# PRESENCE OF POTENTIALLY TOXIC HEAVY METALS IN COW MILK AROUND WESTERN MAHARASHTRA MIDC (INDUSTRIAL) ZONES IN INDIA

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**ABSTRACT:**

There is a group of heavy metals that are mainly known for their fatal toxicological nature whenever they get in contact with animal, human and plants' bodies via the ecological entry route. Metals such as Lead (Pb), Arsenic (As), Mercury (Hg), Cadmium (Cd), and Chromium (Cr) have been identified as some of the potential toxic heavy metals. As a result of the large-scale industrialization taking place in Western Maharashtra state of India, studies have revealed that, the mentioned heavy metals are applied in industries for a number of reasons for manufacturing a number of finished products. Unused/unreacted residual are thereafter passed into CETP plants where effluent treatment fails to fully get rid of them. The heavy metals thereby leak to the groundwater where they cause contamination upon being discharge into creek or rivers by the leaching effect. The contaminated ground water is later used by farmers around the industrial zones for crop cultivation which is afterwards used as food for animals and humans for survival. The heavy metals upon contaminating the ground water do find their way into the plants and ultimately into the animals and human bodies through the food chain. In this study, cow milk samples which were collected across Western Maharashtra were studied for presence of potentially fatal heavy metals like Pb, As, Hg, Cd and Cr via the ICP-MS technique. This study concentrates on the quantity of heavy metals being transported from the industry to the body of living organisms on earth via the ecological route of transportation.

**Keywords**: Ecology, Heavy metals, Arsenic (As), Lead (Pb), Cadmium (Cd), Mercury (Hg), Chromium (Cr), ICP-MS, Cow.

## 1.0 INTRODUCTION

During various stages of finished product manufacturing in an industry, a number of metals are applied as process component examples being Mercury which is applied in manufacturing of fluorescent bulbs, electrical switches, mercury vapor lamps, thermometers, thermostat etc. alternatively other, metals like Lead, Chromium. Arsenic and Cadmium are widely applied in industrial processes to manufacture a number of electronics and other items. Even though such metals tend to be very useful in the manufacturing of the required components to support our day to day living, they are also very toxic when in ionic form whenever they enter the bodies of humans, plants and animals. This report focuses on the ecological entry of Lead, Arsenic, Mercury, Cadmium and Chromium type of heavy metals and their toxic impact on the body of living humans, animals and plants.

**Table–1: Toxic heavy metals symbols with atomic number and atomic weight**

| | |
|---|---|
| **$_{82}Pb^{207.2}$ (Lead)** | **$_{33}As^{74.92}$ (Arsenic)** |
| **$_{80}Hg^{200.6}$ (Mercury)** | **$_{48}Cd^{112.4}$ (Cadmium)** |
| **$_{24}Cr^{51.99}$ (Chromium)** | |

**Table–2: Physical attributes of Lead, Arsenic, Mercury Cadmium, and Chromium**

| Property »» | Atomic No. | Atomic mass. | M. P. (°C) | B.P. (°C) | Density ($g.cm^2$) |
|---|---|---|---|---|---|
| Lead (Pb) | 82 | 207.2 | 327 | 1749 | 11.34 |
| Arsenic (As) | 33 | 74.92 | 817 | 613 | 5.72 |
| Mercury (Hg) | 80 | 200.6 | -38.8 | 356.7 | 13.53 |
| Cadmium (Cd) | 48 | 112.4 | 321 | 767 | 8.65 |
| Chromium (Cr) | 24 | 51.99 | 1907 | 2671 | 7.19 |

### 1.1 The area of cow milk sampling and the procedure

Area selection (Table-3) for cow milk sampling was done in the following zones. In Western Maharashtra region villages namely Nawade, Mumbra, Navapur and Ranjangaon were selected those are located around MIDC zones and this region surrounded by CETP plants. From each selected village, 5 cows were identified and 1 liter of milk from them taken for analysis of the above-mentioned metals using the ICP-MS technique.

**Table–3: Details of the sampling area**

| Sr. No. | Sampling area (Village) | Nearest CETP | Area coordinates |
|---|---|---|---|
| 1. | Mumbra (5 Cows) | Dombivali | Latitude - 19° 10’36″, Longitude - 73° 1’20″ |
| 2 | Nawade (5 Cows) | Taloja | Latitude - 19° 03’ 12.14″, Longitude - 73° 06’ 11.46″) |
| 3 | Navapur (5 Cows) | Tarapur | Latitude - 19° 46’ 48″, Longitude - 75° 40’ 48″) |
| 4 | Ranjangaon (5 Cows) | Waluj | Latitude - 19° 50’ 36″, Longitude - 75° 12’ 36″ |

A total of 20 samples from 20 cows were collected ***(Breed - Khillari, Sub species – Bos indicus)*** from the 4 villages as mentioned above those are located in western Maharashtra MIDC region. Sample identification was allotted as indicated in Table - 4 below**.**

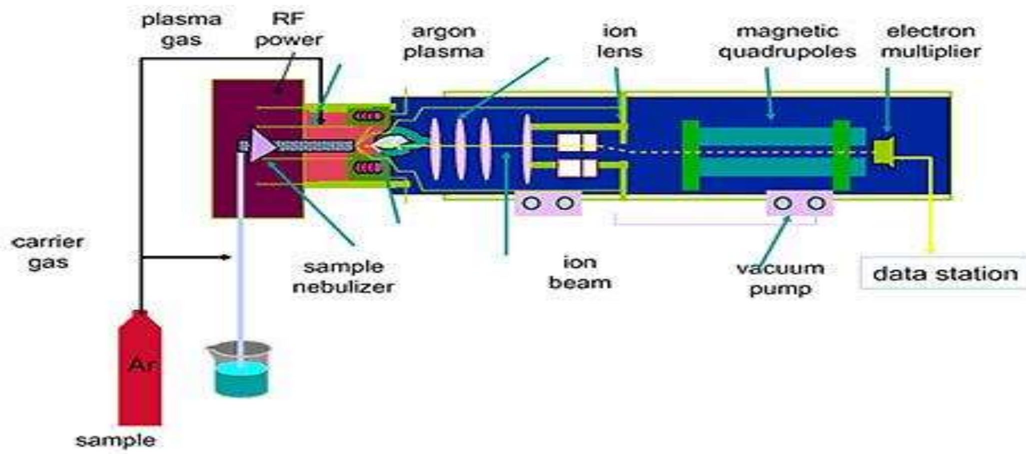
**Table–4: Identification of milk samples**

| Sr. No. | Sampling area | No. cows | Sample identification code |
|---|---|---|---|
| 1. | Mumbra | 5 | MC-1, MC-2, MC-3, MC-4, MC-5, |
| 2. | Nawade | 5 | NC-1, NC-2, NC-3, NC-4, NC-5, |
| 3. | Navapur | 5 | NaC-1, NaC-2, NaC-3, NaC-4, NaC-5, |
| 4. | Ranjangaon | 5 | RC-1, RC-2, RC-3, RC-4, RC-5, |

Where

M – Mumbra, Na – Navapur, N – Nawade, C – Cow, R – Ranjangaon, & 1, 2, 3, 4, & 5 - Cow no.

### 1.2 ICP – MS Instrument




**Figure–1: ICP – MS instrument depiction** (Source- Merck, Water for ICP-MS, Application overview, High purity water for ICP-MS)

A typical ICP- MS instrument is composed of a sampling station, carrier gas, sample nebulizer, plasma gas, argon plasma, RF power, ion lens, magnetic quadrupoles, electron multiplier detector, vacuum pump and data station.

## 2.0 METHOD AND MATERIALS

### 2.1 Materials:

ICP -MS instrument from Agilent make 7800 model was applied in the estimation of Pb, As, Hg, Cd and Cr in the 20 samples of cow milk collected from the selected villages as shown in Table-3 and Table-4 [Source-Agilent Technologies, Inc. (2016)].

**Figure-2: ICP–MS Agilent model 7800** (Agilent Technologies, Inc. 2016)

**Figure-3: ICP–MS Agilent model 7800** (Agilent Technologies, Inc. 2016)

## 3.0 TEST METHOD

### 3.1 ICP-MS Test Parameters

ICP – MS test parameters applied in quantitative estimation of Pb, As, Hg, Cd and Cr are as indicated in Table–5.

**Table-5: ICP-MS test method parameters**

| | |
|---|---|
| Make | Agilent |
| Seeps/Replicates | 10 |
| Stabilization time | 0 sec. |
| Model | 7800 |
| Replicates | 3 |
| Peak pattern | 3 points |
| Gas | He |
| Quick scan | Off |
| Acquisition Mode | Spectrum |

### 3.2 Preparation of sample

Each of the samples of milk collected was thoroughly mixed as part of the homogenization process in order to make it homogenous sample. A 10 mL of milk was transferred through volumetric pipette (Class-A) into 100 mL separate vortex clear glass test tubes. In it a 10 mL of water was added to dilute the sample viscosity. Then 5 mL of 0.2M trichloroacetic acid aqueous solution was added to the sample through glass measuring cylinder to precipitate out fats from the milk. The milk sample was thereafter shaken and the precipitate allowed settling down at the bottom of test tube. The solution was centrifuged at 5000 RPM for 10 minutes in glass centrifuge tubes and the cellar solution carefully decanted to prevent the precipitate from mixing with the decanted solution. The decanted solution was afterwards transferred into a digestion crucible, initially it evaporated at 100°C temperature to remove water and then sample digested at 600°C till solid ash remained at the bottom of the crucible (Rashmika R. Desai et al., 2018). The left over ash was dissolved with an aqueous solution of 20 mL 10% Nitric acid and the resultant solution diluted to 25 mL with purified water. The solution was afterwards aspirated into ICP-MS instrument for determination of Pb, As, Hg, Cd and Cr in the sample of milk. The remaining 19 samples were also prepared in a similar manner and each solution aspirated in ICP-MS instrument as per the test parameters mentioned in Table–4 for the ICP–MS.

### 3.3 Standard preparation:

Series of standard preparation of each of the metals to be tested were uniformly prepared as per the instructions given under 'Sample Preparation' though the digestion part of the procedure was excluded. Below mentioned concentration of each of the metals was prepared as a standard preparation.

**Table-6: Standard preparation with series of concentrations for all metals**

| Metal | Concentration [mg/L] |
|---|---|
| Lead (Pb) | 0.02, 0.04, 0.06, 0.08, 0.10, 0.20 |
| Arsenic (As) | 0.02, 0.04, 0.06, 0.08, 0.10, 0.20 |
| Mercury (Hg) | 0.02, 0.04, 0.06, 0.08, 0.10, 0.20 |
| Cadmium (Cd) | 0.02, 0.04, 0.06, 0.08, 0.10, 0.20 |
| Chromium (Cr) | 0.2, 0.4, 0.6, 0.8, 2.0, 4.0 |

## 4.0 EXPERIMENT RESULTS'

### 4.1 Results

Table–7, 8, 9 & 10 summarizes the experimental outcome for all the toxic heavy metals after the 20 milk samples collected were analyzed as described in method above. Results are presented in below Table-7, 8, 9 & 10 with area wise samples collected.

**Table-7: Cow milk sample results from Mumbra area**

| Results of cow milk samples collected from Mumbra area [mg/L] | | | | | |
|---|---|---|---|---|---|
| | MC-1 | MC-2 | MC-3 | MC-4 | MC-5 |
| (Pb) | 0.057 | 0.040 | 0.127 | 0.080 | 0.030 |
| (As) | 0.090 | 0.053 | 0.070 | 0.030 | 0.100 |
| Hg) | 0.004 | 0.000 | 0.001 | 0.006 | 0.002 |
| (Cd) | 0.026 | 0.052 | 0.013 | 0.073 | 0.034 |
| (Cr) | 2.100 | 3.090 | 1.410 | 0.697 | 1.850 |

**Table-8: Cow milk sample results from Nawade area**

| Results of cow milk samples collected from Nawade area [mg/L] | | | | | |
|---|---|---|---|---|---|
| | NC-1 | NC-2 | NC-3 | NC-4 | NC-5 |
| (Pb) | 0.020 | 0.007 | 0.053 | 0.037 | 0.000 |
| (As) | 0.047 | 0.067 | 0.110 | 0.037 | 0.027 |
| (Hg) | 0.001 | 0.000 | 0.005 | 0.003 | 0.007 |
| (Cd) | 0.082 | 0.020 | 0.024 | 0.046 | 0.023 |
| (Cr) | 1.353 | 1.663 | 0.963 | 1.187 | 0.667 |

**Table-9: Cow milk sample results from Navapur area**

| Results of cow milk samples collected from Navapur area [mg/L] | | | | | |
|---|---|---|---|---|---|
| | NaC-1 | NaC-2 | NaC-3 | NaC-4 | NaC-5 |
| (Pb) | 0.023 | 0.073 | 0.017 | 0.113 | 0.057 |
| (As) | 0047 | 0.140 | 0.027 | 0.007 | 0.087 |
| (Hg) | 0.003 | 0.026 | 0.005 | 0.007 | 0.960 |
| (Cd) | 0.020 | 0.024 | 0.041 | 0.070 | 0.017 |
| (Cr) | 0.747 | 1.223 | 0.630 | 0.960 | 1.340 |

**Table-10: Cow milk sample results (Ranjangaon area)**

| Results of cow milk samples collected from Ranjangaon area [mg/L] | | | | | |
|---|---|---|---|---|---|
| | RC-1 | RC-2 | RC-3 | RC-4 | RC-5 |
| (Pb) | 0.140 | 0.073 | 0.113 | 0.167 | 0.063 |
| (As) | 0.093 | 0.113 | 0.133 | 0.023 | 0.053 |
| (Hg) | 0.004 | 0.003 | 0.002 | 0.006 | 0.010 |
| (Cd) | 0.040 | 0.037 | 0.027 | 0.050 | 0.030 |
| (Cr) | 0.410 | 0.783 | 1.327 | 0.990 | 1.497 |

**Acceptance rule-** (Guidelines for Drinking-water Quality, World Health Organization, Geneva – 2008), **(**Indian standard, 2012).
Maximum permissible exposure for the specified fatal heavy metal (for drinking water in accordance with the WHO and BIS standard) is illustrated in the Table-11 below. Values mentioned in below table for each metal is maximum permissible exposure of respective heavy metal per day per person.

**Table–11: Heave metals acceptance rule**

| Source | Pb | As | Hg | Cd | Cr |
|---|---|---|---|---|---|
| | (mg/L) | | | | |
| WHO/ BIS | 0.01 | 0.01 | 0.001 | 0.003 | 0.05 |

In order to confirm the identity of all five heavy metals, specificity study was performed on each heavy metal on ICP-MS instrument and molecular weight is determined. Further experimental atomic mass is compared with theoretical molecular weight of each metal and identity of all five metals is confirmed. This methodology explained below under Specificity study.

### 4.2 Specificity

**(**ICH Harmonised Tripartite Guideline, 2005), (ICH Guidance on Analytical Method Validation 2002).

To ascertain that the developed ICP–MS method has the capacity to detect all the 5 stated fatal heavy metals both qualitatively and quantitatively, atomic mass of each of the metal element that is recorded on the ICP-MS instrument was compared to that of the theoretical atomic mass. Data derived from the experimental mass of all the elements is presented in Table-11. From the data, it could be confirmed that the ICP-MS developed method is specific and selective for detection of all the mentioned 5 metals separately or individually in all the samples of milk.

**Table–11: Comparison of the theoretical and experimental (observed) atomic mass of each of the elements on ICP-MS instrument**

| Element | Theoretical Atomic mass | Observed Atomic mass |
|---|---|---|
| **(Pb)** | 207.2 | 208 |
| **(As)** | 74.92 | 75 |
| **(Hg)** | 200.6 | 201 |
| **(Cd)** | 112.4 | 111 |
| **(Cr)** | 51.99 | 52 |

For quantitative determination of each metal in all 20 milk samples, calibration curve technique was used. Calibration curve drawn from the standard preparations done by serial dilutions of standard solution of each metal for obtaining desired range of concentrations as shown in Table-6. Calibration curve obtained for all 5 metals is illustrated in Table-13, 14, 15, 16 & 17.

### 4.3 Calibration Curves for metal quantitation in milk samples

**(**ICH Harmonised Tripartite Guideline, 2005), (ICH Guidance on Analytical Method Validation 2002).

#### 4.3.1 Lead calibration curve

**Table-12: Lead calibration curve**

| Sr. No. | Concentration (mg/L) | Corrected concentration (mg/L) | Detector response (CPS) |
|---|---|---|---|
| 1. | 0.02 | 0.019 | 1260 |
| 2. | 0.04 | 0.038 | 2352 |
| 3. | 0.06 | 0.061 | 3541 |
| 4. | 0.08 | 0.083 | 4789 |
| 5. | 0.10 | 0.111 | 6000 |
| 6. | 0.20 | 0.202 | 7156 |

### 4.3.2 Arsenic calibration curve

**Table – 13: Arsenic calibration curve**

| Sr. No. | Concentration (mg/L) | Corrected concentration (mg/L) | Detector response (CPS) |
|---|---|---|---|
| 1. | 0.02 | 0.019 | 244 |
| 2. | 0.04 | 0.038 | 483 |
| 3. | 0.06 | 0.061 | 712 |
| 4. | 0.08 | 0.083 | 955 |
| 5. | 0.10 | 0.099 | 1240 |
| 6. | 0.20 | 0.198 | 1450 |

### 4.3.3 Mercury calibration curve

**Table – 14: Mercury calibration curve**

| Sr. No. | Concentration (mg/L) | Corrected concentration (mg/L) | Detector response (CPS) |
|---|---|---|---|
| 1. | 0.02 | 0.019 | 1943 |
| 2. | 0.04 | 0.038 | 4128 |
| 3. | 0.06 | 0.061 | 6060 |
| 4. | 0.08 | 0.083 | 8761 |
| 5. | 0.10 | 0.101 | 10276 |
| 6. | 0.20 | 0.212 | 12579 |

### 4.3.4 Cadmium calibration curve

**Table – 15: Cadmium calibration curve**

| Sr. No. | Concentration (mg/L) | Corrected concentration (mg/L) | Detector response (CPS) |
|---|---|---|---|
| 1. | 0.02 | 0.019 | 1660 |
| 2. | 0.04 | 0.038 | 2944 |
| 3. | 0.06 | 0.061 | 4110 |
| 4. | 0.08 | 0.083 | 6310 |
| 5. | 0.10 | 0.121 | 7982 |
| 6. | 0.20 | 0.222 | 9789 |

### 4.3.5 Chromium curve calibration

**Table – 16: Chromium curve calibration**

| Sr. No. | Concentration (mg/L) | Corrected concentration (mg/L) | Detector response (CPS) |
|---|---|---|---|
| 1. | 0.02 | 0.019 | 1420 |
| 2. | 0.04 | 0.038 | 2769 |
| 3. | 0.06 | 0.061 | 4309 |
| 4. | 0.08 | 0.083 | 5566 |
| 5. | 1.00 | 1.01 | 7129 |
| 6. | 1.20 | 1.22 | 8340 |

By observing all the calibration curves and the correlation coefficient results from the drawn calibration curve, it could be observed that the detector response (CPS) of the ICP–MS instrument has a linear relationship with the respective heavy metal concentration across the working range. It can thereby be affirmed that the system is effectively working for quantitative estimation of the 5 stated metals from the collected cow milk samples. The formula used for the quantitative estimation of each of the metals is

$\boldsymbol{Y = mX + c}$

This an equation of a straight line where,
Y – Response of the detector (CPS)
X – Concentration of the metal to be determined
m- Slope (obtained from the calibration curve) and
c- Intercept (read from the calibration curve)

## 5.0 DISCUSSION

According to the results of the analysis presented in Table-6 to Table-9, it can be deduced that all the 5 heave metals that is Lead Arsenic, Mercury, Cadmium & Chromium are present in all the 20 cow milk samples that was collected to ascertain extent are estimated accurately and precisely. The mean values of 5 cow samples for each heavy metal are detailed in Table-17.

**Table-17: Average Results of five Heavy Metals**

| Mean Results of five Heavy Metals from milk samples obtained from cows in 4 locations [mg/L] | | | | |
|---|---|---|---|---|
| **Location»** | Mumbra | Nawade | Navapur | Ranjangaon |
| Lead (Pb) [0.01] | 0.067 | 0.023 | 0.057 | 0.111 |
| Arsenic (As) [0.01] | 0.069 | 0.057 | 0.061 | 0.083 |
| Mercury (Hg) [0.001] | 0.002 | 0.003 | 0.011 | 0.005 |
| Cadmium (Cd) [0.003] | 0.039 | 0.039 | 0.034 | 0.037 |
| Chromium (Cr) [0.05] | 1.829 | 1.167 | 1.980 | 1.001 |

Discussion on above mean results of 5 metals content in Cow milk samples obtained from 4 locations is given below.

### 5.1 Lead:

The contents of Lead in milk samples across the screened area ranges from 0.023 – 0.111 mg/L, this is above the limit that has been set by WHO/BIS of 0.01mg/L for drinking water. However, upon the consideration of the average milk consumption of 200 mL per individual in a day, the daily intake of lead will go down by five times from the derived average result to 0.013 mg/L. **It could therefore be inferred that the content of Lead in cow milk across the studied area is equal to the limit set by WHO/BIS and hence, the detected Lead quantity in the milk samples is not safe for consumption.**

### 5.2 Arsenic:

The content of Arsenic in milk samples across the screened area ranges from 0.057–0.083 mg/L for drinking water. Upon the consideration of the average daily milk consumption of 200 mL per person in a day, the daily intake of Arsenic goes down by 5 times from the obtained average value to 0.013mg/L. **It could therefore be inferred that the content of Arsenic in cow milk across the studied area is equal to the limit set by WHO/BIS and hence, the detected Arsenic quantity in the milk samples is not safe for consumption.**

### 5.3 Mercury:

The content of Mercury in milk samples across the screened area ranges 0.002 – 0.011 mg/L. This value is above the limit set by WHO/BIS which is 0.01 mg/L for drinking water. After considering the average daily intake of milk per individual which is 200 mL in a day, the daily Mercury intake reduces by five times from the obtained mean consumption to 0.001mg/L. It can thus be concluded that mercury content in cow milk for the investigated region is less than the limit set by WHO/BIS. Hence, milk is safe for consumption from Mercury intake point of view.

### 5.4 Cadmium:

The content of Cadmium in milk samples across the tested area ranges from 0.034 – 0.039 mg/L which is above the limit set by WHO/BIS of 0.01 mg/L for drinking water. Upon consideration of the mean quantity of milk consumed by an individual in a day which is 200 mL, the daily intake of Cadmium falls by 5 times from the average value to 0.007 mg/L. **This value is still above the limit set by WHO/BIS. Thus the Cadmium level is not safe for consumption. To mitigate any health effect that may arose due to this, it is necessary for the regulators to take on precautionary measures to reduce the Cadmium content leaching in the nearby groundwater from the effluent discharged from the surrounding CETP.**

### 5.5 Chromium:

The contents of Chromium in milk samples across the screened region ranges from 1.001 to 1.980mg/L, this quantity is well above the limit set by WHO/BIS of 0.01 mg/L for drinking water. Upon taking the average daily milk consumption of 200 mL per individual in a day, the daily Chromium intake falls five times from the average value to 0.298 mg/L. This value is still way above the set limit. However, the content of Chromium in cow milk is explained by diet supplement that contain Chromium as one of the essential ingredients for healthy development of cows. The higher Chromium level in the sampled milk is therefore not harmful to the cow nor is it dangerous to the people who consume the cow milk (Nirgude, N. T. et al., 2015).

Details of the nature of diseases that are triggered by toxic heavy metals are highlighted in table 18 below.

**Table-16: Diseases caused by excessive consumption of heavy metals**

| Sr. No. | Metal name | Disease caused |
|---|---|---|
| 1. | Arsenic | Cancer, Liver damage |
| 2. | Chromium | Respiratory tract diseases |
| 3. | Cadmium | Cancer, heart disease |
| 4. | Lead | Brain damage |
| 5. | Mercury | Kidney failure |

## 6.0 CONCLUSION

From the findings of the above research study, it can be deduced that the toxic heavy metals that are Lead, Arsenic, Mercury, Cadmium, & Chromium are detected in cow milk samples from all the regions studied across western Maharashtra regions where MIDC zones and industrialization is prominent. Out of the listed five metals, Lead and Arsenic contents in the milk are equal to the WHO/BIS limit for drinking water while Mercury content is less than the set limit. Cadmium content recorded more than the set limit hence its presence in milk samples is a matter of concern. Though Chromium content is also more than the set limit, being essential nutrient for Cow and also for human growth, its presence is considered to be not harmful.

**Based on above conclusion, finally it could affirmed that, the presence Lead, Arsenic, Mercury and Cadmium type of heavy metals in the milk should be of matter of health concern to both people and animals habiting in the screened area. There is a likelihood that those heavy metals will accumulate in the body as a result of continuous consumption of milk for a long time that will pose serious health related problems to the peoples of studied locality.** H**ence there is need to take urgent precautionary measures in the treatment of waste industrial effluent to minimize leak of studied heavy metals into the underground water.** Chromium level even though found to be higher than the set limit is not harmful to the animals or plants. Its high level is attributed to supplements which are meant to improve the development of the cows. **At the end of this study, it is recommendable that MPCB board of Maharashtra state in India put in place rigorous and innovative steps to assist remove heavy metals from effluents before industrial waste are discharged to the environment. This will assist maintain safe level of heavy metals that does find its way to the human body via the ecological route.**

## 7.0 ACKNOWLEDGMENT

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## 8.0 ABREVIATIONS

- As – Arsenic
- BIS – Bureau of Indian Standards
- Cd – Cadmium
- Cr - Chromium
- CETP – Common Effluent Treatment Plan
- CPS- Counts per second
- He – Helium
- Hg – Merury
- ICP-MS- Inductively coupled plasma mass spectrometry.
- MIDC – Maharashtra Industrial Development Corporation.
- MPCB – Maharashtra Pollution Control Board

- Pb – Lead
- WHO – World Health Organization

## 9.0 STATEMENT OF CONFLICT OF INTEREST-

On behalf of all authors, the corresponding author states that there is no conflict of interest.

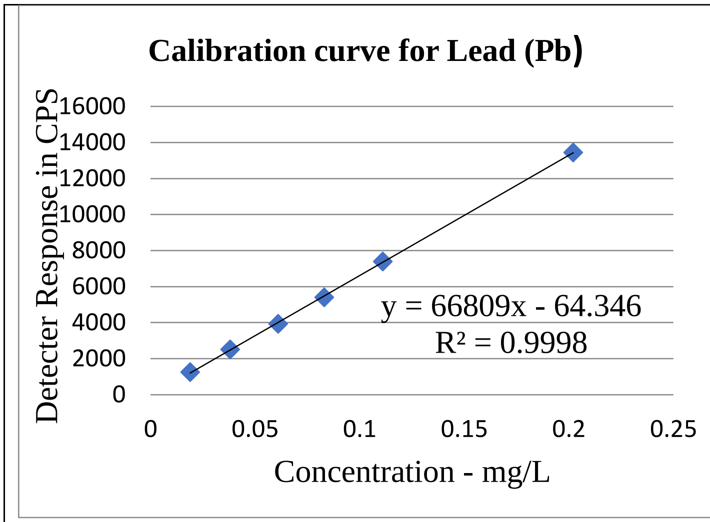


**Figure – 4 : Calibration curve for Lead (Pb)**

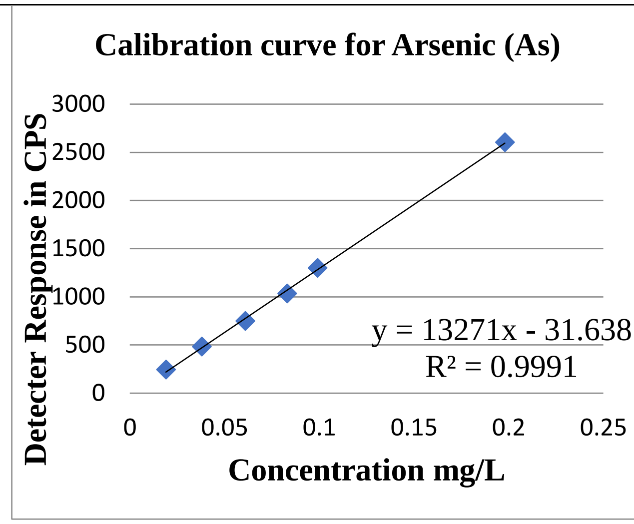


**Figure – 5 : Calibration curve for Arsenic (As)**

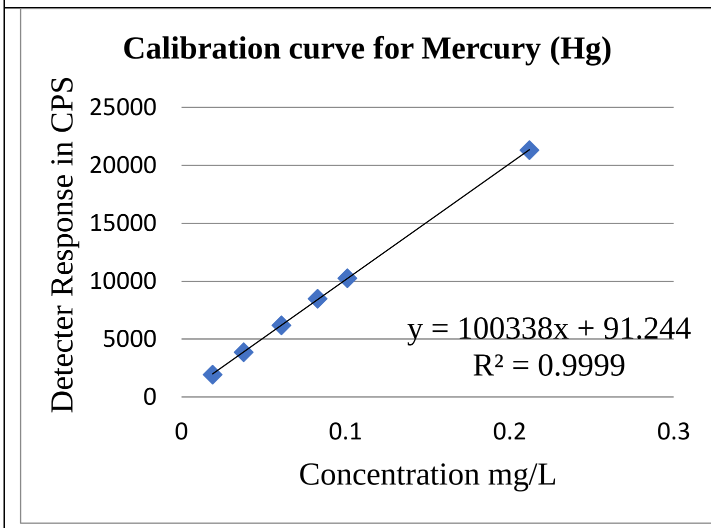


**Figure – 6 : Calibration curve for Mercury (Hg)**

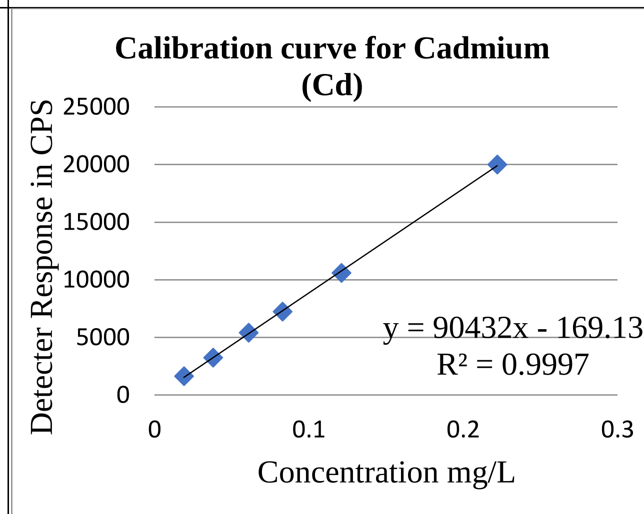


**Figure – 7 : Calibration curve for Cadmium (Cd)**

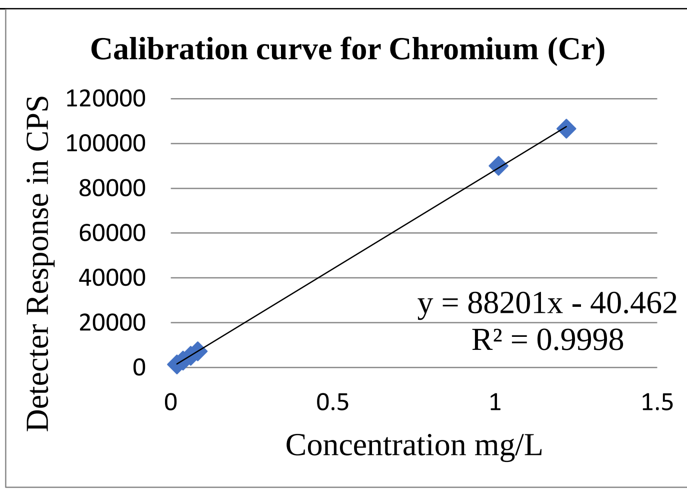


**Figure – 8 : Calibration curve for Chromium (Cr)**

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