CHAPTER IV
MATERIAL AND METHODS

The trace element analysis of environmental and biological samples have been performed using Energy Dispersive X-Ray Fluorescence technique. The details of the set-up, data analysis and the methods of sample preparation are given below.

4.1 Energy Dispersive X-Ray Fluorescence Technique

4.1.1 Experimental set-up

The energy dispersive X-ray fluorescence spectrometer used in the present work is described in two parts. In the first part, details of low energy photon excitation system are given, while in the second part, details of fluorescent x-ray detection system are described.

a) Low energy photon excitation system

A low energy photon excitation system formed an important part of the x-ray fluorescent spectrometer. It consisted of various radioisotopes, secondary exciters and the exciter system. Photons emitted from the radioisotopes or secondary exciters were used to excite the characteristic x-rays of the samples. The geometrical arrangements for low energy photon source and exciter system are shown in Figure 4.1. This system can be operated in two modes.

1) Direct fluorescence mode

In the direct fluorescence mode (Figure 4.1a), photons emitted from radioactive sources viz. Fe-55 (50 mCi), Cd-109 (25 mCi) and Am-241 (300 mCi) were used to directly excite
Fig. 4.1 SAMPLE, EXCITOR AND DETECTOR GEOMETRY SET UP
(a) DIRECT FLUORESCENCE MODE (b) SECONDARY FLUORESCENCE MODE
the characteristic x-rays from the sample.

ii) Secondary fluorescence mode

In the secondary fluorescence mode (Figure 4.1b), photons from the annular source of Am-241 (300 mCi) excited the x-rays of secondary exciters of different elements which in turn were used to excite the characteristic x-rays of the sample.

The photon sources (Am-241, Cd-109, and Fe-55), the exciter system model (NER-496) and the secondary exciters were procured from New England Nuclear, USA. A modified arrangement of different components of exciter system for both the modes of excitation is shown in Figure 4.1. It consisted of shielding and holder assembly as given below.

A primary shield made of tungsten alloy as shown in Figure 4.1, provides shielding of the XRF detector from the unwanted radiations and also collimated the fluorescence x-ray beam upon the detector at a high angle of incidence. The aluminium lining at the collimator eliminates the background due to tungsten L X-rays.

A tungsten alloy spacer shield defines the secondary fluorescence target cavity when used in the secondary fluorescence mode and is used as a spacer in the direct fluorescence mode. In both the cases it serves as a peripheral shield to minimize stray radiations.

A tungsten alloy ring shield permits the exciter system to be "shut off" in the secondary fluorescence mode. In the direct fluorescence mode, it provides maximum peripheral
shielding of the assembly aperture when necessary, but can be eliminated for higher yield when its use is not necessary. The aluminium lining eliminates the tungsten L X-rays background.

An aluminium annular holder provides a convenient method of mounting the shield assembly and the source on the detector. The detector-housing cavity permits the aluminium holder to be slipped over the housing and retained by hand-tightening screws.

A retainer aluminium ring (not shown in the diagram) is screwed over the assembly-cavity to hold it in a proper position.

The sample holder consists of a perspex cup with a central hole of one inch diameter. This cup can be screwed over the retainer ring. In the central hole there is a step of 2mm depth. In this step the sample to be studied is placed. This sample holder provides a repeatable geometry.

Low energy photon sources

Annular sources of Fe-55, Cd-109, and Am-241 have been used in the present study. The energies of photons emitted from Fe-55 and Cd-109 radioisotopes were taken as the weighted average of their $K_{α}$ and $K_{β}$ x-ray energies, according to their intensity ratios. To calculate the weighted average, the $I_{K_{β}} / I_{K_{α}}$ intensity ratios were taken from the tables of Khan and Karimi (1980). The weighted average of the photon energies for the Fe-55 and Cd-109 radioisotopes came out to
be 5.96 and 22.6 keV respectively. A gamma-ray of energy 88.2 keV emitted by Cd-109 radioisotope contributed insignificantly to the production of fluorescent x-rays because of its low photoionisation cross-section and low intensity as compared to that of 22.6 keV photon. Am-241 radioisotope emitted Np L x-rays in addition to 59.5 keV gamma ray. To suppress the Np L x-rays and any other low energy photons present in the Am-241 source, a graded shield of Al and Cu, similar to the one described by Arora et al (1981) was used.

In the secondary fluorescence mode (Figure 4.1b) the annular source of Am-241 (300 mCi) was used to excite the x-rays of the secondary targets namely Cu, Se, Y, Mo, Ag, Sn, Sm and Dy. The purity of these secondary exciters was better than 99.9%. These secondary exciters consisted of thick elemental foils (thickness 0.01") fixed on aluminium annulas. The outer diameter of the annulas was 1.5 inch and the internal diameter was 0.625 inch. The secondary exciters namely Cu, Se, Y, Mo, Ag, Sn, Sm, and Dy provided the weighted average photon energies of 8.14, 11.4, 15.2, 17.8, 22.6, 25.8, 41.0 and 46.9 keV respectively. In this way photons of wide range of energies became available for the excitation of fluorescent x-rays.

b) X-ray detection system:

Characteristic x-rays emitted from the sample were detected by the x-ray detection system, which consisted of a Si(Li) detector (resolution 165 eV at 5.9 keV) coupled to an ND 100 multichannel analyser, through a spectroscopy amplifier.
A block diagram of the x-ray detection system is shown in Figure 4.2. The main parts of this x-ray detection system are briefly discussed below.

**Si(Li) detector**

The Si(Li) detector model (SLP-06165), manufactured by EG & G ORTEC, U.S.A. has been used. This x-ray detector has a lithium drifted silicon diode which performs the sensitive function of semiconductor detector. A low noise cryogenic preamplifier, a cryostat and a liquid nitrogen dewar are the other main parts of the detector. Both the Si(Li) chip and the FET used in the first stage of preamplifier are mounted in a cryostat and are operated at a temperature close to that of liquid nitrogen.

The sensitive volume of the planer Si(Li) detector is located behind the Be window of thickness 0.0254 mm. The detector has an active area of 23.27 mm$^2$ and sensitive depth of 5.5 mm. The recommended operating voltage of the detector is 1500 volts with negative polarity. It has got good efficiency when used in the energy range 3 to 60 keV, but it can also be used beyond this range where the detector efficiency is not considered to be important.

**Preamplifier:**

A preamplifier (Model No. 139 of EG & G ORTEC), consists
Fig. 4.2 BLOCK DIAGRAM OF THE X-RAYS DETECTION SYSTEM
of hybrid components. This device is used to match the output impedance of the detector and the input impedance of the spectroscopic amplifier. The proper performance of this device is very important because the overall output of the detector system depends upon the amplification of the signal by pre-amplifier. At this stage the signal level is very low so the contribution towards the pulse width in the signal processing comes largely from the preamplifier itself. This pre-amplifier provides optimum input coupling and good operational characteristics. The preamplifier includes a charge sensitive input loop with a very high open loop gain resulting in a ultra low noise performance.

The output pulse is a negative pulse with rise time < 200 ns and exponential fall with a 50 μs time constant. The change in gain is less than 50 ppm per degree centigrade over 0 to 50°C temperature range.

The preamplifier accepts d.c. power from a standard power output connector. The amplitude of output signal is proportional to x-ray energy and is of negative polarity. The output signal is fed to the spectroscopy amplifier.

**Spectroscopy Amplifier**

The output from the preamplifier is further amplified by a spectroscopy amplifier, and is then fed to the pulse height analyzer. In the present work, the spectroscopy amplifier (ORTEC model 572) supplied by EG & G ORTEC, has
been used. This amplifier includes the pile up rejector also. It is a single bin model with versatile combination of switch selectable pulse shaping and output characteristics.

As per requirements it has got extremely low noise performance, wide range of gain and an excellent overload response for universal amplification in high resolution spectroscopy. The input impedance of this amplifier is 500 ohms. It has the capability to accept both positive and negative pulses having rise time less than 600 ns and fall time greater than 40 micro seconds.

**Bin and Power Supply:**

All the units were of modular type and could be plugged into a common power bin, which supplied various voltages required for these units. In this study ORTEC model 4001-A bin and power supply has been used. It provides d.c. outputs of ±12 and ±24 volts with noise and ripple less than 3 mV, peak-to-peak. Its power output is limited to 72 VA with regulation of ±0.05 percent. The temperature coefficient is less than 0.01 percent per °C over a range 0 to 60°C. The output impedance is 0.3 ohms for frequency upto 100 KHz. The circuit is protected against accidental over-loadings by means of a thermal protection switch which is activated when the ambient temperature approaches 20°C. There are twelve module connectors for standard supply voltages.
Detector-bias supply:

ORTEC model 459 detector-bias supply capable of giving ±3 KV has been used to provide a bias voltage of -1.5 KV to the Si(Li) detector. This bias supply has noise and ripple <10 mV peak-to-peak for 2 Hz to 50 MHz frequencies. The temperature stability is better than 0.02 percent per °C through 0 to 50 °C. Available voltage stability is <0.1 percent per hour output voltage with constant input voltage from bin supply.

Multichannel analyser:

In the present study, a 4 K multichannel analyser (ND-100) supplied by Nuclear Data, USA has been used. This analyser consists of a bench top console containing data handling, control circuit, solid state memory, display oscilloscope, input/output interfaces, analog to digital converter (ADC) and a power supply. The data acquisition selection, storage, display, manipulation and input/output are totally controlled by the front panel controls. All the operations are controlled by a set of just 15 push buttons.

The MCA has got a solid state memory having a cycle of 2 micro-seconds which ensures the high speed data acquisition. The standard 1024 channel memory field is expandable to 2048 or 4096 channels. Its counting capacity is 1,048,575 counts per channel. The analog-to-digital converter (ADC), Model ND Gen II digitises the pulses obtained from the output of spectroscopic amplifier. The ADC enhances the acquisition
rate by its 100MHz digitising unit.

For the output purpose, there is one flat face CRT display having 100 KHz display rate providing optimum, vibration free viewing of both alpha numeric characters and data spectrum. Data for analysis were transferred either on the magnetic tape through magnetic transport system coupled to the MCA or were taken on paper through the fast printer supplied by CENTRONIX (model 781).

4.1.2 Calibration of the Si(Li) detector system

a) Energy Calibration

Before doing the energy calibration, the linearity of the detection system was measured with the help of an electronic pulser. For energy calibration, most intense x-ray peak of Fe(\(K_\alpha\)) having energy of 6.400 keV observed in the decay of Co-57, and a \(\gamma\)-ray peak of Co-57 of 14.390 keV have been used. The slope and intercept of the linear calibration curve were obtained analytically from the above two points. With the help of the slope and intercept the energy of various photo-peaks were then determined.

b) Efficiency Calibration

The efficiency calibration of the detector in the energy range 3 to 60 keV was done by using the standard radio-active sources of Fe-55, Cs-137 (NBS standards), Co-57, Am-241 and a mixed NBS standard sources, containing Eu-155, Eu-154, Sb-125 and Te-125m. The Co-57 source was prepared by drying
a drop of radio-active solution on perspex disc of one inch diameter. Sources of Fe-55 and Am-241 were in evaporated form on steel backing, while Cs-137 and mixed NBS standard sources were in sandwich form between the two pieces of mylar sheets. These sources were thin enough to avoid any absorption of the photons in the source itself. The sources were placed in front of the detector in identical position one after the other. This position was later on used for the placement of the unknown samples to take care of the effect of the geometry.

The intensities of the radiations from Am-241 source were taken from Cohen (1980) and for Co-57 from an NCRP report (1978). For NBS standards the actual photon intensities were provided by NBS, Washington, D.C., U.S.A. The photon intensities alongwith the uncertainties in the paranthesis for all the sources are given in Table 4.1.

It was not possible to calibrate the detector properly below 12 keV energy by using radio-isotopes only, because there were not sufficient number of calibration points in that range. Further, there were large energy gaps, in the energy calibration curve, corresponding to which the efficiency was not known. The efficiency values at these intermediate energies and below 12 keV were therefore obtained by using thin standard foils of Ti, Cu, Zn, Ge, Se, and Y. The method described by Kumar et al (1983) for measuring K x-ray yields and knowledge of the K x-ray cross-sections has been
**TABLE 4.1:** Relative photon intensities in the decay of various radio-nuclides.

<table>
<thead>
<tr>
<th>Energy of photon in keV</th>
<th>Kind of radiation</th>
<th>Name of isotope</th>
<th>Intensity as quoted</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.895</td>
<td>Mn-Kα X-ray</td>
<td>Fe-55</td>
<td>189.6(60)</td>
<td>NBS standard*</td>
</tr>
<tr>
<td>6.490</td>
<td>Mn-Kβ X-ray</td>
<td></td>
<td>25.5(8)</td>
<td></td>
</tr>
<tr>
<td>6.400</td>
<td>Fe-Kα X-ray</td>
<td>Co-57</td>
<td>49.4(9)†</td>
<td>NCRP Report** (1978)</td>
</tr>
<tr>
<td>7.058</td>
<td>Fe-Kβ X-ray</td>
<td></td>
<td>6.63(21)†</td>
<td></td>
</tr>
<tr>
<td>14.390</td>
<td>γ-ray</td>
<td></td>
<td>9.54(13)†</td>
<td></td>
</tr>
<tr>
<td>13.940</td>
<td>Np-Lα X-ray</td>
<td></td>
<td>132(3)‡</td>
<td></td>
</tr>
<tr>
<td>17.750</td>
<td>Np-Lβ X-ray</td>
<td></td>
<td>194(4)‡</td>
<td></td>
</tr>
<tr>
<td>20.810</td>
<td>Np-Lγ X-ray</td>
<td></td>
<td>49.6(20)‡</td>
<td></td>
</tr>
<tr>
<td>26.305</td>
<td>γ-ray</td>
<td></td>
<td>23.6(1)‡</td>
<td></td>
</tr>
<tr>
<td>59.54</td>
<td>γ-ray</td>
<td></td>
<td>355(9)‡</td>
<td></td>
</tr>
<tr>
<td>27.40</td>
<td>Kα X-ray(Te)</td>
<td>Mixed</td>
<td>105(2)</td>
<td>NBS standard*</td>
</tr>
<tr>
<td>42.8</td>
<td>Kβ X-ray(Gd)</td>
<td></td>
<td>122(2)</td>
<td></td>
</tr>
<tr>
<td>86.0</td>
<td>γ-ray of Eu-155</td>
<td></td>
<td>49.5(5)</td>
<td></td>
</tr>
<tr>
<td>32.1</td>
<td>Kα X-ray</td>
<td>Cs-137</td>
<td>556(13)</td>
<td>NBS standard*</td>
</tr>
<tr>
<td>36.6</td>
<td>Kβ X-ray</td>
<td></td>
<td>135(6)</td>
<td></td>
</tr>
</tbody>
</table>

* For NBS standards, the actual photon intensities were provided by the National Bureau of Standards (NBS), Washington, DC, U.S.A.

** A handbook of radioactivity measurement procedures, Report no. NCRP 58 (1973).

† Intensity values are given per 100 decays.

‡ Intensity values are given per 1000 decays.
used. According to this method, the efficiency \( \varepsilon_{K\alpha(j)} \) of the detector at the K\( \alpha \) x-ray energy of a given element \( (j) \) is given by the relation:

\[
\varepsilon_{K\alpha(j)} = \frac{N_{K\alpha(j)}}{\text{IoG} \, \sigma_{K\alpha(j)} \, \beta_{K\alpha(j)} \, m_j}
\]  

(4.1)

where \( N_{K\alpha(j)} \) is the number of counts per unit time for K\( \alpha \) x-ray of the \( j \)th element, \( \text{IoG} \) is the flux of incident radiation falling on the sample, \( \sigma_{K\alpha(j)} \) is the x-ray fluorescence cross-section for the K\( \alpha \) x-ray of the \( j \)th element, \( m_j \) is the mass in g cm\(^{-2} \) of the \( j \)th element and \( \beta_{K\alpha(j)} \) is the absorption correction of the sample which accounts for the absorption in the sample of the incident photons and the emitted characteristic x-rays of the \( j \)th element. \( \text{IoG} \) factor was calculated with the help of Y foil by taking the efficiency for this foil from the curve and using the method described in section 4.1.3(d). Thus knowing the value of \( \text{IoG} \), the relative detection efficiency for the K x-rays of all the other elements, was evaluated. The efficiency points, obtained with the help of the above mentioned sources and metallic foils, were least square fitted to a function (eqn. 4.2) in order to reduce the error in interpolating the efficiency curve

\[
\log \varepsilon = \frac{A(-3)}{E^3} + \frac{A(-2)}{E^2} + \frac{A(-1)}{E} + A(0) + A(1)E
\]  

(4.2)

where \( \varepsilon \) is the efficiency at energy \( E \) and \( A(-3), A(-2), A(-1), A(0) \) and \( A(1) \) are constants the values of which are obtained by least square fitting of the experimental points.
The efficiency curve was fitted in three parts over different energy regions. The fitted curve deviated from the experimental points by less than 3% over the energy region 5-15 keV. The fitted efficiency curve is shown in Figure 4.3.

4.1.3 Data Analysis
a) Quantitative elemental analysis of the samples using EDXRF method:

To overcome the difficulties of the external standard method in quantitative elemental analysis of samples using EDXRF technique, a fundamental parameter approach has been developed. This approach has been described, but briefly, by Gedcke et al (1982).

The measured x-ray counting rate for the given element is related to its concentration by equation 4.3.

\[
m_j = \frac{N_i(j)}{I_0 G \sigma_1(j) \varepsilon_1(j) \beta_1(j)}
\]

(4.3)

where

- \( m_j \) = concentration of the jth element in g/cm², present in the sample
- \( N_i(j) \) = net counts per unit time for the ith group of x-ray of element J (normalized photo-peak area)
- \( I_0 \) = Intensity of the incident photon
- \( G \) = geometry factor
- \( \sigma_1(j) \) = x-ray fluorescence cross-sections of ith group of x-rays of element j.
Fig. 4.3 RELATIVE EFFICIENCY CURVE FOR 6mm φ x 5.5mm Si(Li) DETECTOR USING RADIOISOTOPES AND METALLIC FOILS FOR THE GEOMETRY USED IN ACTUAL EXPERIMENTS
\( \xi_{i(j)} \) - efficiency of the detector for the \( i \)th group of x-rays of element \( j \). The evaluation of this parameter is given in detail in the previous section.

\( \beta_{1(j)} \) - self-absorption correction factor of the target material which accounts for the absorption in the target, both for the incident photons and the emitted characteristic x-ray lying under the \( i \)th x-ray peak of element \( j \).

The various parameters involved in this relation have been evaluated independently as described below:

b) Photo-peak area evaluation

The characteristic x-rays of the various elements were analysed graphically using the total-peak-area (TPA) method (Yule, 1968 and Baedecker, 1971). In this method, the following equation was used to find the area \( A \) under the photopeak.

\[
A = G - B \quad (4.4)
\]

where

\[
G = \sum a_i, \text{ which is obtained by adding the counts } (a_i) \text{ recorded in channels under the x-ray peak. The number of channels } (n_a) \text{ under the x-ray peak were selected from the graph.}
\]

\[
B = \frac{n_a}{n_b} (N_{B1} + N_{B2})
\]

Here, \( N_{B1} \) and \( N_{B2} \) are the total counts in two equal background regions on the left and right hand limits of the peak, and \( n_b \) is the total number of channels on both sides of the peak, taken for calculating the background.
The error ($\Delta A$) in the area evaluation was calculated using the relationship:

$$\Delta A = \sqrt{G + \left( \frac{n_a}{n_b} \right) B}$$

(4.5)

To improve the counting statistics minimum of three spectra were taken for each sample and the weighted average alongwith the standard deviation was calculated as described in section 4.4(a).

The normalized photopeak area ($N_{i(j)}$) was calculated, from the knowledge of total peak area ($A$) and the live time for which spectrum is accumulated.

c) **Determination of self-absorption correction factor**

For thin samples ($<1 \text{ mg/cm}^2$), the self-absorption of the characteristic x-rays is normally very low but as the sample thickness is increased, the self-absorption also increases correspondingly up to certain thickness. It is because of the fact that a fraction of the incident photons are absorbed by the target as they penetrate deep into the target material. Similarly a fraction of the fluorescent x-rays emitted by the target are also absorbed. This correction has to be applied to take care of these absorption
effects. This was accomplished by the following method.

Let "\( t \)" be the actual thickness of the target of uniform thickness in \( g/cm^2 \). To make the correction for self-absorption, thickness "\( t \)" is replaced by an effective thickness "\( t_{\text{eff}} \)" where

\[
t_{\text{eff}} = t \beta
\]  

(4.6)

where "\( \beta \)" is the self-absorption correction factor which is a function of the linear absorption coefficients \( U_1 \) and \( U_2 \). Here \( U_1 \) is the linear absorption coefficient of the target material for the incident photon and \( U_2 \) is the linear absorption coefficient of the target material at the characteristic x-ray energy. \( \beta \) is always less than unity.

The geometry for the exciter source, sample and detector was considered in general to be such that the incident photons fall at an angle \( \Theta_1 \) with the normal to the target and the characteristic x-rays leave at an angle \( \Theta_2 \) with the normal as shown in Figure 4.4.

The target of thickness "\( t \)" is assumed to be made of very thin layers each of thickness "\( dx \)". Let the interaction take place at a depth "\( x \)" within target in a layer of thickness "\( dx \)".

The intensity of gamma rays reaching the elementary layer \( dx \), \( x \) distance deep in the target is:

\[
I_1 = I_0 \exp \left( - U_1 \frac{x}{\cos \Theta_1} \right)
\]  

(4.7)

where \( I_0 \) is the intensity of the incident radiation.
Fig. 4.4 SCHEMATIC DIAGRAM SHOWING THE ARRANGE-MENT OF SOURCE TARGET AND DETECTOR IN REFLECTION GEOMETRY SET UP
The number of x-rays produced from the layer as a result of photoelectric interaction is given by

\[ I_2 = I_o \exp\left(-\frac{\mu_1 x}{\cos \theta_1}\right) \sigma_{ij} (ij) \omega_K \]  \hspace{1cm} (4.8)

where \( N = \text{Aogadro's number} \)

\( \sigma_{ij} \) = Photoelectric cross-section of \( i \)th x-ray of \( j \)th element.

\( \omega_K \) = average fluorescence yield for \( K \)th shell

(\text{where} \, K = K,L,M, \ldots \, \text{shell})

The intensity \( dI \) of x-rays emerging out of the target at an angle \( \theta_2 \) is given by

\[ dI = I_2 \exp\left(-\frac{\mu_2 x}{\cos \theta_2}\right) \]  \hspace{1cm} (4.9)

\[ dI = I_o N \sigma_{ij} (ij) \omega_K \exp\left[-\left(\frac{\mu_1}{\cos \theta_1} + \frac{\mu_2}{\cos \theta_2}\right) x\right] dx \]  \hspace{1cm} (4.10)

The intensity \( I \) of the x-ray emerging out from the whole of the target is found by integrating the above equation w.r.t. \( x \) varying from zero to \( t \), i.e.

\[ I = I_o N \sigma_{ij} (ij) \omega_K \int_0^t \exp\left[-\left(\frac{\mu_1}{\cos \theta_1} + \frac{\mu_2}{\cos \theta_2}\right) x\right] dx \]  \hspace{1cm} (4.11)

\[ = I_o N \sigma_{ij} (ij) \omega_K \left[1-\exp\left[-\left(\frac{\mu_1}{\cos \theta_1} + \frac{\mu_2}{\cos \theta_2}\right) t\right]\right] \frac{1}{\left(\frac{\mu_1}{\cos \theta_1} + \frac{\mu_2}{\cos \theta_2}\right)} \]  \hspace{1cm} (4.12)

\[ = I_o N \sigma_{ij} (ij) \omega_K t_{\text{eff}} \]  \hspace{1cm} (4.13)
For the geometry used in the present measurements, the emitted radiations are perpendicular to the detector surface and therefore \( \Theta_2 \) is zero. The effective angle of incidence for the incident photons (\( \Theta_1 \)) will be different for direct and secondary fluorescence modes. This comes out to be 32° for the secondary fluorescence mode in the present set up. In the direct excitation mode \( \Theta_1 \) is 30°, 72° and 66° for Fe-55, Cd-109 and Am-241 radio-isotopes respectively. Values of \( \mu_1 \) and \( \mu_2 \) have been taken from the tables of Storm and Israel (1970). To calculate the values at the required energy (E), the interpolation of \( \mu \) values between the two known points has been done using the relation:

\[
\mu(E) = \mu(E_2) \left( \frac{E}{E_2} \right)^\eta
\]  

(4.15)

\[
\eta = \frac{\log \left( \mu(E_1) \right) - \log \left( \mu(E_2) \right)}{\log (E_1) - \log (E_2)}
\]  

(4.16)

where \( E_1 \) and \( E_2 \) are the two energy limits between which the energy \( E \) lies. Overall error in \( \beta \) depends upon the error in \( \mu \) values and thickness (t) of the foil. The error in \( \mu \) is estimated to be about 3%. 

\[
d - \exp \left[ - \left( \frac{\mu_1}{\cos \Theta_1} + \frac{\mu_2}{\cos \Theta_2} \right) t \right]
\]  

(4.14) 

or \( \beta = \frac{1 - \exp \left[ - \left( \frac{\mu_1}{\cos \Theta_1} + \frac{\mu_2}{\cos \Theta_2} \right) t \right]}{\left( \frac{\mu_1}{\cos \Theta_1} + \frac{\mu_2}{\cos \Theta_2} \right) t}
\]
This theoretical method for applying the $\beta$ correction was possible in those cases where the sample matrix was known, like that in the evaluation of fundamental parameters namely: $\sigma_{ij}$, IoG and $\epsilon$, where standard single element foil were used. In the case of air and water samples, the matrix was not known and therefore no such correction was applied. In case of thick biological samples of unknown matrix, an experimental method for the determination of $\beta$-value, as described in section 5.2.2, was applied.

d) Incident X-ray intensity and the Geometry factor (IoG)

The IoG factor represents the effective intensity of exciting x-rays falling on the sample. This has been evaluated separately by running the K x-ray spectra of atleast five standard single element foils. The standard foils had the thickness varying from 70 to 160 $\mu$g/cm$^2$ evaporated on 6.3 $\mu$m thick mylar foil. These target materials were obtained from MicroMatter (USA). For each foil three spectra were taken for time intervals ranging from 3,000 to 20,000 seconds depending upon the counting statistics. The weighted average of the area per unit time under the $K\alpha$ x-ray peak was taken and the value of IoG was evaluated for each foil, using the relationship.

$$\text{IoG} = \frac{N_{K\alpha} (j)}{\sigma_{K\alpha} (j) \beta_{K\alpha} (j) \epsilon_{K\alpha} (j) \ m_j}$$  (4.17)

where various factors have the same meanings as for eqn.(4.1).
The final value of IoG was computed by taking the weighted average of IoG values obtained from different foils. The error in the IoG value has been evaluated based on the uncertainties in various parameters involved in relation 4.17. The statistical error in the area evaluation is $\leq 1.5\%$. The uncertainty in the detector efficiency is estimated to be $3\%$ as explained in section 4.1.2. The error in $m_j$ is about $2\%$. The error in absorption correction factor ($\beta$) for thin foils is fairly small. Thus the overall error in the value of IoG is estimated to be about $5\%$.

e) X-ray fluorescence cross-sections ($\sigma_{ij}$)

The x-ray fluorescence cross-sections can be evaluated theoretically or measured experimentally using standard single element foils as described in detail, in section 5.1. It has been found (section 5.1) that the theoretical and experimental values of the x-ray fluorescence cross-section agree with each other within the experimental errors. Therefore in the trace element analysis of environmental and biological samples, either theoretical or experimental values of $\sigma_{ij}$ have been taken depending upon the errors involved in these values.

4.1.4 Methods of Sample preparation

Environmental Samples

a) Water samples

The water samples were collected from the Chandigarh water supply system, which normally supplies the ground water. For EDXRF study the samples were prepared by

† The ground water samples were taken from the tap.
drying the water on a Whatman filter paper (No-42), having pore size of 2.5 \( \mu \text{m} \), using a multidrop technique. In order to avoid the contamination, the samples were prepared in a dust free atmosphere. The glass-ware used were first cleaned with chromic acid \((\text{H}_2\text{CrO}_4)\) solution and then washed in double distilled water and finally dried in an oven at 70\( ^\circ \text{C} \).

Ten samples of ground water were collected. Each water sample was spreaded uniformly over a filter paper having an area of 5 cm\(^2\) and dried by maintaining the temperature below 90\( ^\circ \text{C} \). Drying of the sample and dispensing of the liquid was done simultaneously till 15-25 ml of water got dried on the filter paper. The weight of the water residue deposited was measured by weighing the filter paper before and after drying the water sample over it. To avoid the curling of the sample, a perspex ring having diameter of one inch was mounted on each sample.

For Proton Induced X-ray Emission (PIXE) study, the water samples were prepared by rotation method as described by Kivits et al (1979). The water samples were evaporated on cellulose acetate (Selectron filter) paper having an area of 5 cm\(^2\). To prepare the samples of uniform thickness, a set-up was designed and fabricated in this laboratory (Figure 4.5). In this apparatus a microdispenser is centred above a table which is capable of rotating at a speed of 18000 rpm. Liquid samples from the microdispenser are allowed to fall on the selectron filter, clamped to the
Fig. 4.5 BLOCK DIAGRAM OF THE SAMPLE PREPARATION UNIT
BY DRYING SAMPLE SOLUTION ON ROTATING SUBSTRATE
rotating table. Air drying is done before the removal of the foil, which proves effective in preventing the curling of the foil. Parameters adjusted, in order to obtain the optimum uniformity, were: the frequency of rotation, the distance between the tip of the dispenser and the Selectron filter and speed of dispensing the liquid. Best uniformity was obtained by rapid dispensing (<0.2 seconds); slow dispensing resulted in a ring shaped distribution. The targets prepared with a rotation speed less than 18000 rpm were not uniform. Parameters concerning the liquid are the viscosity and surface tension, which could be varied by the addition of certain chemicals. To investigate the influence of the surface tension, different kinds of synthetic detergents or alliphatic alcohols were added. Most successful were ethanol and propanol. Although the surface tension decreased more by propanol than ethanol, but the disadvantage of adding propanol was that it gave precipitates. Therefore in the present study, the ethanol was used. The ratio of water to ethanol was taken as 2:1.

Because of the constant amount of liquid deposited (35 μl), the constant wetted area (400 mm²), the constant porosity of the selectron foil and its low area density (5 mg/cm²), a good uniformity of the deposited samples were obtained. All the samples were put in air tight plastic boxes and preserved in a vacuum desiccator.

The samples prepared by rotation method are not suitable for EDXRF study, because the amount which can be deposited
by this method is not sufficient for the detection of the elements present in the water sample at ppm level. This method can be used for EDXRF study only if the water samples are preconcentrated. In that case, there will be more chances of error in measuring the amount of water evaporated on the filter. Secondly there is a possibility of loss of elements due to adhesion to sides of the tube.

On the other hand, the rotation method found to be better for PIXE analysis as compared to that of multidrop technique. This is because in case of PIXE technique, the exposed area is about 0.5 cm² and it is possible to prepare uniformly distributed samples at this small exposed area by using rotation method.

Keeping the above points in view, the water samples were prepared by multidrop technique for study, by EDXRF method. The results of this study are compared with the results obtained by PIXE technique.

b) Air particulate samples:

The air particulate samples† were collected by drawing the air at a constant rate with the help of a rotary pump, through ashless Millipore filter* (4.7 cm diameter and 0.1 μm pore size) placed in a perspex sample holder. The geometrical arrangement of the sample holder for collecting the air

* For Pb, present in exhaust particulate, Seeley and Skorerbee (1974) has reported 95% collection efficiency when collected on 0.3 μm Millipore filter.
† The air particulate samples were collected at the height of 3 meters above the ground.
Sample holder for collecting the aerosol samples

The sample holder to collect the air particulate samples has been designed and fabricated in the laboratory, and is shown in Figure 4.6. It consists of a perspex holder having a central hole of 4.7 cm diameter. Leak proof wire gauze is placed inside the holder on which the Millipore filter is placed. To avoid the leakage, two O-rings are used, one below the wire gauze and the other above the Millipore filter and are screwed with a perspex cup. To avoid the contamination of the wire gauze, Whatman filter no. 42 having pore size 2.5 \( \mu \)m, was placed inbetween the wire gauze and Millipore filter. Below the wire gauze, a connector of perspex, having outer diameter 1 cm and inner diameter of 0.6 cm is made to connect the sample holder with the rotary pump, with the help of a suction tube made of rubber having inner diameter of 6 mm.

Calibration of the air suction apparatus:

Free air displacement rate was supplied by the manufacturer of the rotary pump. The air displacement with Millipore filter was calculated by measuring the pressure inside the filter with the help of a manometer designed in this laboratory. The ratio of the pressure inside the filter to the atmospheric pressure (76 mm Hg) was calculated. The suction rate with Millipore filter in position was then
FIG. 4.6 SAMPLE HOLDER FOR THE COLLECTION OF AEROSOL SAMPLES
calculated as given below.

\[
\text{Suction rate with filter} = \frac{\text{Pressure with filter}}{\text{Pressure without the filter}} \times \text{free air displacement of the rotary pump.}
\]

(4.18)

Using the eqn. (4.18), the suction rate of the rotary pump through Millipore filter came out to be 1.46 m³/hr. The flow rate was measured by taking the average value of the flow rate evaluated in the beginning, at middle and the end of the collection period. The weight of the air particulate collected on each sample was measured by weighing the Millipore filter paper before and after collecting the sample. A perspex ring having diameter of one inch was mounted on each sample. All the samples were kept in air tight plastic boxes and were preserved in a vacuum desiccator.

c) Biological samples:

Male albino mice (weighing 20-25 gram each) were used for trace element study. Animals were procured from central animal house, Panjab University, Chandigarh, and inbred in the animal house of the department. The animals were kept in plastic cages under hygienic conditions and were provided feed and water \textit{ad libitum}. The standard animal feed was obtained from Hindustan Lever Limited, Bombay. As reported by the manufacturers of this feed, it contained crude protein 24 percent, ether extract 4 percent, crude fibre 4 percent, ash 8 percent, calcium 1 percent, phosphorus 0.6 percent and nitrogen free extract 50 percent. The vitamins
and minerals present in this feed were: vitamin A/D3, vitamin E, vitamin K, vitamin B₁₂, thiamin, riboflavin, pantothenic acid, niacin, pyridoxine, choline chloride and folic acid. The mineral contents of this feed was determined in this laboratory by neutron activation method (Mangal and Gulati, 1981).

Various organs/tissues of the animals were studied for trace element concentrations using EDXRF set-up as described in section 4.1. Before taking out the organs, blood was drawn with the help of a fine heparinized glass capillaries from the orbital sinuses of the mice. Ethylene-diamine tetra-acetic acid (EDTA) was added in the tubes to avoid coagulation of the blood. After taking out the blood for haematological studies, packed cells and plasma were separated by centrifuging the blood at 3000 rpm (1508 g) for 20-30 minutes. The animals were then anaesthetized using petroleum ether. The anaesthetized animals were fixed on a dissection board and the abdomen was cut open. Perfusion of the animal was done by Tyrode's ringer solution. After perfusion, nine organs namely liver, kidneys, lungs, heart, brain, femur, muscles, spleen and pancreas were taken out. All these organs along with blood-cells were lyophilized and then grinded with the help of agate pestle mortar.

The samples of plasma were made by mixing in agate pestle mortar, one ml of the plasma dropwise in 200 mg of cellulose powder. The drying of the sample was done simultaneously under infra-red lamp.
From each organ self-supporting thick pellets having one inch diameter and thickness varying from 40-70 mg/cm² were made under a pressure of 20,000 lbs/inch², using hydraulic press.

All the samples were kept in air tight plastic boxes and were preserved in a vacuum desiccator.

4.2 Haematological Studies

The following haematological parameters have been studied using a techniques of Dacie and Lewis (1969)

a) Total leukocyte count
b) Differential leukocyte counts
c) R B C count
d) Haematocrit.

a) Total Leukocyte counts

Dilution of blood was made in Turks solution (2% acetic acid with a pinch of crystal violet) in the ratio of 1:20(V/V) using haemoglobin pipette. One drop of the diluted mixture was released into Neubauer chamber of the haemocytometer and the cells in the large squares were counted. The total-leukocyte-number was calculated using the formula.

\[
\text{Total Leukocyte counts} = \frac{\text{No. of cells} \times \text{dilution factor}}{\text{volume of squares} \times \text{No. of squares}} \quad (4.19)
\]

b) Differential leukocyte counts

For this purpose, the blood films, prepared on clean glass, were air dried and further fixed in methanol for one
minute. The blood films were stained with freshly diluted Gimesa stain (1:9 in 0.2 M phosphate buffer pH 7.0) for 40 minutes. Thereafter these smears were differentiated in phosphate buffer. Finally the blood smears were air dried and different cell types viz. neutrophils, lymphocytes, monocytes, eosinophils and basophils were counted. Minimum 100 cells were counted for determining percentage of different cell types.

c) Total RBC Counts

Dilution of blood was made in formal citrate solution (1% formalin in 31.3 g/l trisodium citrate) in the ratio of 1:20 (V/V) and the solution was spread on haemocytometer. The RBC were then counted in the central small squares of the haemocytometer and total number was calculated using the formula

\[
\text{Total RBC counts} = \frac{\text{Number of cells} \times \text{dilution factor}}{\text{Volume of squares} \times \text{number of squares}}
\]  

(4.20)

d) Haematocrit

The macromethod popularly called as Wintrobe's method was employed for determining the haematocrit value.

Wintrobe tube of internal diameter 3 mm and length 110 mm was used.

The tubes were filled with blood up to 100 mm mark and centrifuged at 3,000 rpm for 30 minutes. The height of the column of red blood cells was taken as packed cell volume or haematocrit.
4.3 Radiotracer technique

Uptake, distribution and elimination of lead in various mouse organs/tissues has been studied using radiotracer technique. In this technique lead containing known fraction of radioactive Pb-210 was administered to the animals intraperitoneally. The amount of administered lead in various organs/tissues was monitored by measuring the characteristic $\gamma$-ray from Pb-210. This method is considered to be advantageous because of the high sensitivity, freedom from contamination, ease of sample preparation and less time consumption.

4.3.1 Experimental Set-up

A brief discussion of detector set-up and compatible electronics (Figure 4.7) used for monitoring the $\gamma$-ray of Pb-210 is given below.

a) The NaI(Tl) detector:

The NaI(Tl) detector was preferred due to its high efficiency and the fact that Pb-210 is a monoenergetic $\gamma$-ray source thereby causing no resolution problem. For the present measurements, a cylindrical NaI(Tl) well-type detector (make HARSHAW) of length 25 mm and well dimensions 17.5 mm x 22 mm was used. The crystal has an efficiency of about 60% for Pb-210 $\gamma$-ray. The NaI(Tl) crystal is optically coupled to photomultiplier tube (RCA 6199) by using viscous silica fluid. This combination is encapsulated in aluminium case to protect it from its exposure to humidity, as NaI(Tl) is a hygroscopic material. A highly regulated voltage supply
FIG. 4.7 BLOCK DIAGRAM OF THE GAMMA-RAY SPECTROMETER
and a resistive voltage divider network provides voltage to various dynodes and anode of the photomultiplier tube.

The NaI(Tl) crystal converts a single photon of high energy into large number of photons of low energy called scintillations, to which photomultiplier tube is sensitive. The scintillations are conducted to photocathode of photomultiplier tube through the silica fluid coupling.

Scintillations falling on the photocathode causes the emission of photoelectrons which are accelerated by a chain of dynodes. In this process, the number of electrons is increased by a factor of about $10^6$, due to secondary electron emission at each dynode. These electrons are collected by the anode leading to a negative polarity fast voltage pulse at the output.

b) **Amplifier:**

The voltage pulse from the anode is fed to the spectroscopy amplifier through a cathode follower. The polarity of anode pulse is inverted before amplification takes place. The amplifier shapes the pulse in a predetermined manner. The output of the amplifier is always positive.

c) **Single channel analyser (SCA)**

The amplified positive pulse is fed to a single channel analyser (SCA) operated in differential mode with upper and lower level discriminators preset to allow the detection of pulses corresponding to energy region 10-70 keV. The SCA allows only those pulses whose amplitude lie between two discriminators levels.
d) **Timer and Counter:**

The selected pulses from the SCA are counted by a digital counter which has a provision of visual display up to six digits. A timer is used for the preselection of counting time (seconds) for the digital counter.

### 4.3.2 Sample preparation

Radioactive source of Pb-210 in the form of lead nitrate solution (half-life 22 years, specific activity 40 μCi per mg of Pb) was obtained from Bhaba Atomic Research Centre, Bombay, India. The partial decay scheme of Pb-210 is given in Figure 4.8. Pb-210 undergoes β⁻-decay followed by the emission of single γ-ray of energy 47 keV. The purity of Pb-210 was checked by taking its spectrum using high resolution HPGe detector and no radioactive impurity was detected.

Forty male albino mice (weighing 20 to 25 gram each) were divided into eight groups of five animals each. The animals of all the groups were provided, ad libitum drinking water and standard animal feed supplied by the Hindustan Lever Ltd., Bombay. Before the administration of Pb-210, the animals were fasted for 18-20 hours. Each animal was administered intraperitoneally 0.2 ml of Pb-210 having an activity of 2 μCi. The animals were sacrificed in groups of five each at intervals of 1, 3, 6, 12, 24, 72, 168 and 360 hours post Pb-210 administration.
Fig. 4.8 DECAY SCHEME OF $^{210}\text{Pb}$
Blood was drawn with the help of heparinized glass capillaries from the orbital sinuses of the mice and was centrifuged at 3000 rpm for 20–30 minutes to separate the packed cells and plasma. After collecting the blood, an intraperitoneal injection of pentobarbitone (4 mg/100 g body weight) and heparin (200 unit/100 g body weight) was given to each animal. Penobarbitone was used to anaesthetize the animals (Conrad and Barton, 1978). The heparin was used to avoid the clotting of the blood. Perfusion of the animals was done by Tyrode's ringer solution. After perfusion, eleven organs namely liver, kidney, lungs, heart, stomach, small-intestine, large-intestine, pancreas, thigh muscles, spleen and femur were removed and washed with physiological saline solution (0.89 % NaCl). Stomach, small-intestine and large intestine were cleaned with the help of a scalpel and their inner contents were removed. In case of femur, the extraneous material surrounding the bone was removed with a sharp scalpel and it was extracted out by cutting the femur at both ends. These tissues were weighed and then transferred to the counting tubes containing potassium hydroxide (30%) solution, except femur, making the volume equal to 3 ml in each case. The femur was dissolved in 10 N HNO₃ solution instead of KOH. The test tubes were kept for 3 days so that the organs could dissolve completely.

The test tubes were run on gamma ray counter for a time interval of 1000 seconds. Three sets of readings were taken for each sample. The background was run, separately, and subtracted from the sample counts.
0.2 ml solution of Pb-210 radioisotope (activity 2 μCi) was transferred to a test tube and made to 3 ml with distilled water. In order to avoid the dead time correction, this solution was divided into two parts so that the counts did not exceed about 500 counts/sec. Five runs of 1000 seconds each were taken and the weighted average was worked out. The background counts were taken separately and subtracted from the standard counts.

In case of whole body the counting was done just before the dissection of the animals. Animal was immobilized in a cylinder of 1.5 inch diameter with the help of a plunger and kept close to the detector. The counting was done on a scintillation counter as described above. The data was taken for a time interval of 60 seconds each. The counting of the standard was carried out under identical geometry by taking 0.2 ml solution of Pb-210 radioisotope (activity 2 μCi) in a vessel similar to the one used in whole body counting. The volume was made equal to the volume occupied by the mice, in the vessel, by adding distilled water. Background counts were taken separately and subtracted from the sample and standard counts.

4.3.3 Data Analysis
a) Evaluation of Percentage Uptake:

The resultant counts were divided by the tissue weight to get the normalized counts/g of the wet tissue. The fraction of the injected Pb-210 taken up by each organ
was calculated using a relationship

\[
\% \text{ dose/gram} = \frac{\text{tissue counts/g}}{\text{counts in the standard}} \times 100
\]  

(4.21)

The percentage dose/g of wet weight for each organ was calculated for the various time intervals post Pb-210 administration. Weighted average as well as standard deviation were calculated by taking the organs/tissues of five animals in each group.

b) **Evaluation of elimination rates**

The decay data was fitted to a sum of two exponential function of the type:

\[
N(t) = N_1 \exp(-\lambda_1 t) + N_2 \exp(-\lambda_2 t)
\]  

(4.22)

where \(N(t)\) is the percentage uptake at time \(t\), \(N_1\) and \(N_2\) are the percentage uptakes of fast and slow decay components at zero time and \(\lambda_1\) and \(\lambda_2\) are the corresponding decay constants. Since the function \(N(t)\) is not linear w.r.t. constants \(N_1, N_2, \lambda_1, \lambda_2\) and no transformation can reduce it to a linear equation, therefore, the usual method of least square fitting cannot be applied. To fit non-linear function an iterative "least square method" as reported by Scarborough (1966), was applied in this study.

The essential difficulty inherent in the fitting of eqn. (4.22) by iterative method is that it is necessary to prepare initial estimates for calculating the exact decay parameters. In case these initial estimates are poor,
the iteration does not converge. To overcome this difficulty, the initial estimates of $N_1$, $N_2$, $\lambda_1$ and $\lambda_2$ required in the iterative calculation (Scarborough, 1966) were found out by fitting the decay points at regular intervals (obtained by polynomial interpolation of the experimental points) to a double exponential function by a non-iterative method of Mukoyama (1981) as described in appendix A-I. Starting from a set of these estimated values of the parameters $N_1,N_2,\lambda_1$ and $\lambda_2$, iterative method (appendix A-II) was applied and the calculations were continued using a computer programme till the desired degree of convergence was obtained.

Applying this method, the values of the four coefficients were obtained to work out the biokinetics parameters of Pb in various mouse organs/tissues.

4.4 Statistical analysis

a) Weighted average

The weighted average of the values obtained from different samples of the same population was found out as follows

If $X_1 \pm \Delta X_1$, $X_2 \pm \Delta X_2$ ..... $X_n \pm \Delta X_n$
are 'n' independent measurements of a given quantity, $\Delta X_1$ being the uncertainty in $X_1$, then the weighted average of these measurements is given by $\bar{X} \pm \Delta \bar{X}$, where

$$\bar{X} = \sum \frac{X_i}{(\Delta X_i)^2}$$

(4.23)
Here \( W = \frac{1}{\sum (\Delta x_i)^{-2}} \)

Then

\[
\text{Statistical error} \ = \ (W)^{1/2}
\]

(4.24)

And

\[
\text{Standard error} \ = \ \left[ W \sum (\Delta x_i)^{-2} \frac{(\bar{x} - x_i)^2}{n-1} \right]^{1/2}
\]

(4.25)

The value found to be maximum, out of the two obtained from equations (4.24) and (4.25) was taken as the uncertainty \( \Delta \bar{x} \) in the average value \( \bar{x} \).

Standard deviation was calculated using the equation:

\[
\text{Standard deviation} \ = \ \text{S.E.} \times \sqrt{n}
\]

(4.26)

where S.E. is the standard error and \( n \) is the number of values taken for calculating the weighted average.

b) Uncertainty determination

The uncertainty in the measurement of XRF cross-sections has been calculated using the relationship

\[
\Delta \sigma_{ij} = \sigma_{ij} \sqrt{(\frac{\Delta A}{A})^2 + (\frac{\Delta B}{B})^2 + (\frac{\Delta C}{C})^2 + (\frac{\Delta D}{D})^2}
\]

(4.27)

where \( \Delta \sigma_{ij} \) is the error in XRF cross-sections of \( i \)th x-ray and \( j \)th element

\( \sigma_{ij} \) = XRF cross-section of \( i \)th x-ray and \( j \)th element

\( A \) = area under the x-ray peak.

\( \Delta A \) = error in the area evaluation

\( B \) = IoG Value

\( \Delta B \) = error in the IoG value
C = relative efficiency value
ΔC = error in the relative efficiency value
D = thickness of the foil.
ΔD = error in the thickness of the foil.

c) Student "t" test

To estimate whether the difference between the mean values of two groups are statistically significant or not, the student "t" test was applied. The value of "t" was calculated by using the expression

\[
t = \frac{\bar{X} - \bar{Y}}{\sqrt{\frac{S^2}{n_1} + \frac{1}{n_2}}}
\]

where

\[
\bar{X} = \text{Arithmatic mean value of group I of } n_1 \text{ samples}
\]
\[
\bar{Y} = \text{Arithmatic mean value of group II of } n_2 \text{ samples}
\]
\[
S^2 = \frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{(n_1+n_2-2)}
\]

S_1 and S_2 are the respective standard deviations.

The calculated values of "t" were compared with the theoretical values taken from the standard tables (Biostatistics by Goldstein, A., 1964) and the corresponding "p" values were determined.
d) **Correlation coefficient**

The interdependence between the analysed elements was examined by calculating the correlation coefficients. The coefficient of correlation is defined as

\[
\text{r}(X,Y) = \frac{\text{Covariance}}{(\text{Standard deviation in } X)(\text{Standard deviation in } Y)}
\]

(4.29)

Where \( X \) and \( Y \) are any two data sets each having 'n' values, the mean values of which are \( \bar{X} \) and \( \bar{Y} \) respectively, thus;

\[
\text{r}(X,Y) = \frac{\frac{1}{n} \sum (X_i - \bar{X}) (Y_i - \bar{Y})}{\frac{1}{n} \left[ \sum (X_i - \bar{X})^2 \sum (Y_i - \bar{Y})^2 \right]^{1/2}}
\]

(4.30)

where \( \bar{X} \) and \( \bar{Y} \) are the mean values for the two groups of samples, \( X_i \) and \( Y_i \) are the ith members of group I and II respectively.

The significance of these coefficients has been determined by using the expression which follows a student t-test i.e.

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

(4.31)

where \( n \) is the sample size in either group and \( r \) is the coefficient of correlation.

This test is derived from a test for the significance of the correlation between any two samples drawn from normal population. Assuming the null hypothesis that there is no
correlation in the bivariate population \( (p=0) \) against the alternative that a significant correlation exists \( (p \neq 0) \) was applied.

e) **Chi-Square \( (\chi^2) \) Test**

\( \chi^2 \) test was applied to test the goodness of the fitted function to various sets of data points, using the equation:

\[
\chi^2 = \frac{1}{N-(n+1)} \sum_{i=1}^{N} \left[ \frac{1}{\sigma_i^2} (Y_{\text{obs.}} - Y_{\text{fitted}})^2 \right]
\]

Where \( N-(n+1) \) is the degree of freedom left after fitting \( N \) data points to the \( n+1 \) parameters and \( \sigma_i^2 \) is the standard deviation for the \( i \)th data points.

The value of \( \chi^2 \leq 1 \) indicates that the fitted function is appropriate to describe the set of data points.

The value of \( \chi^2 \) was compared with the theoretical values taken from the standard table (Statistical Methods by Snedecor and Cochran, 1967) and the corresponding "\( p \)" value was determined.