Chapter 3

EXPERIMENTAL METHODOLOGY

Water is a very good servant,
but it is a cruel master.

C.G.D. Roberts
3. Experimental Technique

We had commenced physico-chemical analysis and monitoring of selected heavy metals including Fe, Mn, As and Zn, except Al in water on monthly, pre-monsoon, monsoon and post-monsoon basis, in two consecutive sessions i.e. 2009 and 2010. The obtained experimental results were subjected to statistical investigation such as mean, SD, SE, % CV, correlation matrix and WQI.

3.1 Sampling and Preservation Procedure:

For this purpose experiment was conducted in the following steps:

A. Choosing appropriate sampling spots:

Sampling spots had been selected as the background of the degree of pollution around the BALCO industrial area. The locations of sampling spots are given in Figure no.1 named from BS1 to BS10. These were orderly Parsa – Bhata (BS1, GW), Bhadra – Para (BS2, GW), Rumghara (BS3, SW), Dondro (BS4, GW), Dhengu – Nullah (BS5, SW), Kesla – Ghat (BS6, SW), Bela – Kachar (BS7, SW), Rugbaheri (BS8, SW), Lal – Ghat (BS9, GW), Belgahni – Nullah (BS10, SW).

B. Method for sample collection:

Grab water samples were collected at fixed time viz. 1st date of every month between 9:00 am to 4:00 pm in stopper polyethylene bottles of 2L capacity, for physico-chemical analysis while glass bottles were used for heavy metals. Before sampling containers were washed in order of 6N HNO₃, Tap water, DD water and finally with water samples.

C. Preservation Technique:

For physicochemical analysis water samples were preserved by keeping in refrigerator at 4°C and adding 3 – 4 drops of conc. HNO₃ to minimize the pH below 2 to avoid precipitation or degradation of dissolved and suspended metallic elements.

3.2 Experimental Method:

In order to develop a clear perception, water samples were analyzed into three sections viz. Physical, Chemical and Heavy metals. These parameters were determined by performing relevant experiment in sampling spots and in laboratory.
i. **Temperature**: Temperature of the collected water sample was measured on the sampling spot as it changes frequently using analyzer kit manufactured by Electronic India Model 172.

ii. **pH (Potentia Hydrogenii)**: Regulatory guidelines given by BIS IS : 10500:2004, Sec – 3025, Part – 11 was used for computation of pH. Hydrogen ion concentration of the contaminated water sample was recorded at the time of sampling in situ using analyzer kit. The instrument had been calibrated before testing by using buffer solution of pH 4, pH 7 and pH 9 in order to minimize errors.

iii. **Electrical Conductance**: It was measured by the analyzer kit using a conductivity cell on the sampling spot. The instrument was first calibrated according to the instruction given by the manufacturer and then measurement was being taken.

iv. **Turbidity** (IS : 10500:2004, Sec – 3025, Part – 10): Turbidity sampler embedded in analyzer kit was used for the observation of turbidity of the polluted sample. The turbidity meter was calibrated using stock turbidity suspension of hydrazine sulphate [(NH₂)₂H₂SO₄] and hexamethylene tertamine [(CH₂)₆H₄]. Mixing in the ratio of 1 : 1, the above suspension, standard solution was prepared whose standard value is 1.0 ml = 40 NTU, subsequently reading was recorded from collected samples from different locations.

v. **Total Solids (TS)**: It was determined gravimetrically in laboratory within 24 hour of sampling.

*Principle*: Total residue was the material left in the vessel after evaporation of a sample and subsequent drying in oven at appropriate temperature. Well – mixed sample evaporated in weighted dish and dried to a constant weight in an oven at 103°C – 105°C. The increase in weight over that of the empty dish represents the total solids.

*Requirements*: Measuring flask and Measuring Cylinder, Evaporating China dish (200 mL), Muffle Furnace, Desiccators, Analytical Balance with weight box, Steam Bath.

*Procedure*: Cleaned evaporating dish was ignited at 103°C – 105°C C for 1 hr in a muffle furnace. Cooled and stored in desiccators. Weight the dish in the desiccators prior to the use label as (B). Measured sample (100 mL) was transferred to a pre – weighted dish and evaporated to complete dryness in over and then in steam bath. Dry the
evaporated sample for an hour in oven at 103 – 105°C. Cool in desiccators to balance temperature. Repeat the above process until a constant weight (A) is obtained.

**Calculation:**

\[
\text{Total Solid in mg/L} = \frac{(A - B) \times 1000}{V}
\]

Where

- A = Final weight of the china dish in grams.
- B = Initial weight of the dried dish in grams
- V = Volume of sample water taken in ml.

**vi. Total Dissolved Solid**\(^{26}\) (TDS): It was determined with the help of Indian standard method of BIS (IS : 10500:2004, Sec – 3025, Part – 16)

**Principle:** Well – mixed water sample was filtered through standard filter paper, evaporated to dryness in a pre – weighted china dish and dried at 180°C. The increase in the weight represents the TDS.

**Requirements:** Measuring flask and Measuring Cylinder, China dish (200 ml), muffle furnace, Desiccators, chemical Balance with weight box and filtration apparatus (membrane filter funnel & gooch crucible)

**Procedure:** Filtered sample of 100 ml was taken in dried pre – weighed (B grams) in evaporating dish washed with 10 mL distilled water for 3 consecutive attempts, then heated on hot plate at 100°C. Now dish was kept on oven and heated at 105°C up to dryness. After cooling, the final weight was recorded.

**Calculation:**

\[
\text{Total Dissolved Solid in mg/L} = \frac{(A - B) \times 1000}{V}
\]

Here,

- A = Final weight of the dish in grams.
- B = Initial weight of the sidh in grams.
- V = Volume of the sample taken in ml.
vii. Total Suspended Solid\(^{21}\) (TSS):

It was determined mathematically as a difference of value of total solids and total dissolved solids as follows:

\[
\text{TSS} = \text{TS} - \text{TDS}
\]

Total suspended solid = Total Solid – Total Dissolved Solids

viii. Total Acidity\(^9\):

It may be defined as the power of the water to neutralize or to assimilate hydroxyl ion\(^{28}\). Titrimetric method had been preferred due to its simplicity. It is expressed as CaCO\(_3\) in mg/L\(^{22}\).

Principle: Hydrogen ions presents in mineral acids such as carbonic acid, acetic acid, hydrolyzing salts such as iron, aluminum sulfates reacts with hydroxyl ions of the alkali added in the process of titration\(^{19}\). Mineral acid present can be calculated at pH 4.3 while CO\(_2\) and carbonic acid can be determined at pH 8.3\(^{16}\).

Requirement: Volumetric flask, Burette, Pipette, Burette stand, Glass rod, Conical flask, Beakers, Chemical – balance with weight box, Magnetic stirrer etc.

Reagents: Reagents were prepared using chemicals of A.R. Grade using standard methods.\(^9\)

- **Standard NaOH 0.05N Solution**: 2g of NaOH pellets was suspended in CO\(_2\) free water (boiled water for 15 min, cooled, measure final pH) and dilute to 1000ml. Store in air-tight, rubber stopper, pyrex/corning glass bottle to protect from atmospheric CO\(_2\) and standardized against 0.05N HCl solution.

- **Phenolphthalein indicator**: 0.5g of solid phenolphthalein was dissolved in 500 ml of 95% ethyl alcohol. Add 500ml of de-ionized and DD water.

- **Methyl Orange indicator**: 50mg methyl orange powder was put in CO\(_2\) free distilled water and dilute to 100 ml.

Procedure: Acidity was determined in two steps:

- **Methyl orange acidity**: 20 ml of aliquot of the sample was placed in 100 ml conical flask. Added 2 drops of methyl orange indicator solution and titrated with standard solution of 0.05N NaOH till color change to faint orange at pH 4.3. Record the reading as (A).

- **Total Acidity (Phenolphthalein acidity)**: Few drops of the phenolphthalein indicator was added to the above sample and same titration procedure was
repeated further with same titrant i.e. 0.05 N NaOH. Note down the reading of burette at the end point as (B). Three readings were taken from each sample.

- **Calculation:**

  \[
  \text{Methyl Orange acidity in mg / L of CaCO}_3 = \frac{A \times N \text{ of NaOH} \times 50,000}{V}
  \]

  \[
  \text{Phenolphthalein acidity in mg / L of CaCO}_3 = \frac{B \times N \text{ of NaOH} \times 50,000}{V}
  \]

  Where,

  A = Volume of NaOH used with methyl orange in indicating the sample of pH 3.7.

  B = Volume of NaOH used with phenolphthalein in titration from pH 3.7 to 8.3.

- **ix. Total Alkalinity**: Alkalinity is mainly due to carbonates, bicarbonates, borates, phosphates, silicates hydroxide and other bases, as a result of dissociation or hydrolysis of solutes reacts with addition of standard acids. It was determined volumetrically using customary method of BIS, IS : 10500:2004, Sec – 3025, Part – 23.

  **Principle**: Alkalinity of the sample can be estimated by titrating with standard solution of HCl. Titration to pH 8.3 or decolorization of phenolphthalein indicator will indicate complete neutralization of OH\(^{-}\) and \(\frac{1}{2}\) of CO\(_3^{2-}\) while to pH 4.3 or sharp changes from yellow to orange of methyl orange indicator will indicate total alkalinity.

  **Requirements**: Volumetric flask, Burette, Pipette, Burette Stand, Glass rod, Conical Flask, Beakers, Chemical – balance with weight box.

  **Reagents**: Standard A.R. grade chemicals were used for solution preparation.

  - **Sodium Carbonate Solution**: Weigh accurately 13.25 g anhydrous Na\(_2\)CO\(_3\) (previously dried at 140°C for 2 hr) and was suspended in CO\(_2\) free DD water, diluted to 250 ml in volumetric flask.

  - **Hydrochloric Acid**: 0.1N HCl solution was prepared by diluting 8.3 ml of conc. HCl to 1000ml. Standardize it against Na\(_2\)CO\(_3\).

  - **Phenolphthalein Indicator**: 0.5 g powder was dissolved in 500 ml of 95% ethyl alcohol. Added 500 ml distilled water.
• **Methyl Orange Indicator:** 0.5g of methyl orange powder was suspended to 100 ml CO₂ free DD water and dilute to 1000ml.

**Procedure:** Course of action for determination of alkalinity was followed in two parts Phenolphthalein alkalinity and Methyl Orange alkalinity:

100 ml of aliquot was placed in 250ml conical flask. Phenolphthalein indicator was added with the help of glass rod. If no pink color appears, phenolphthalein alkalinity was absent. If a pink color appears, then titrate with 0.1N HCl water sample was taken and two - three drops of phenolphthalein indicator was added, was then titrated with the titrant 0.1 N HCl upto the end point, which shows phenolphthalein alkalinity as (A). Now, 2 - 3 drops of methyl orange was added to the same sample and continued titration further until the yellow colour changes to pink at the end point as (B).

**Calculation :**

\[
 PA \text{ as } CaCO_3 \text{ mg } / L = \frac{A \times N \text{ of } HCl \times 50,000}{V}
\]

\[
 TA \text{ as } CaCO_3 \text{ mg } / L = \frac{B \times N \text{ of } HCl \times 50,000}{V}
\]

A = Volume of standard HCl solution required when phenolphthalein indicator is used.
B = Volume of Standard HCl solution required when methyl orange indicator is used.
V = Volume of water sample used in experiment.

**x. Total Hardness**\(^{36}\): Hardness in water is caused by dissolved calcium and, to a lesser extent, magnesium. It is usually expressed as the equivalent quantity of calcium carbonate\(^{34}\). EDTA Complexometric Titration Method approved by BIS (IS : 10500:2004, Sec – 3025, Part – 21) was used as guiding principle for estimation.

**Principle:** EDTA and its sodium salts form a chelated soluble complex when added to a certain metal cations. If small amount of dye such as Eriochrome black T (EBT) added to an aqueous solution containing Calcium and Magnesium ions at a pH of 10.0 ±0.1 the solution becomes wine red\(^{16}\) due to the formation of Mg – EBT complex.. During titration with EDTA \((Na_2H_2Y)\), \(Ca^{2+}\) first reacts to form relatively stable \(CaY^{2-}\) followed by \(Mg^{2+}\) to five \(MgY^{2-}\) which is less stable\(^{31}\). Excess EDTA finally reacts with Mg – EBT complex (wine red) liberating the free indicator (blue).\(^{27}\)
Experiment Methodology

\[ \text{Ca}^{2+} + H_2Y^{2-} \rightleftharpoons \text{CaY}^{2-} + 2H^+ \]

\[ \text{Mg}^{2+} + H_2Y^{2-} \rightleftharpoons \text{MgY}^{2-} + 2H^+ \]

\[ \text{Mg}^{2+} - D(\text{red}) + H_2Y^{2-} \rightleftharpoons \text{MgY}^{2-} + HD^-(\text{blue}) + H^+ \]

Reagents: Prescribed A. G. grade chemical were used for assessment.

a. Buffer Solution: \(\text{NH}_4\text{Cl} + \text{NH}_4\text{OH} + \text{Mg – EDTA}\)

16.9g \(\text{NH}_4\text{Cl}\) was dissolved in 143 mL concentrated \(\text{NH}_4\text{OH}\) solution. Added 1.25 Mg salt of EDTA and diluted to 250 mL with distilled water.

b. Complexing Agent (Inhibitor)

Na – EDTA salt was added 250mg/mL till the sample was dissolved completely.

Precaution: This was added before the addition of buffer solution. Generally, this complexing agents are not required, until and unless water contains interfering ions require adding an appropriate complexing agent to give a clear sharp change in color at the end point.

c. Indicators

**Eriochrome black T** (Sodium salt of 1-(1-hydroxy -2-napthyl – azo) -5 nitro, 2-napthol 4- sulphonic acid)

0.5g of powder dye was suspended in 100g of 2,2,2 – nitrotriethanol or 2 – methoxy methanol.

d. EDTA Std. Solution(0.01M): Weighed 3.723g analytical reagent grade Di sodium EDTA was dissolve in distilled water, diluted to 1000mL.

e. STD. Calcium Solution: Prepared standard calcium solution using \(\text{CaCO}_3\) as

1mL = 1mg.

Procedure: Diluted 25mL sample to 50mL with distilled \(\text{H}_2\text{O}\). Complexing agent was introduced with subsequently 0.5 mL buffer solution to give pH 10. Add 1-2 drops of indicator solution – in case of dull color change, and then more indicators was added. Added std. EDTA titrant slowly with continuous stirring, till the last reddish color disappears. Add last few drops at 3-5sec intervals till blue color as an end point. Three concurrent burette reading was noted and hardness was calculated.
Calculation:

\[ \text{Total Hardness in mg/L} = \frac{\text{Volume of 0.01N EDTA (in ml)} \times 1000}{\text{Volume of sample in ml}} \]

3.3 Metal Analysis using ICP – AES\textsuperscript{26}:

For measurement of under consideration Heavy metals Fe, Mn, As, Zn and Al (light metal), Inductively Coupled Plasma – Atomic Emission Plasma (ICP – AES) Spectrophotometer method was used. BIS 10500 : 2004, Sec – 3025 (part – 2) describes the procedure to determine the dissolved, particulate and trace metals in raw, potable and waste water with the help of Inductively Coupled Plasma – Atomic Emission Plasma (ICP – AES) Spectrophotometer.

3.3.1. Principle: The basis of the method is the measurement of atomic emission by optical spectroscopic technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where excitation occurs. Characteristic atomic – line emission spectra are produced by a radio – frequency inductively coupled plasma. The spectra are produced are dispersed by a grating spectrometer and the intensities of the lines are produced and controlled by a computer system. A background correction technique is used to compensate for variable background contributions to the determination of trace elements.

3.3.2. Apparatus:

- Inductively coupled plasma – atomic emission spectrometer including
  - Computer – controlled atomic emission spectrometer with background correction.
  - Radiofrequency generator.
  - Argon gas supply.

- Sample bottles, 250ml or 500 ml for collection and storage. Containers should be previously cleaned with 10% volume fraction nitric acid before use.

- Glassware (beakers, filters, volumetric flasks and pipettes) reserved for elemental preparation only. All glassware shall be soaked in 10% volume fraction nitric acid overnight, before use.
• Acid dispensers, variable volume.

• Membrane filtration equipment and filters of pore size 0.45 µm reserved for element preparation.

3.3.3. Calibration: Standard calibrations were applied using one or more calibration solution and calibration blanks covering the measurement range and calibration function was determined.

3.3.4 Reagents: All the reagents were prepared using A.R. grade chemicals.

• Nitric Acid, ρ(HNO₃) = 1.40 g/mL

• Dihydrogen dioxide (hydrogen peroxide), H₂O₂, Volume fraction 30%.

• Sulphuric acid, ρ(H₂SO₄) = 1.84 g/mL.

• Hydrochloric acid, c(HCl) = 0.2 mol/L

• Ammonium Sulphate, (NH₄)₂SO₄

• Trace elements can be determined by using multielement stock solution.

  ➢ Multielement reference solution I

  ρ (Fe, Mn, Al, Zn)= 10mg/L

  This solution was prepared by adding (10±0.1) ml of each of the commercial 1.000 mg/L stock standard solution of reference elements in a 1-litre volumetric flask. Add 50 ml HNO₃ and make up the volume with water.

  ➢ Multielement reference solution II

  ρ (As)= 10mg/L

  This solution was prepared by adding (10±0.1) ml of each of the commercial 1.000 mg/L stock standard solution of reference elements in a 1-litre volumetric flask. Add 50 ml HNO₃ and make up the volume with water.

3.3.5. Analysis using ICP - AES:

The main steps followed in the procedure are:

** Preliminary treatment and Enrichment of Water samples:

Water samples were collected in 2L glass bottles previously rinsed with HNO₃ and followed by de – ionised water. Sample water is enriched
by adding 0.5mL conc. nitric acid to reduce to pH < 2 for the determination of total metal content.

*** Determination of Metal Content:

Metal content was detected Inductivcel coupled Plasma Atomic Emission Spectrophotometer (Thermo iCAP 6300 ICP UK make OES radial view spectrometer) in collaboration with ANACON laboratories, Nagpur. The procedure undertaken was as follows:

- The system was initiated with the appropriate operating configuration of spectrometer and installed computer system.
- It was flushed with the reagent blank between each standard.
- Two identical aliquots of same sample labeled A and B, each of volume $V_x$ was taken. To A, small volume of $V_s$ of standard analyte solution of concentration $\rho_s$ of the solvent was added. Analytical signals was measured in A and B. Now concentration $\rho_x$ of unknown sample was calculated using the formula

$$\rho_x = \frac{S_A V_s \rho_s}{(S_A - S_B) V_x}$$

Where,
- $S_A, S_B$ are analytical signals of solutions A and B respectively.
- $V_s$ is the added volume of standard analyte, in mm.
- $V_x$ is the volume of identical aliquots of sample solution, in mm.
- $\rho_s$ is the mass concentration of standard analyte solution, in mm/L.

- The technique is based on the measurement of the emission of one wavelength, which is highly selective for a specific element. Calibration matrix should match as possible the sample matrix. Recommended wavelengths for element detection are -
  a. Iron : 259 – 940 nm
  b. Aluminum : 308 – 215 nm
  c. Manganese : 257 – 610 nm
  d. Arsenic : 193 – 696 nm
  e. Zinc : 213 - 856 nm
3.3.6. **Interferences**: Spectral interferences, background emission, stray light and spectral overlaps, physical, chemical and memory interferences were properly eradicated during the tenure of measurement.

3.3.7. **Expression of Results**: Results were expressed according to precision, in mg/L, or not more than three significant figures.

3.4. **Assessment of Statistical Parameters**:

Statistical calculation were done using M.S Excel\textsuperscript{25} using various standard inbuilt function. Correlation Matrix was prepared with special Data Analysis Tool in Excel.

3.4.1. **Mean**\textsuperscript{23}: The value obtained by dividing the sum of a set of quantities by the number of quantities in the set

\[
\text{Mean} \mu = \frac{\sum x}{N}
\]

\(x = \text{Value of Observation,}\)
\(N = \text{Number of Observation}\)

3.4.2. **Standard Deviation**\textsuperscript{5}: The standard deviation is a measure of how widely values are dispersed from the average value. The following was used for calculating SD.

\[
\text{Standard Deviation} \sigma = \sqrt{\frac{N \sum x^2 - (\sum x)^2}{N(N-1)}}
\]

\(x = \text{Values of Parameter.}\)
\(N = \text{No. of Observations.}\)

3.4.3. **Std. Error**\textsuperscript{24}: The standard error of the mean can refer to an estimate of that standard deviation, computed from the sample of data being analyzed at the time. It can be calculated by the formula:

\[
\text{Standard Error} = \frac{S}{\sqrt{N}}
\]

\(S = \text{Standard Deviation}\)
\(N = \text{No. of Observation}\)

3.4.4. **Coefficient of correlation**: Correlation is a method used to evaluate the degree of interrelation and association between two variables\textsuperscript{29}. The greater the association between variables, the more accurately we can predict the outcome of events\textsuperscript{17}.
Karl Pearson’s Coefficient of Correlation 

\[ r = \frac{N \sum xy - \sum x \sum y}{\sqrt{N \sum x^2 - (\sum x)^2} \sqrt{N \sum y^2 - (\sum y)^2}} \]

\( x, y \) = the values if array 1 and array 2 respectively.
\( N \) = Number of Observations.

3.4.5. T – Test : The significance of correlation coefficient was determined using t – Test\(^{13}\). If \( r \) wasthe coefficient of correlation of the above given \( n \) pair of values then calculated the statistic \( t \) given by,

\[ t = \frac{r \sqrt{N - 2}}{\sqrt{1 - r^2}} \]

\( t \) = test for significance
\( r \) = correlation coefficient
\( N \) = Number of Observations.

3.4.6. Coefficient of variation\(^{20}\) : The coefficient of variation (CV) was measured as the ratio of the standard deviation \( \sigma \) to the mean \( \mu \):

\[ \% \text{ variation of CV} = \frac{\sigma}{\mu} \times 100 \]

3.4.7. Water Quality Index : WQI is the most effective tool\(^{13,38}\) to communicate information on the quality of water to the concerned citizen and policy maker. For estimation of WQI, the following water quality parameters were analyzed: Temperature, pH, Specific Conductance, turbidity, TS, TDS, TSS, T. Alkalinity, T. Hardness, Fe, Mn, Zn, As and Al. Mathematically, WQI was calculated using Weighted Arithmetic Index Method\(^{36,37}\). The course of action was divided into three steps :

I. Evaluation of Quality rating : \( q_i \) is the quality rating scale for each parameter, assigned by dividing its concentration \( V_i \) in the sample by its relevant \( S_i \) i.e. Indian drinking water standard for each parameter in mg/L specified in guidelines IS 10500 : 2004. The result multiplied by 100.

\[ q_i = \frac{V_i}{S_i} \times 100 \]

II. Calculation of Relative Weight (\( w_i \)): It is inversely proportional to standard value as prescribed by BIS, individually for each parameter.
\[ w_i = \frac{k}{S_i} \]

Where, \( k = 4.9 \) is constant of proportionality.

**III. Computation of overall Water Quality Index (WQI):**

\[ WQI = \frac{\sum q_i w_i}{\sum w_i} \]

Where \( \sum q_i w_i \) is the sum of the product of individual, quality scale and relative weight.

\( \sum w_i \) is the sum of relative weight of particular water quality parameter.

**3.5 References:**

11. Hem, J. D.; *Study and Interpretation of chemical characteristics of Natural water*, USGS, water supply paper, 1985, **3**, p2054.

12. Trivedi, R. K. and Goel, P.K.; *Chemical and Biological methods for water pollution studies*, Environomedia, Karad, Maharashtra, India, **1986**.


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*A waster of water is a waster of better.*

*Old Irish Adage*

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*The Waters are Nature's storehouse in which she locks up her wonders.*

*Isaac Waltonn*