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ICP AES assessment of lead, mercury and determination of impact on animal productivity

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Abstract

This study aims to assess the contamination of heavy metals (Arsenic, Mercury, Cadmium, and Lead) in buffalo milk and water from farms located near industrial areas in India, where pollution risks are higher due to nearby industrial developments. Agriculture, particularly the dairy sector, plays a significant role in sustaining poor farmers and the broader farming community in India, which is one of the largest and fastest-growing markets for milk and milk products. However, environmental pollution, including heavy metals, poses a potential threat to public health and animal well-being. Long-term exposure to these metals in feed or food can cause toxicological effects, as these metals accumulate in the tissues of dairy animals and are excreted in milk.

A total of 150 samples (milk and water) were collected from farms near industrial zones and analyzed for heavy metals using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The farms were screened for subclinical mastitis using the California Mastitis Test (CMT), and only animals with negative results were included in the study. Buffaloes were categorized based on their milk yield into high (14-16 liters/day) and average (8-10 liters/day) groups. Three water samples from each farm were collected from the main supply, storage tank, and drinking water tank.

The study found heavy metals in milk and water samples, highlighting the need for continuous monitoring of pollution levels and their impact on both animal and human health. The data generated could guide the formulation of preventive measures to mitigate the health risks posed by heavy metal contamination in dairy products.

Keywords: Buffalo milk, heavy metals, water samples, contamination, ICP-AES, industrial areas, mastitis, public health, environmental pollutants, dairy products

Introduction

Agriculture and animal husbandry in India have been intricately woven into each other since the time immemorial. India holds the second-largest agricultural land in the world with a land area of 179.9 million hectares and contributes to 61 percent of the land area to the total area (World Bank Report, 2013) ^[25]. For the sustenance of poor farmers and the overall prosperity of the farming community the dairy sector is very important. In India, more than 1000 million tones milk produced per year. The country has a long tradition of milk production and consumption. India is one of the world's largest and fastest-growing market for milk and milk product. The demand for value-added milk products such as cheese and probity drinks is increasing at a double-digit rate in India (Vinola R *et al.*, 2018) ^[24].

The environment is continuously being loaded with diverse chemical substances and pollutants released via anthropogenic activities. As a result, wildlife and human populations are exposed to a chemical substance which might also cause adverse effects (EPA, 2000) ^[8]. Nowadays, pollution is a worldwide trouble which occurs due to the presence of contaminants into environment, which produces adverse effects. Air, soil and water pollution are the major forms of pollution, introducing of particulates matter, organic molecules, and different dangerous substances into Earth's surroundings which in all likelihood inflicting ailment and damage to different dwelling organisms.

Long term exposure of metalloid, heavy metals, other chemical substances etc. in feed or food produces toxicological effects.

Heavy metals are widely spreading in the environment. Due to excessive levels of elements, such as chromium (Cr), cadmium (Cd), lead (Pb) and mercury (Hg) produces toxicity (Llobet JM *et al.*, 2003) [33]. During manufacturing process and particular environmental condition gives entry to the toxic metals in milk and milk products. Heavy metals gather in tissues of dairy animals and the long run excrete in milk due to their non-biodegradable and chronic nature (Burger & Elbin 2015) [4]. For this cause, environmental research is often completed to come across traces of these metals inside the organs and body fluids of animals living in a unique habitat.

The regions selected in the trials are the one where risk of heavy metal toxicity is more due to industrial developments. In addition, these areas have many buffalo farms spread in close vicinity with the industrial areas, and these animals get exposed to the pollution of industries. Milk produced in these farms are supplied to the surrounding areas. Therefore, it is necessary to know the status of heavy metals in the milk produced amongst the farms in selected zones.

This study aimed to determine and quantify the residues of heavy metals in buffalo milk and water samples by ICP AES. An assessment was also done to provide a comparison between the level of metals from the water source and the milk sample from the region. The generated data would help us know the status of heavy metals present in milk posing potential threat to public health including animals and further guide us towards any preventive measures that need to be undertaken, for animal health as well as from the public health aspect.

Study design

Total 150 samples of buffalo milk and water from respective farms were analyzed for heavy metals like Arsenic, Mercury, Cadmium and Lead. The farms were selected which was present in close vicinity of industrial area, road side, near to industrial plants. Animals were screened for sub clinical mastitis by performing California Mastitis Test (CMT) on their milk sample. Animals whose milk samples was detected negative for subclinical mastitis were included in the study. The milk samples were collected from selected animals and water samples were collected from respective farms. On an average 70 to 80 animals were present at selected farm. From each farm, 17 animals at "1 month post calving stage" were randomly selected from total number of animals. The selected animals were divided into two groups - high milk producing (milk yield 14 to 16 liters per day) group which included 10 animals and seven animals were selected in average milk producing (milk yield 8 to 10 liters per day) group.

Three water samples were collected from the same selected farms. Water samples were collected from three sites i.e. main water supply, storage tank, from drinking water tank of the animal. After collection, samples were carried on ice during transport and stored in deep freezer (-20 °C) until further analysis.

Samples were then processed by acid digestion method and their aliquots were sent for detection of heavy metals to Indian Institute of Technology (IIT), Powai.

Selection of Farms

Farms were selected from different area of Mumbai suburbs and Konkan region based on following criteria

- Farms which were in close vicinity of industrial area,

- Farms which were located along the road side area
- Farms located near the industrial areas where contamination of water with effluent was expected.

Selection of animals

Farm which was present in close vicinity of industrial area, road side, near to industrial effluent of that farm animals were selected for milk and water sample collection. Seventeen buffaloes were randomly selected from each farm.

Animal inclusion criteria

- Animals which were healthy (On physical examination and body confirmation).
- Animals which were tested negative for subclinical mastitis by California mastitis test.
- Milk samples were collected from buffaloes which were at one month post calving stage

Sampling Procedure

Milk sample

Udder was washed with clean water before milking. Then first four to five strips of milk were discarded. Milk samples were taken from each teat in CMT paddle for testing subclinical mastitis by California Mastitis Test. Animals tested negative for subclinical mastitis by CMT test were selected for sampling of milk. The milk samples were randomly collected from udder in sterilized 50 ml polypropylene sampling tube. Approximately 30ml milk was collected from each buffalo. Tubes were labeled as name of the area, sample name, sample number and buffalo number. Sample were carried on ice during transport to the laboratory, wherein they were kept in a deep freezer (-20 °C) until further analysis.

Water sample

Three water samples were collected from the same selected farms. Water samples were collected from three sites i.e. main water supply, storage tank, from drinking water tank of the animal. Water samples were collected after mixing water for 2-3 times from storage tank, drinking water tank and directly from main water supply. 100ml water sample were collected in sterile polypropylene tubes. After collection, samples were carried on ice during transport and stored in deep freezer (-20 °C) until further analysis.

Sample digestion

Digestion procedure

Wet digestion method using acid was used to digest milk samples. A 1ml of each milk sample was added into crucibles containing 10 ml 65% nitric acid. They were kept on a hot plate and contents were allowed to evaporate until 1 ml of solution was left. The solution was left to cool down and later, 5 ml of perchloric acid was added to the same solution and again kept on the hot plate. White fumes were let to evaporate until 1ml solution was left in the crucible. The digest was finally diluted with deionized water up to 25 ml (Richards, 1968) [27]. The same method was followed for the analysis of water samples.

Heavy metal analysis

In this study, detection of heavy metals was carried out using Inductively Coupled Plasma Atomic Emission

Spectrophotometer (ICP-AES), available at IIT, Powai, and Mumbai [13].

Inductively Coupled Plasma Atomic Emission Spectrometer (ICP- AES)

Principle of ICP-AES

ICP-AES is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its atomic character. The energy transfer for electrons when

they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital. The energy transfer is inversely proportional to the wavelength of electromagnetic radiation. Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is very common to select a single wavelength (a multiple) for respective element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the sample being analyzed. Thus, by determining which wavelengths are emitted by a sample and by determining their intensities, we can qualitatively and quantitatively find the elements from the given sample.

Table 1: Comparison between ICP- AES, ICP-MS, GF-AAS

Parameters	ICP-AES	ICP-MS	GF-AAS
Detection limit quality	Excellent for all elements	Excellent	Good for few elements
Wavelength	130nm to 800nm	130nm to 770nm	228.8nm
Sample usage	Low	Low	High
No. of elements	>100	>75	>50
Isotopes and its analysis	Yes	Yes	No

General specification of ICP-AES

R. F. generator: maximum of 1.6KW, 27.12MHz

Spectrometer-wavelength range: 130 to 770 nm, Resolution- approx.9 Pico meter, having capability to scan full spectrum to have qualitative information about the content of the sample.

Detector: Charge Coupled Device (CCD)

Vertical torch assembly having full demonstrable quartz torch with individual tubes as well as a ceramic fully demountable torch for HF based solution.

Nebulizers: concentric, cross flow, organic nebulizer (hydrocarbons, solvents) as well as cross flow nebulizer for HF containing solutions.

Spray chamber: HF resistant cyclonic chamber and hydrocarbon solution spray chamber. Spray chamber suitable for cross flow nebulizer are available.

Different spectral lines used for the detection of various metals:

The wavelength used for detection in ICP-AES is generally unique for each element. The spectral lines used for Lead, Arsenic, Mercury, Copper and Cadmium and their detection lines are shown in (Table.2)

Preparation of series of standard solutions of the respective metal ion

The selected heavy metals were Lead, Arsenic, Mercury and Cadmium. Calibration curves for each of the selected metals were prepared using three standard solutions. The usual procedure in quantitative analysis method is to prepare a series of standard solutions over a concentration range suitable for the sample being analyzed i.e. such that the expected sample concentrations are within the range established by the standard. These standards prepared by dilution from 1000ppm stock solution were as follows:

Table 2: Wavelength of the spectral line and detection limit of ICP-AES

Element	Wavelength(nm)	Detection limit (ppb)
Arsenic	189.042	10
Lead	220.353	10
Mercury	184.950	10
Cadmium	214.438	10
Copper	328.000	10

0.5ppm, 1ppm, and 10 ppm for all the above metals. Calibration curves were drawn for Pb, As, Hg and Cd by plotting absorbance versus metal ion concentration.

Standard solutions

The standard metal solutions of Arsenic, Cadmium, Mercury and Lead were used with the following concentrations for calibration and linear regression analysis.

- Arsenic standard solution:** 1000 mg/L in deionized water.
- Cadmium standard solution:** 1000 mg/L Cadmium in 0.5M HNO₃.
- Lead standard solution:** 1000 mg/L Lead in 0.5M HNO₃

- Mercury standard solution:** 1000mg/L Mercury in 0.5M HNO₃

Stock standard solutions

- Arsenic:** 1mg/L (10 µl standard solution was taken in 10 ml volumetric flask and diluted with 8M HCl to make the volume of 10 ml).
- Cadmium:** 1 mg/L (10 µl standard solution was taken in 10 ml volumetric flask and diluted with 0.1M HNO₃ to make the volume).
- Lead:** 1mg/L (10 µl standard solution was taken in 10 ml volumetric flask and diluted with 0.1M HNO₃ to make the volume).

4. **Mercury:** 1mg/L (10 μ l standard solution was taken in 10 ml volumetric flask and diluted with 0.1M HNO₃ to make the volume).

Calibration Curve and Measurement of Metal Concentrations

The calibration curves were drawn for Hg, As, Cd and Pb using linear regression analysis of the concentrations of the standard solutions versus absorbance values. Four series of working standard solutions of metals were prepared by diluting the intermediate standard solution (10 mg/L) with deionized water.

A blank and standards were run in ICP-AES and four points of calibration curve were established. Each standard solution was measured three times and the mean was plotted. The correlation coefficients of calibration curves are given in Table 4. The correlation coefficient 0.99 and more showed that there is strong linear relationship between concentrations and absorbance. Each of the sample solutions were aspirated into the instrument and the absorbance values of the metals was recorded. Concentrations of each metal were determined by interpolation from the calibration curves. Triplicate determinations were carried out on each sample.

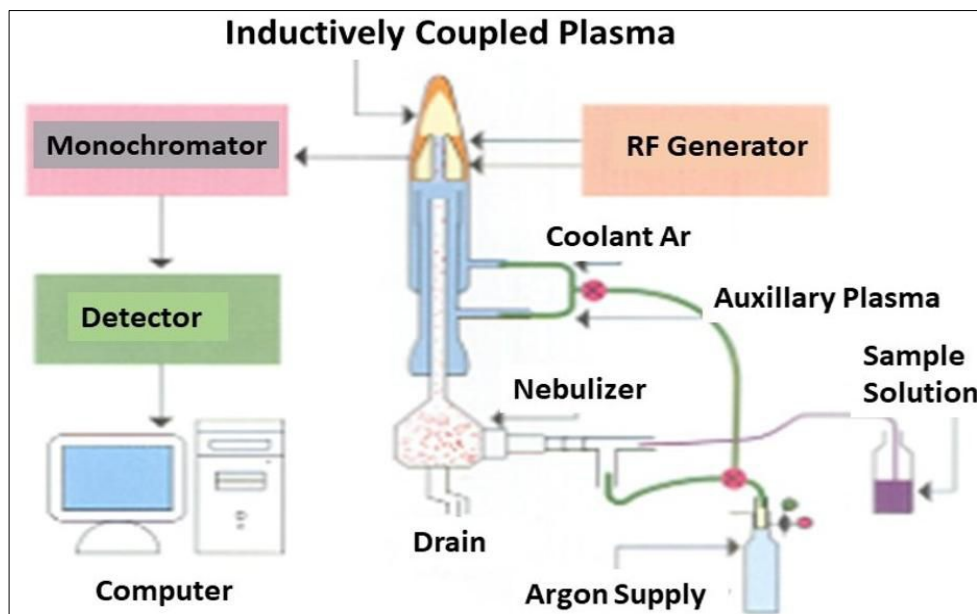


Fig 1: Working Principle of ICP-AES

Data Analysis

Data was analyzed using Sigma Stat software 4.0. ANOVA, t-test, and Pearson correlation method were used for data interpretation. The data were expressed in term of descriptive statistics while the figures were presented with Mean values as (Mean $\pm$ SD). A P-value less than 0.05 was considered as Significant. When the results are non-significant the difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability.

Result and Discussion

The present study was undertaken to evaluate the heavy metals Mercury, Lead, in milk samples of industrialized area in Maharashtra. The total 160 samples were collected from 8 different regions viz SZ-1, SZ-2, SZ-3, SZ-4 SZ-5, SZ-6, SZ-7, and SZ-8. All regions were closely situated to industrial areas and highways. In addition 17 water samples were collected from every sampling point. Samples were processed in the Department of Veterinary Pharmacology and Toxicology Mumbai veterinary College, Parel. Samples were analyzed for heavy metals viz Mercury, Lead, Copper, and Cadmium at their specific wavelength 184.950, 220.353, respectively by using Inductively Coupled Plasma Atomic Absorption Spectrometer (ICP-AES) in the department of Sophisticated Analytical Instrument Facility (SAIF) at Indian Institute of Technology (IIT), Powai.

Instrumental Calibration: Quality of data obtained from ICP- AES are mainly depending on the calibration and preparation of standard solutions. Instrument was calibrated by making freshly intermediate standard solution. Standard solutions were prepared from stock solution of every metal. Different concentrations of standard solutions of the respective metal were analyzed to get the coefficient of correlation above 0.995. The standard was analyzed as a sample to confirm the standardization of detection procedure. Dilution factor was taken into consideration while calculating the results. Three replicates of each sample were analyzed and their mean was taken as final result for each metal.

Concentration of lead

The lead values found in present investigation in milk samples of buffalo of different regions of Maharashtra suburbs indicated that the concentration of lead is below the detection level and these findings were compared with National and International scenario of different areas in support of the result found in the present study.

Indian Scenario

Patel *et al.* (2017) ^[16] determined the concentration of lead (cow milk 0.124 $\pm$ 0.063, Buffalo milk 0.016 $\pm$ 0. 356) in milk of dairy animals in Mehsana district of Gujarat. Result revealed that the concentration of lead was below

the permissible limit whereas another study conducted by Nirgude (2015) ^[15] in the area of Boisar- Tarapur area of Palghar district of Mumbai. Milk samples were analyzed by using Inductively Coupled Plasma Atomic Emission Spectroscopy for the determination of heavy metals. Result found that the concentration of lead ranges between 0.065 to 0.137 ppm with a mean concentration of 0.089 ppm. This range is within permissible limit of food safety and standard authority of India. In another study investigation carried out by Kambli *et al.* (2019) ^[11] Mumbai suburb region was selected for determination of heavy metals in industrialized zones from milk samples. They found that the concentration of lead was high in Borivali and Bhiwandi region. Zodape *et al.* (2012) ^[26] collected 15 samples of cow milk of different brand collected from different sites of Mumbai. They evaluated level of copper, zinc, chromium, mercury, lead. They found that the highest mean concentration of lead in milk obtained between 5.904ppm and 0.139. This concentration is above MRL of standards given by International Dairy Federation (1997). The method of samples analysis in studies conducted by Nirgude 2015 ^[15], Kambli *et al.*, 2019 ^[11], Zodape *et al.*, 2012 ^[26] were same as used in present investigation

International Scenario

Belete *et al.* (2014) ^[3] evaluated concentrations of selected heavy metals in cow's milk in Ethiopia. The concentration of Cr, Cu, Mn and Zn were detected whereas Pb, Ni, Co and Cd were not found. Also in present investigation the concentration of lead was below the detection level. Hence present study findings were in accordance with the study conducted by Belete *et al.* (2014) ^[3]. The investigation was conducted by Dizaji *et al.* (2012) ^[7] for the evaluation of the concentration of lead in milk samples. Samples were collected from different zonal east Azerbaijan Province: Tabriz, Sarab, Maragheh, Meyaneh, Azarshahr, Oskou, Malekan and Ahar. The concentration of lead found highest in the Meyaneh city (182.08 ± 20.25 pbb) and the lowest ones in the Ahar city (12.82 ± 1.91 pbb). Out of total samples 28% samples were found below minimum residual limit by FAO/WHO standard (Pb: 20 μ g/kg). The heavy metals, lead and cadmium in incoming raw milk in milk production factories in Arak city, Iran were evaluated by Delavar *et al.* (2012) ^[5]. They found that the mean concentration of lead was lower than the standard level. Shahriar *et al.* (2014) ^[22] conducted study to evaluate metals in milk in Bangladesh. They found that the lead concentration in the range of 0.008 to 0.020 mg/kg. This range does not exceed the permissible limit. Milk samples of three lactating animals including cows, buffalos and goats during July 2014 to February 2015 from four districts of Punjab Province viz Dera Ghazi Khan, Sahiwal, Rahim Yar Khan and Bahawalnagar and were analysed for heavy metals (Ismail *et al.*, 2017) ^[29]. They found higher level of lead (0.007-0.041) which exceed the permissible limit of the Codex Alimentarius Commission (2011) (0.02 μ g/g). Sayed *et al.* (2011) ^[30] conducted the study to assess the content of selected essential metals (Fe, Zn, Cu and Cr) and some heavy metals (Pb and Cd) in raw cow's milk in different areas of Egypt. They found the higher level (0.327 μ g/g) of lead than the than the

permissible limit. Contamination of heavy metals like Pb and Cd in fresh milk and pasteurized milk of Bandung and surrounding area were analysed by Harlia *et al.* (2018) ^[9]. They found that 83% samples were contaminated by lead while 53% of samples exceed the permissible.

Concentration of Mercury in milk samples

The Konkan and Mumbai suburbs were investigated for mercury in milk samples of dairy farm present in industrialized area where the concentration of mercury is below the detection level and these findings were compared with National and International scenario of different areas in support of the result found in the present study. However, the national and international scenario for lead in milk samples of different area is as follow:

National Scenario

The present laboratory of Mumbai veterinary college has conducted the survey of milk samples for heavy metal analysis in Mumbai suburb region. in this survey Kambli *et al.* (2019) ^[11] found that level of mercury was alarming in the Bhiwandi and Borivali region. Zodape *et al.* (2012) ^[26] also determined level of mercury in 15 branded cow milk samples collected from different areas of Mumbai. His findings is not in accordance with result obtained in present study as mercury not detected in any sample analysed. This may be due to the difference in sampling area, collection sites and time of collection.

International Scenario

Aquino *et al.* (2017) ^[28] evaluated concentration of mercury in whey protein to monitor exposure of Brazilian population. They found that the lower concentration of mercury than the permissible limit. Qin *et al.* (2009) ^[17] collected commercial milks from China, Japan and raw milk from Inner Mongolia of China and analysed for different metals. The analysed data showed that no any samples were the positive for heavy metals. Study on determination of level of heavy metals (arsenic, lead, cadmium and mercury) in tin milk produced in Ghana was conducted by Amposh *et al.* (2014) ^[2]. They found that the higher concentration of mercury 0.03 ppm in milk above the recommended permissible limit. Jolly *et al.* (2017) ^[10] conducted study to determine concentration of heavy metals (arsenic, cadmium, mercury, lead, copper) in powdered milk, raw milk, and packed milk. The reports said that the mercury concentration was higher in all samples.

Concentration of Heavy Metals in Water

In the present investigation, three water samples were taken from all farms of selected regions. The water supply to all farm is from the different sources (Municipal Corporation, lake, well, etc.) The samples were collected from three different sites (Main source of water, Storage tank, and waterer) of a farm. Reason behind the water collection from different site was to understand the main source of contamination. Total five metals (lead, mercury, arsenic, and cadmium, copper) were analysed. No any heavy metal detected in any sample of the water samples.

Table 3: Concentration of heavy metals in water of different Region

Region	Mercury (ppm)	Lead (ppm)
SZ-1	ND	ND
SZ-2	ND	ND
SZ-3	ND	ND
SZ-4	ND	ND
SZ-5	ND	ND
SZ-6	ND	ND
SZ-7	ND	ND
SZ-8	ND	ND

One way ANOVA was done on Sigma Stat Software (4.0). In case of Copper, significant difference was found between control (MRL) and Water samples in Ram mandir and Mahape regions ($P < 0.05$). There was a significant difference found among the eight regions ($P < 0.001$). Significant difference found at 5% level of significance. ND (not detected) means less than 0.01ppm

National Scenario

Sandeep *et al.* (2012) ^[21] analysed the drinking water samples for heavy metals (cadmium, copper, lead) of Uttar Pradesh region. They found that higher concentration of cadmium and lead which exceeded the permissible limit given by BIS whereas the concentration of copper was within the permissible limit of BIS. In present study the concentration of copper obtained from different sampling points is within the permissible limit which is in line with

the findings of Sandeep *et al.* Rathod *et al.* (2014) ^[20] conducted a study to evaluate the concentration of heavy metals in eight different lakes of Thane region. The result revealed that the concentration of copper and arsenic was below the permissible limit. The results of this study is in accordance with the observations noted in present investigation. The concentration of mercury exceed the permissible limit.

Table 4: Permissible limit value of different standard

Metals	Water		
Metals	WHO Limit (ppm)	Indian Standards (ppm)	ICMR (ppm)
Arsenic	0.01	0.05	0.05
Lead	0.01	0.05	0.05
Mercury	0.001	0.001	0.001
Cadmium	0.003	0.01	0.01
Copper	2.0	0.005- 1.5	1.5

Raju *et al.* (2014) ^[18] evaluated heavy metals (arsenic, lead, mercury, cadmium) concentration in groundwater of villages situated near the coastal area of Nellore district, Andhrapradesh. The obtained data showed that the concentration of arsenic, lead, mercury, cadmium were below the permissible limit of Indian standard and WHO. Singare and Fern. (2014) ^[23] assessed heavy metals (lead and mercury) in the water samples of Mahim creek, Mumbai. They found that the higher concentration of lead

and mercury in the analysed samples set for inland surface water by Central Pollution Control Board (CPCB) of India. Ramchander *et al.* (2015) ^[19] evaluated the heavy metals in water samples of Balapur, Jeedimetla, Patancheru and Tarnaka areas of Hyderabad city. The result showed that the concentrations of lead and cadmium are moderately high in the water samples of Balapur and Patancheru area whereas copper had no significant differences of concentrations compared to standard given by WHO.

Table 5: Comparison between three different sites of water collection.

Region	Concentration of different Sources of Water (ppm)		
	Main water source	Water storage tank	Waterer
SZ-1	0.027	0.048	0.043
SZ-2	0.046	1.289	1.191
SZ-3	0.000	0.000	0.000
SZ-4	0.000	0.000	0.000
SZ-5	0.041	0.051	0.035
SZ-6	0.018	0.031	0.010
SZ-7	0.090	0.014	0.035
SZ-8	0.036	0.076	0.021

One way ANOVA was done on Sigma Stat Software (4.0). In case of three different sites of water collection, significant difference was found between control (MRL) and water samples of all regions ($P < 0.05$). There was a statistical significant difference found among the eight regions ($P < 0.001$). Significant difference found at 5% level of significance. ND (not detected) means less than 0.01ppm

International Scenario

Kantoma *et al.* (2017) ^[12] analysed the water samples for heavy metals in the area of Kaduna state of Nigeria. The result obtained showed that the concentration of copper, lead, and cadmium was below the permissible of WHO. The result of this study is in line with the result obtained in the present study. Mohankumar *et al.* (2016) ^[14] was aimed to determine the pollution of water due to heavy metals in industrial region and residential area in Coimbatore of

Tamilnadu state. They found that the concentration of heavy metals in industrialized area was below the permissible limit of WHO whereas concentration of heavy metals were higher in some area of residential region. Khan *et al.* (2011) ^[31] decided to evaluate concentration of cadmium in water samples of Peshwar city. The result showed that the concentration of cadmium exceeds the permissible limit of WHO.

In view of the above discussed reports and result obtained in present study it can be concluded that though the heavy metals concentration was not detected in all the regions except the copper, the possibility of other contamination of milk and water cannot be ruled out. The monitoring of milk samples and water should be the continuous process as the results vary according to time of sample collection, processing of samples, human error etc.

Correlation of Milk yield and Heavy metals level

In the present investigation, 17 milk samples and three water samples were collected from each sampling point of the study area. Out of 17 samples, 10 milk samples were collected from the high milk yield animal's Of 14-16 litters/day and 7 samples from the animals having an average milk yield of 8-10 litters/ day.

Table 6: Heavy metal concentration in the milk of high milk yield VS average milk yield buffaloes

Region	Concentration of Metals (ppm)									
	High milk production (N= 10)					Average Milk Production (N=7)				
	As	Hg	Pb	Cu	Cd	As	Hg	Pb	Cu	Cd
SZ-1	ND	ND	ND	0.136	ND	ND	ND	ND	0.087	ND
SZ-2	ND	ND	ND	0.0192	ND	ND	ND	ND	0.020	ND
SZ-3	ND	ND	ND	0.0113	ND	ND	ND	ND	0.000	ND
SZ-4	ND	ND	ND	0.0118	ND	ND	ND	ND	0.003	ND
SZ-5	ND	ND	ND	0.0167	ND	ND	ND	ND	0.029	ND
SZ-6	ND	ND	ND	0.0270	ND	ND	ND	ND	0.021	ND
SZ-7	ND	ND	ND	0.025	ND	ND	ND	ND	0.027	ND
SZ-8	ND	ND	ND	0.025	ND	ND	ND	ND	0.027	ND

Analysis was done using t-test in Sigma Stat Software (4.0). For copper no significant difference ($P = 0.574$) was found in the values from high milk yield vs average animals at 95% confidence level. ND (not detected) means less than 0.01ppm.

The comparison was done between the average and heavy yield animals with respect to level of the heavy metals level in their milk to observe the effect of heavy metals on milk yield if any. The data is given in Table no 4.8

The concentration of arsenic, mercury, lead and cadmium were below the permissible limit (<0.01). The level of copper in different region viz SZ-1, SZ-2, SZ-3, SZ-4, SZ-5, SZ-6, SZ-7, SZ-8 was 0.136, 0.0192, 0.0113, 0.0118, 0.0167, 0.027, 0.025, respectively in high milk yield animals whereas in average milk yield animals the concentration of copper from different regions viz SZ-1, SZ-2, SZ-3, SZ-4, SZ-5, SZ-6, SZ-7, SZ-8 was 0.087, 0.02, ND, 0.003, 0.029, 0.021, 0.027, respectively.

Based on the statistical results, it proves that there was no strong relationship found between level of milk yield and level of heavy metals. Using t- test, for copper, no significant difference was found in the values of average and high milk yield at 95% confidence level.

The Study conducted by Kambli *et al.* (2019) [11] were they evaluated heavy metals (arsenic, mercury, lead, copper, cadmium) and effect of heavy metals on milk yield in the Mumbai suburbs region. They found that no statistical difference between the heavy metals and milk yield whereas these findings are in consistent with the above study.

Comparison of heavy metals between milk and water samples

Statistics showed no strong relationship between the level of metals and the milk yield of animals (Table No. 4.9). Using t-test, for copper, lead, mercury, arsenic, cadmium no significant difference was found in the values from average vs high milk yield animals at 95% confidence level. Thus, no strong inference can be made from the results obtained in the present investigation. The reports said that, chronic toxicity of heavy metals like arsenic is associated with reduced milk yield.

The study conducted by Abdalla *et al.* (2013) [1] to evaluate the concentration of heavy metals in waste water and milk (cow, goat, sheep) of animals grazing around sugar cane plants in Sudan. They analysed milk and water samples for copper, zinc, lead, cadmium, chromium, cobalt. They found that the higher concentration of metals in milk than the

concentration of metals in water. They concluded that the contamination was not mainly from waste water, but from sources such as soil containing fertilizers, pesticides, contaminated feed, traffic road intensity, etc, might have played the role.

In another study conducted by Yeotikar *et al.* (2018) [32] for heavy metal (chromium, arsenic, nickel, lead) contamination in buffalo blood and milk samples of Ludhiana district, Punjab. They concluded that the concentration of heavy metals was higher in milk and blood, and this might have resulted from ingestion of contaminated water.

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Conflict of Interest

The authors declare that they have no competing interests.

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