INTRODUCTION

Milk is a good source of nutrition and a part of daily diet, especially vital for the growth and development of children. Accordingly, milk is consumed widely in almost all parts of India; urban as well as rural (Vinola et al., 2018). Such widespread use in daily food requires its analysis for any toxic substance. Among all the potential toxic materials that could be present in the milk, heavy metals are the ones that are of significant importance since they exhibit a wide array of hazardous impacts on human as well as animal health. The potential toxicity of metals is well understood. Acute and chronic toxicity data of all the heavy metals are well established. Moreover, several metals have emerged as human carcinogens (Farahmandkia et al., 2017).

Presence of metals in air, water, soil, and food is due to natural as well as anthropogenic activities (Beiger and Jernelev, 1986). Some heavy metals like Cu, Fe and Zn are essential to maintain proper metabolic activity in living organisms; others like Pb and Cd are non-essential and have no biological role (Qin et al., 2009). However, at high concentrations, even essential metals also cause toxicity to living organisms (Li et al., 2005).

The metals present in the milk are a result of exposure of the animal to certain factors like eating grass contaminat-
ed with metals, drinking water from contaminated sources and even the dust that settles on the grass that the animal consumes (Amponsah, 2014). The presence of heavy metals in various farm inputs, including feed, fertilizer, water and environment leads to excretion of the residues in animal's milk (Younus et al., 2016). Heavy metals accumulate in tissues of dairy animals and ultimately excrete in milk because of their non-biodegradable and persistent nature (Burger and Elbin, 2015).

Mumbai and its surrounding regions have many buffalo farms spread in close proximity with the industrial areas, and hence have a high likelihood of exposure to environmental pollution. The produced milk is consumed locally and has a wide distribution across the city and adjacent areas. Therefore, it is imperative to know the status of heavy metals in the milk produced amongst the farms in Mumbai. Very few reports are available so far of such investigation. The aim of this study was to detect and quantify the residues of heavy metals in buffalo milk. An assessment was also done to provide a comparison between the level of metals from the water source and the milk sample from the respective farm. The generated data would help us know the status of heavy metals present in milk posing potential threat to human consumers and further guide us towards any preventive measures that need to be undertaken, for animal health as well as from the public health aspect.

MATERIALS AND METHODS

STUDY AREA AND ANIMALS

The study area included five regions in Mumbai suburbs: Goregaon, Borivali, Thane, Bhiwandi and Kalyan (as shown in Figure 1). Area selection was done due to their close proximity to industries, highway, and construction sites responsible for heavy pollution. The dairy farms located in these areas mainly supply raw milk to many local dairies in Mumbai city and its suburbs. Five arms were selected, one from each region. From each farm, milk was taken from twenty buffaloes and the study included a total of 100 buffaloes. Detection of heavy metal concentration was carried out to assess the environmental quality for the same.

SAMPLING

Sterilized sampling tubes were used to avoid sample contamination. Out of twenty samples, one sample was a water sample representative of that farm, 15 samples were from the animals having average milk yield of 8-10 liters/day, and remaining 4 samples belonged to the buffaloes having a high milk yield of 14-16 liters/day. Approximately 30 ml of sample was collected from each cow and kept in an ice box during transport to the laboratory, wherein they were kept in a deep freezer (-20°C) until further analysis.

PREPARATION OF MILK SAMPLE

Wet digestion method using acid was used to digest milk samples. 1ml of 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 per chloric 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). The samples were further analyzed by ICP-AES (Inductively coupled plasma- Atomic Emission Spectrophotometry). The ICP-AES was available in Indian Institute of technology (IIT, Mumbai), in SAIF (Sophisticated Analytical Instrument Laboratory), where the analysis was performed. Detection limit for all the metals was 10ppb.

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±SD). A P-value less than 0.05 was considered as Significant.

RESULTS AND DISCUSSION

The concentrations of heavy metals in the fresh buffalo’s milk from the five regions of Mumbai suburbs are present-
ed in Table 1. Among the four elements analyzed (As, Cd, Pb, Hg), Arsenic was below the detection limit (10ppb) of the instrument for all areas. As per the report, the highest concentration was that of Lead (0.08ppm) above the MRL (Maximum Residual Limit) (0.02ppm) from Borivali area, and Mercury (MRL=0.015ppm) level was found to be highest (0.032ppm) from Bhiwandi area. Cadmium was found in only one sample from Borivali area and its concentration (0.014ppm) was slightly above the permissible limit (0.009ppm), but not significant. Kalyan region shows levels of Lead and Mercury within limits. Samples from Goregaon contain only Lead, that too not in significantly higher concentration.

Table 1: Mean heavy metal levels in milk of buffaloes (Mean ±SE)

| Region (N=20) | Arsenic (ppm) | Lead (ppm) | Cadmium (ppm) | Mercury (ppm) |
|---------------|---------------|------------|---------------|---------------|
| Borivali      | ND            | 0.030±0.003 | 0.001         | ND            |
| Kalyan        | ND            | 0.014±0.003 | ND            | 0.002±0.001   |
| Goregaon      | ND            | 0.026±0.001 | ND            | ND            |
| Bhiwandi      | ND            | 0.001±0.003 | ND            | 0.009±0.002   |
| Thane         | ND            | 0.008±0.003 | ND            | 0.010±0.002   |

One way ANOVA was done on Sigma Stat Software (4.0). In case of Lead, significant difference was found between control (MRL) and milk samples from Borivali regions (p < 0.05). For both Lead and Mercury, there was a significant difference among the five regions (p <0.001). “Significant difference found at 5% level of significance, ND = not detected, means less than 0.01ppm.

For the present study, the average concentration of the heavy metals can be presented in the following order: Pb (0.015) > Hg (0.004) > Cd (0.002) > As (ND). The maximum permissible limit for the heavy metals under study is given in the Table 2.

Table 2: Recommended levels of heavy metals in water and milk as per WHO and Indian standards (Singh et al., 2002; Duruibe et al., 2007)

| Element | WHO standard (in ppm) | Indian standard (in ppm) |
|---------|-----------------------|--------------------------|
|         | MILK      | WATER       | MILK        | WATER       |
| Lead    | 0.01      | 0.02        | 0.05-1.13   |             |
| Cadmium | 0.003     | 0.01        | 0.001-0.009 |             |
| Mercury | 0.001     | 0.001       | 0.001-0.015 |             |
| Arsenic | 0.01      | 0.05        | 0.001       |             |

There is no exposure level below which Lead appears to be safe. One of the major sources of Lead contamination in milk is water, especially in more contaminated areas (Codex Alimentarius Commission, 2003); so, regular water testing should be one of the important topics for future study in order to corroborate the findings of present investigation. In present study, however, contaminated feed, soil or air seems to be the most probable causes because water sample tested from the all the farms did not show high levels of lead (Table 3).

Table 3: Concentration of Mercury in Milk Samples

| Mercury (ppm) |
|---------------|
| Samples from Mumbai City, India. The level is the same as found in the present study, which is slightly higher than the Indian standards. Mercury concentration of raw cow’s milk wa
Table 3: Comparison of heavy metal levels between water sample and milk sample from the respective areas

| Region      | Water (ppm) | Milk (mean in ppm) |
|-------------|-------------|--------------------|
|             | Lead | Arsenic | Mercury | Cadmium | Lead | Arsenic | Mercury | Cadmium |
| Thane       | 0.028 | ND | ND | ND | 0.030 | ND | 0.010 | 0.014 |
| Bhiwandi    | ND | ND | ND | ND | 0.001 | ND | 0.009 | ND |
| Kalyan      | 0.021 | ND | ND | ND | 0.014 | ND | 0.002 | ND |
| Goregaon    | ND | ND | ND | ND | 0.008 | ND | ND | ND |
| Borivali    | 0.029 | ND | ND | ND | 0.026 | ND | ND | ND |

*The values are Mean. Correlation coefficient between milk samples and water sample was calculated using Sigma Stat Software (4.0). A sample of water from each respective farm is compared with its milk sample (n=19). Water samples from Goregaon, Borivali and Kalyan contained traces of Lead, the values of which are consistent with those found in the milk samples from these regions. For all the metals tested (Pb, Cd, Hg, As), no strong relationship was found between the two groups. ND = not detected, means less than 0.01 ppm.

Concentration of Arsenic in Milk Samples
None of the milk samples collected from the five regions showed any traces of Arsenic. Water samples, too, were negative for this metal.

Concentration of Heavy Metals in Water
In the same study, a representative sample of water was collected from the respective farms to conclude whether water is the source of contamination. The results for the same are given in Table 3, and shows that water contains only traces of Lead and no other heavy metals.

Mercury was below detection limit (<0.01 ppm) in the water samples from all the farms. Therefore, it can be said that water may not be the source of contamination of this toxic metal in the milk samples studied under this investigation.

Concentration of Cadmium in Milk Samples
Cadmium was found in only one sample from Borivali region. No Cadmium was found in the water sample. Studies have showed that Cadmium concentrations in the milk of cows which have been raised in industrial areas and next to the highways or animals which are fed with food contaminated with heavy metals is much higher than of animals that are bred in cleaner areas (Pavlović et al., 2004; Patra et al., 2008). The area under present study was close to various industries, though the Cadmium was found in only one sample and the concentration (0.014 ppm) was not too high. This finding is inconsistent with the literature.
of heavy metals between the water sample and the milk samples. Therefore, it cannot be said that water is the only source of heavy metal exposure in these animals.

**Correlation of Milk Yield and Level of Heavy Metals**

The results showing comparison between the average and heavy yield animals with respect to the heavy metal levels in their milk, to see the effect of these metals on milk yield, if any, are given in the following Table 4. Arsenic and Cadmium were not considered for comparison since they were either not detected or found in very few samples.

According to statistics, no strong inference can be made regarding effect of heavy metals on milk yield, because both high and low yielding animals are showing variable level of toxic metals. No literature can be found on this particular topic. However, chronic toxicity of heavy metals like Arsenic is associated with reduced milk yield. Future studies with large sample size, focusing solely on relation between level of toxic metals and milk yield can prove beneficial.

Table 4: Heavy metal concentration in the milk of high milk yield vs. average milk yield buffaloes

| Region    | Element concentration (in ppm) | High milk yield (n=4) | Average milk yield (n=15) |
|-----------|--------------------------------|-----------------------|---------------------------|
|           | Mercury | Lead | Mercury | Lead |
| Thane     | 0.016   | 0.006| 0.009   | 0.010 |
| Bhiwandi  | 0.019   | 0.005| 0.009   | ND   |
| Kalyan    | 0.007   | 0.009| 0.001   | 0.015|
| Goregaon  | ND      | 0.023| ND      | 0.026|
| Borivali  | ND      | 0.023| ND      | 0.032|

*Analysis was done using t-test in Sigma Stat Software (4.0). For both Mercury and Lead, no significant difference was found in the values from average vs. high milk yield animals at 95% confidence level. Number of animals needs to be taken into consideration. Nevertheless, in a normal farm setting, high milk yield animals are generally less in number than the entire farm population. ND = not detected, means less than 0.01ppm.

**CONCLUSION**

Buffalo milk samples from Mumbai suburbs were found positive for Mercury and Lead, which indicate the level of environmental pollution around the respective farms as the source of their exposure. As the sampling was done from the farms situated near the industrialized zones, speculation can be made that the industrialization might be one of the routes of the heavy metal exposure in these animals. Though few samples were positive for heavy metals, the correlation between the water sample and milk samples was negative which indicate different sources of heavy metals and their exposure.

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**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

**AUTHORS CONTRIBUTION**

All authors contributed equally.

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