Chapter-III

Sampling
And
Analytical Methodology
SAMPLING AND ANALYTICAL METHODOLOGY

This chapter is divided into two sections and will provide the complete information on sampling methodology and analytical methodology executed for this present study.

- First section explains sampling methodology, sample preservation and transportation of ground water samples from sampling site to laboratory.
- Second section explains analytical methodology used for the estimation of 22 metals concentrations like Beryllium (Be), Boron (B), Aluminum (Al), Antimony (Sb), Manganese (Mn), Molybdenum (Mo), Iron (Fe), Copper (Cu), Zinc (Zn), Indium (In), Tin (Sn), Barium (Ba), Thallium (Tl), Silver (Ag), Arsenic (As), Nickel (Ni), Chromium (Cr), Lead (Pb), Cobalt (Co), Selenium (Se), Mercury (Hg) and Cadmium (Cd) levels in Ground Water samples which are collected from 35 coastal villages of SPSR Nellore district by using different analytical techniques named by Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) & Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES).

3.1 SAMPLING METHODOLOGY

Estimation of metals in ground water samples at trace levels with accuracy is one of the major challenges in the water studies. Sampling part will play key role for estimation metals in ground water. There might have number of chances for elemental contamination at trace level studies. It is very important to take special precautions during water sampling. In the present study, it has taken proper precautions during the collection of ground water samples in 35 villages of coastal area of SPSR Nellore district for elemental analysis.

There might be chances to get contamination from sample collection container. Before collecting the samples, the sample containers are soaked overnight with 2% nitric acid and washed with double distilled water and dried in clean metal free area.

The sampling was carried out as per the Bureau of Indian Standards: 3025 (Part – 1) / American Public Health Association 22nd Edition. After drying the sample containers stored dust free area. At each sampling location, water samples were collected in two pre-
cleaned containers for duplicate measurement and also to maintain counter sample for repeatability analysis.

The bottles were rinsed three times with the groundwater sample of the particular location and collected the final sample to avoid the contamination. The collected groundwater samples acidified and preserved by the addition of 0.5 ml of supra pure grade nitric acid to the each samples to prevent the loss of metals. All the collected groundwater samples are duly packed and preserved at 4°C by using thermo-coal box with ice packs. The preserved samples are transported to laboratory for metal analysis.

The following Quality Control / Quality Assurance measures are taken during the sampling of groundwater samples:

- It has ensured that all necessary required sampling requirements of the sampling study meet all the selection criteria identified.
- It has ensured prior to starting the water sampling that proper sampling requirements are arranged.
- Before commencement of the study, we have ensured that all the items required are available for sampling at site and in laboratory. In case of any missing items, suitable alternate arrangements have been made and required materials were procured.
- Proper Labeling of Samples to easily identify the samples at the laboratory. An unique sample codes have given for each sample collected in each site to identify the samples easily.
- Owing the transportation of samples, all the samples are preserved in thermocol box to maintain uniform temperature, to minimize the cross contamination.

3.1.1 SUMMARY OF SAMPLING STUDY:

In most of the villages groundwater is using for drinking purpose. It is necessary to study the quality of groundwater in rural areas to give the awareness to the villagers. Studying of groundwater quality with respect to the elemental contamination is very important. Most of the groundwater studies are concentrating on physico-chemical analysis of ground
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water quality. It is also essential to study the metals in ground water quality because some of the metals at ppb levels act as a toxic and carcinogenic and some of the metals upto certain levels act as nutrients but beyond the specific levels these metals acts as a toxic and cause several health problems to the public.

SPSR Nellore district is one of the major coastal area in the south Andhra Pradesh region. Majority part of SPSR Nellore district is covered coastal region. Major fish and fisheries products are exported to other countries from Nellore district. Nearly 1,60,000 – 2,00,000 tons of fishery products are exporting from SPSR Nellore district. The majority of the villagers of coastal area of SPSR Nellore district are depending on the fishery activities.

Most of the villagers are depending on the ground water for drinking and house hold purpose. The villagers do not have awareness on the quality of the ground water which they are using for drinking purpose. It is very essential to study the ground water quality in village areas to give the awareness on the ground water quality. In this point of view the study has selected 35 different villages of coastal area of SPSR Nellore District to study the 22 metal concentration levels ground water.

The present study has focused on estimation of 22 metals in rural areas of coastal area of SPSR Nellore district, Andhra Pradesh. For this present study 35 rural areas selected for estimation of 22 metals named by Beryllium (Be), Boron (B), Aluminum (Al), Antimony (Sb), Manganese (Mn), Molybdenum (Mo), Iron (Fe), Copper (Cu), Zinc (Zn), Indium (In), Tin (Sn), Barium (Ba), Thallium (Tl), Silver (Ag), Arsenic (As), Nickel (Ni), Chromium (Cr), Lead (Pb), Cobalt (Co), Selenium (Se), Mercury (Hg) and Cadmium (Cd) by using two consecutive years different sophisticated analytical techniques named by ICP-MS and ICP-OES.

The schematic diagram of study area showing in India map is shown in Chapter-1, Figure-1.3. The schematic diagram of ground water sampling location in study area is shown in Chapter-1, Figure-1.4. The details of sampling locations have been summarized in Chapter-1, Table -1.1. The schematic ground water collection photographs at coastal village area of SPSR Nellore district are shown in Figure- 3.1 and Figure-3.2.
Figure- 3.1 - Sample Collection at Momidi by the Researcher

Figure- 3.2 - Sample Collection at Thummalapenta by the Researcher
3.2 ANALYTICAL METHODOLOGY:

This section will explain brief notes on analytical methodology followed for estimation of 22 metals like Beryllium (Be), Boron (B), Aluminium (Al), Antimony (Sb), Manganese (Mn), Molybdenum (Mo), Iron (Fe), Copper (Cu), Zinc (Zn), Indium (In), Tin (Sn), Barium (Ba), Thallium (Tl), Silver (Ag), Arsenic (As), Nickel (Ni), Chromium (Cr), Lead (Pb), Cobalt (Co), Selenium (Se), Mercury (Hg) and Cadmium (Cd) levels in ground water by using different analytical techniques named by Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) for the carried present study.

3.2.1 ANALYTICAL TECHNIQUES:

The samples collected from different villages of coastal areas of Nellore district are preserved and transported into the laboratory. The analysis has been carried out based on American Public Health Association (APHA 22nd Edition) – Standard Methods for Examination of Water and Waste Water method Number SM 3125 and SM 3120.


3.2.2.1 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) Technique:

Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) is one of the best and most advanced technique for determination of metal concentrations up to 0.1 ppb levels in different matrices. By using this ICP-MS we can able to determine the elements concentrations (limit of quantification (LOQ) levels) up to 0.5 µg/L and Limit Of Detection (LOD) up to 0.1 µg/L in aqueous medium with good precision and accuracy.

Preparation of standards

A mixed 100 mg/L stock solution is prepared in combination of high pure NIST traceable standard solutions of Beryllium, Boron, Aluminium, Antimony, Manganese, Molybdenum, Iron, Copper, Zinc, Indium, Tin, Barium, Thallium, Silver, Arsenic, Nickel, Chromium, Lead, Cobalt, Selenium, Mercury and Cadmium. By using this 100 mg/L stock solution
series of different working standards are prepared. The diluents sequence involved in the preparation of standard solution is shown in following Table-3.1. The optimum instrumental conditions of ICP-MS are summarized below.

**Table -3.1**

**Preparation of Working Standard Solutions for ICP-MS analysis**

<table>
<thead>
<tr>
<th>Concentration of Stock Solution, mg/L</th>
<th>Volume of Stock, mL</th>
<th>Volume of Diluent*, mL</th>
<th>Final Volume, mL</th>
<th>Final Concentration, mg/L</th>
</tr>
</thead>
<tbody>
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<td>100</td>
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</tbody>
</table>

* The diluents used are 2% supra pure Nitric acid and 1% Hydrochloric acid solution.

**ICP-MS OPERATING CONDITIONS:**

- RF Power : 1550 w
- RF matching : 1.75 V
- Sample Depth : 8.0
- Carrier Gas : 1.03 L/min
- Torch –H : 0.2mm
- Torch-V : 0.2 mm
- Dilution Gas Mode : ON
- S/C temperature : 2 degree C
**Ion Lens**

- Extract 1 : 0.0V
- Extract 2 : -165V
- Omega Bias : -100V
- Omega lens : 7.8V
- Cell Entrance : -40V
- Cell Exit : -60V
- Deflect : -0.2V
- Plate Bias : -60 V

**Pole Parameters:**

- AMU Gain : 128
- AMU offset : 127
- Axis Gain : 0.9997
- Axis Offset : 0.01
- QP Bias : -15 V

**Octopole Parameters:**

- Oct P RF : 200V
- Oct P Bias : -18.0V

**Reaction Cell:**

- Reaction Mode : ON
- H₂ Gas : 0.0 mL/min*
- He Gas : 4.5 ml/min*

* These values may change based on instrument tuning.

**LINEARITY:**

Before conducting sample analysis, a linear curve is prepared by using seven different linear concentration standards ranging from 0.001 mg/L–0.2 mg/L. All metals having good linear graph with correlation coefficients of >0.995 were observed in the preparation of standard curves. After the completion of standard curve preparation, one standard check and one QC check are analyzed. The observed schematic photographs of calibration curves of all twenty two trace metals during the analytical study has given in Figure 3.3 to Figure – 3.24.
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Figure 3.3 Linearity Curve of Beryllium by ICP-MS

Figure 3.4 Linearity Curve of Boron by ICP-MS
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Figure 3.5 Linearity Curve of Aluminium by ICP-MS

Figure 3.6 Linearity Curve of Antimony by ICP-MS
Figure 3.7 Linearity Curve of Manganese by ICP-MS

Figure 3.8 Linearity Curve of Molybdenum by ICP-MS
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Figure 3.9 Linearity Curve of Iron by ICP-MS

Figure 3.10 Linearity Curve of Copper by ICP-MS
Figure 3.11 Linearity Curve of Zinc by ICP-MS

Figure 3.12 Linearity Curve of Tin by ICP-MS
Figure 3.13 Linearity Curve of Barium by ICP-MS

Figure 3.14 Linearity Curve of Thallium by ICP-MS
Figure 3.15 Linearity Curve of Silver by ICP-MS

Figure 3.16 Linearity Curve of Arsenic by ICP-MS
Figure 3.17 Linearity Curve of Nickel by ICP-MS

Figure 3.18 Linearity Curve of Chromium by ICP-MS
Figure 3.19 Linearity Curve of Lead by ICP-MS

Figure 3.20 Linearity Curve of Cobalt by ICP-MS
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Figure 3.21 Linearity Curve of Selenium by ICP-MS

Figure 3.22 Linearity Curve of Mercury by ICP-MS
Figure 3.23 Linearity Curve of Cadmium by ICP-MS

Figure 3.24 Linearity Curve of Indium by ICP-MS
SAMPLE ANALYSIS:

After observing satisfactory calibration curve, the samples are aspirated into the plasma torch of ICP-MS, measure the intensities and compare with the standard solution at the characteristic masses of the respective elements. During the analysis one sample blank for every 25 samples and one standard check within the calibration range are analyzed for verification of the system response in between the samples. For every 20 samples one spike recovery sample is analyzed by using spiked blank matrix for the recovery estimation.

CALCULATION

Analyte concentration (mg/L) = (A – B) / D

Where:

A = Observed sample concentration – ICP-MS (mg/L)
B = Observed blank concentration – ICP-MS (mg/L)
D = Dilution Factor

3.3.2.2 Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) Technique:

Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) is one of the best and most advanced technique for determination of metal concentrations >10.0 ppb levels. The ICP-OES technique is useful and rugged technique for estimation of metals >10 ppb levels. The analytical data obtained for 22 metals by ICP-OES technique are summarized and discussed.

Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) is one of the widely using technique for determination of metal concentrations in different environmental matrices. By using this ICP-OES we can able to determine the elements concentrations (Limit Of Quantification (LOQ) levels) upto 0.01 mg/L in aqueous samples and mercury 0.5 µg/L.
Preparation of standards

A mixed 100 mg/L stock solution is prepared in combination of high pure NIST traceable standard solutions of Beryllium, Boron, Aluminium, Antimony, Manganese, Molybdenum, Iron, Copper, Zinc, Indium, Tin, Barium, Thallium, Silver, Arsenic, Nickel, Chromium, Lead, Cobalt, Selenium, Mercury and Cadmium. By using this 100 mg/L stock solution, series of different working standards are prepared. The diluents sequence involved in the preparation of standard solution is shown in Table-3.2

Table -3.2

<table>
<thead>
<tr>
<th>Concentration of Stock Solution, mg/L</th>
<th>Volume of Stock, ml</th>
<th>Volume of Diluent*, mL</th>
<th>Final Volume, mL</th>
<th>Final Concentration, mg/L</th>
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</table>

* The diluent used is 2% supra pure Nitric acid and 1% Hydrochloric acid solution.
The ICP-OES operating conditions used during the analysis of 22 elements is summarized below.

**ICP-OES OPERATING CONDITIONS:**

- **RF Power**: 1450 w
- **Nebulizer Gas Flow**: 0.6 L/ min
- **Auxiliary Gas Flow**: 0.2 L/ min
- **Plasma Gas Flow**: 15 L/ min
- **Sample Pump Rate**: 2 ml/ min
- **Plasma Viewing**: Radial
- **Processing Mode**: Peak Area
- **Auto Integration**: 1 – 5 Seconds
- **Read Delay**: 60 seconds
- **Replicates**: 60 seconds
- **Background Correction**: 1 point manual

**LINEARITY:**

Before conducting sample analysis, a linear curve is prepared by using seven different linear concentration standards including blank ranging from 0.0 to 0.5 mg/L. All metals having good linear graph with correlation coefficients of > 0.995 were observed in the preparation of standard curves. After the completion of standard curve preparation, one standard check and one blank check are analyzed. In between for every 20 samples analysis one standard check has run as a sample to check the system performance during the analysis. The linearity calibration graphs observed for twenty two trace metals are given in below **Figure -3.25 to 3.41.**
Figure 3.25 Linearity Curve of Beryllium by ICP-OES

Figure 3.26 Linearity Curve of Boron by ICP-OES
Figure 3.27 Linearity Curve of Aluminium by ICP-OES

Figure 3.28 Linearity Curve of Antimony by ICP-OES
Figure 3.29 Linearity Curve of Manganese by ICP-OES

Figure 3.30 Linearity Curve of Iron by ICP-OES
Figure 3.31 Linearity Curve of Copper by ICP-OES

Figure 3.32 Linearity Curve of Zinc by ICP-OES
Figure 3.33 Linearity Curve of Barium by ICP-OES

Figure 3.34 Linearity Curve of Arsenic by ICP-OES
Figure 3.35 Linearity Curve of Nickel by ICP-OES

Figure 3.36 Linearity Curve of Chromium by ICP-OES
Figure 3.37 Linearity Curve of Lead by ICP-OES

Figure 3.38 Linearity Curve of Cobalt by ICP-OES
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Figure 3.39  Linearity Curve of Selenium by ICP-OES

Figure 3.40  Linearity Curve of Mercury by ICP-OES
SAMPLE ANALYSIS:

After observing the satisfactory calibration curve and standard checks data, the samples are aspirated into the plasma torch of ICP-OES, measure the intensities and compare with the standard solution at the characteristic masses of the respective elements.

CALCULATION

Analyte concentration (mg/L) = (A – B) / D

Where:

A = Observed sample concentration – ICP-MS (mg/L)

B = Observed blank concentration – ICP-MS (mg/L)

D = Dilution Factor
3.3.2.2 SPIKE RECOVERY STUDIES:

Spike recovery studies have been carried out by using blank micro glass filter papers and study by spiking of known concentrations of standards in blank matrix. The recovery of spiked concentration has been studied. The spike recoveries are observed minimum of 80% at low level spiking and minimum 90% recoveries observed at middle and higher level spiked blank matrices. The spike recovery data are summarized in Chapter-4, Table-4.7 and Chapter-5, Table-5.7.