in part from external contamination such as the mother's diet, the environment or the manufacturing process [45]. The degree of arsenic toxicity is basically dependent on the form (e.g. inorganic or organic) and the oxidation state of the arsenic compounds. It is generally considered that inorganic arsenic is more toxic than organic arsenicals (WHO 2001), [45] Arsenic in mother and cow milk were not detected in our research region, Bitlis. Concha et al. [46] found arsenic level as 23  $\mu\text{g}/\text{kg}$  in mother's milk collected from Andean. Timtek et al. [47] determined arsenic levels as 50.0  $\mu\text{g}/\text{kg}$ , 9.0  $\mu\text{g}/\text{kg}$  and 0.2  $\mu\text{g}/\text{kg}$ , respectively in milk collected from Bursa's industrial areas, rural areas and areas where the traffic was dense. Data reported in the literature on the as concentration in cow milk varies from 0.2 to 4.93  $\text{ng}/\text{mL}$ . [48] our study, there was no arsenic level and WHO's [35-41-43] threshold value is not exceeded.

### Conclusions

This study concluded that Arsenic, Cadmium, Lead and Zinc levels in mother milk and cow milk replace of pollution. We examine the other literatures that has been observed lead levels increase and zinc levels falls in the urban areas. The study would give an indication of the exposure of mothers and infants in the community to the heavy metals and also ascertain the safety of absolute breast feeding of infants. Levels of lead in breast milk were lower than other studies. The reason for this is that there are not any factories in the province of Bitlis. As well as there is less than traffic and pollution regions in the literature. As is known, milk is an important source of zinc. In our study, zinc level in breast milk and cow milk have shown conformity with other studies. Arsenic and cadmium level in the breast milk could not be determined. However, in the drinking mothers breastfeeding their babies, the damage has been

investigated indirectly. The importance of breastfeeding increased with each passing day, mothers should be educated about these metals. Also, heavy metals contained in the structure of soil can be transmitted to the food. Thus, both human milk and cow's milk expose heavy metal. As well as, the same analysis should be carried out operation in cow's milk, the soil and water samples in the place of residence should be considered exposed to heavy metal contamination and levels of heavy metals should be viewed. Environmental pollutants enter the human body ways such as food, air and water. These pollutants accumulate in the body of mother for years. And these pollution enter transplacentally way and breast milk. This creates a potential risk for baby to be born. Mothers should care feedings to tolerate such contaminations and should be enlightened on this subject. Therefore, this contamination can be tolerated by human; human should care to nutrition. People must be illuminated in this regard.

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