Pot culture experiments were conducted to study the uptake and translocation of chromium with reference to metal-organic acid interactions in variety of the plants grown in hydroponic, sand and soil cultures.

Based on inherent capability of the combination of radiotracer and ion exchange resins like XAD 2, Dowex 1 and Dowex 50, investigations have been made to develop a method for the estimation of organically bound chromium.

*Radiotracer technique used in this work is best suited to plant uptake studies because of its conveniences and sensitivity. The technique is fast, nondestructive and there are minimum chances of contamination, avoiding the use of huge amount of concentrated acids in digestion processes.*

*Hydroponic, sand and soil culture experiments were designed to highlight the possibility of the existence of metal-organic acid interactions. Comparison of sand and soil uptake studies is expected to explain the role of various species present in soil in modifying the metal-organic acid interactions and their subsequent uptake by plants.*

The general methodology and the technique employed in the study, have been described as follows:

**INSTRUMENTATION**

**RADIATION DETECTION AND MEASUREMENT**

All methods for detection of radioactivity are based on the interactions of the charged particles or the electromagnetic rays with matter traversed. Electromagnetic radiations like X rays and gamma rays lose their energy in a stopping material mainly through three mechanisms namely: (i) Photoelectric effect (ii) Compton scattering, (iii) Pair production. These processes are strongly dependent on the energy of the photon and
atomic number \((Z)\) of the stopping material. Other effects such as Rayleigh scattering and Thompson scattering etc. are much less important and can be ignored in the detection process.

(i) **Photoelectric effect**
In this process, a photon is absorbed in the medium and energy is transferred to one of the electrons (normally tightly bound orbital electron) then the velocity of that electron will be too high to remain in the orbital resulting in its emission. The difference between the incident photon energy \((E_i)\) of the electron and the binding energy of the photon \((E_b)\) appears as the kinetic energy of the ejected electron \((E_e)\).

\[ E_e = E_i - E_b \]

The probability of the emission of the electron is in the order of \(K>L>M>N\)........ electrons if the energy of the photon is high enough. Photoelectric effect is characterised by the total absorption of the photon energy within the medium and is the predominant mode of interaction of low energy gamma rays.

(ii) **Compton Scattering**
In this process, the photon interacts with an electron that may be loosely bound or free. The incoming photon is deflected through an angle \(\theta\) with respect to its original direction and fraction of its energy is transferred to the electron. Energy of the electron and the scattered photon is:

\[ E_g = E_i / \left[1 - \frac{E_e}{E_o}(1-Cos\theta) \right] /mc^2 \]
\[ E_e = E_o - E_g \]

Where \(E_o\) is the energy of the incident photon, \(E_g\) is the scattered photon energy, \(E_e\) is the scattered electron energy, \(m\) is the electron rest mass and \(c\) is the velocity of light. \(\theta\) is angle between scattered and incident gamma ray. Compton scattering is responsible for compton continuum in the gamma ray spectra.

(iii) **Pair production**
This process involves the complete absorption of a photon in the vicinity of an atomic nucleus with the formation of an electron positron pair. In accordance with the momentum conservation, this interaction mainly occurs in the field of the nucleus of the absorber
material. Pair production can not occur when the energy of gamma radiation is less than 1.02 MeV that is equivalent to the rest mass of \(e^- + e^+\) pair with zero kinetic energy. The cross section for pair production is also proportional to \(Z^2\). At high energies, where pair production is the predominant process, gamma ray energies can be best determined by measurements of the total energies of electron-positron pairs. Pair production is always followed by annihilation of the positron, usually with the simultaneous emission of two 0.51 MeV photons. The absorption of quanta by the pair production process is, therefore, always complicated by the appearance of this low energy secondary radiation with its associated Compton.

In summary, all the three processes produce moving electrons in matter that can be detected directly or can initiate other electronic processes to obtain an electric charge pulse that represents the initial photon energy. The recorded pulse is proportional to the energy lost in all the three processes. Full energy photopeak results from the complete energy deposition of the photon in the detector by any one or the combination of the three processes mentioned.

**SIMPLE COUNTING SYSTEMS**

A basic measurement in many physics experiments is a simple counting of the number of pulses from a detector. In this setup, the analog signal from a detector is amplified by the preamplifier, shaped and further amplified by the amplifier. The resulting analog signal is then sent through the low level discriminator that delivers a signal for every input pulse with an amplitude greater than the threshold value. The signal is then sent to the timer/scaler that counts each arriving pulse for a given counting period \(T\) preset in the timer section. Discriminator serves the dual purpose of excluding low level noise in analog suit, and shaping the accepted signal to a form suitable for the scaler to accept.

**GENERAL PRINCIPLE OF DETECTION**

In almost all radiation detectors, deposition of radiation in its volume causes ionization releasing electric charge (in the case of scintillator, emitted light is converted to electric charge in photomultiplier tube) and effective collection of this charge, under the applied electric field forms of the basic signal of the interacting radiation. The ionization results in a very low current or voltage pulse that needs proper amplification. The amplified pulses are further processed to obtain the number of radiation (counting) or the energy and the intensity of the radiations (spectrometry). Interaction time is very small, a few
nano seconds in gases and a few pico seconds in solids. These times are so short that the deposition of the radiation energy can be considered instantaneous. The detector acts like a capacitor and the charge collected in the capacitor can be discharged through a resistance giving a voltage pulse.

**Nal (TI) SCINTILLATION DETECTOR**

Scintillation detectors are based on the conversion of the absorbed energy into light and detection by the use of photomultiplication. Among the inorganic scintillators, Nal, activated with 0.1 to 0.2% thallium is, by far, the most widely used. The high density (3.7g/cm³) of Nal and the high molecular weight of iodine make this a very efficient gamma ray detector. A popular size of the gamma ray measurement is a 7.5 cm diameter and 7.5 cm high cylinder. Another useful type has a re-entrant well in the centre to allow measurement of (liquid or solid) sample in nearly un-geometric.

Approximately 30 eV of energy deposition in a Nal (TI) crystal is required to produce one light photon, and it takes on an average about 10 photons to release one photoelectron at the photocathode of the multiplier. These photoelectrons are then accelerated by a potential of the order of 100 V to the first dynode where each one produces n secondary electrons; these secondary electrons are then similarly accelerated and multiplied n-fold at the second dynode, and so on. With 10 dynodes and with n typically about 3 or 4, the total multiplication factor is n¹⁰ or of the order or 10⁵ or 10⁶. Thus a 0.3 MeV gamma ray absorbed in a Nal (TI) crystal might produce 10⁴ light photons giving 10⁵ photoelectrons and leading eventually to an output pulse of about 10⁸ electrons or 1.6 x 10⁻¹¹ coulomb (C). In an output circuit of about 10⁻¹⁰ F capacity this would be a pulse of about 0.16 V requiring further amplification. There is a good correlation between the energy absorbed in the scintillator and the size of the output pulse.

An additional feature in the Nal(TI) spectra is so-called iodine escape peak about 28 KeV below the photopeak. It results from the absorption of gamma ray near the surface of the detector and subsequent escape of the K-X ray of the iodine. This effect becomes less pronounced with increasing gamma ray energy because fewer of the initial interactions take place near the surface.
(NaI (Tl) GAMMA-RAY DETECTOR COUPLED TO A 4K MCA)
MULTI CHANNEL ANALYSER (MCA)

Multi Channel Analyser (MCA) are used widely for pulse height analysis. The basic function of the MCA is to sort out the incoming pulses from the detector according to the pulse height and keep count of the number of pulses at each pulse height in a multi channel memory. The content of a large number of channels is known as pulse height spectrum that can be displayed on the visual display unit for monitoring.

MCA works by digitizing the amplitude of the detector pulse heights with a Nuclear Analog to Digital Converter (NADC). The MCA then takes this channel address, and increments by one of the corresponding memory channel contents whose address is proportional to the digital number. In this way pulses are sorted out according to the height of the analog pulse and the number at each pulse height stored in the corresponding memory locations. As a result of this histogramming process, one builds up the pulse height spectrum. The total number of channels into which the voltage range is to be digitized is known as the "conversion gain" and it determines the resolution of the MCA.

INTERFERENCE IN GAMMA (γ) COUNTING

There are several factors such as background radiations, sample geometry and dead time that affect the count rate or counting efficiency of the detector.

Background Radiations
Interfering background in gamma spectra originates either from within the sample being counted (Compton produced or due to presence of other radionuclides) or from the environment. The Compton increases if the sample being analysed has a high content of high energy gamma emitting radioisotopes. For extremely weak samples, the environmental background becomes more significant. This can be reduced using massive shielding generally made of lead.

Sample Geometry
The sample size was selected as large as possible for maximum efficiency. The sample was distributed uniformly so as to minimize the distance between the sample volume and the detection itself.
Dead Time
Radiation produces a pulse in the detector subsequent to its interaction. While this pulse is being processed, detector is not available for processing next pulse that might be generated during this interval. Because of the random nature of radioactive decay, there is always some probability that a true event will lost because it occurs too quickly following a preceding event. The minimum time separation required to record two successive events as two separate pulses are usually called the dead time of the counting system. The dead time losses increase with the increase in the count rate. Detector should have a small dead time so that many counts are not lost just because the detector is still processing an earlier pulse. The percentage dead time is given by:

\[
\text{\% Dead time} = \frac{(\text{True Time} - \text{Live Time}) \times 100}{\text{True Time}}
\]

ENERGY AND EFFICIENCY CALIBRATION

The detector was calibrated for the gamma ray energy of particular isotope using Gamma Reference standards (Modules Disc Type, provided by Electronics Corporation of India Ltd.,) Details of gamma reference standards are tabulated below (Table - 2:1)

Table 2:1 Decay characteristics of few radionucloids used for calibration

<table>
<thead>
<tr>
<th>ISOTOPE</th>
<th>GAMMA ENERGY (MeV)</th>
<th>NOMINAL ACTIVITY ((\mu)Ci) as on July, 1991</th>
<th>HALF LIFE (Year/Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO(^{57})</td>
<td>0.123</td>
<td>3.0</td>
<td>273 Days</td>
</tr>
<tr>
<td>CO(^{60})</td>
<td>1.17;1.33</td>
<td>1.0</td>
<td>5.3 Years</td>
</tr>
<tr>
<td>Cs(^{137})</td>
<td>0.662</td>
<td>1.0</td>
<td>30 Years</td>
</tr>
</tbody>
</table>

The gamma ray intensity (A) is given by the expression:
Net Count Rate \( (P) = \text{Abundance (A)} \times \text{Efficiency (E)} \times \text{Disintegration Rate (D)} \)
Where \( P \) is the photopeak area, \( E \), is the efficiency for detection of a gamma ray with energy \( E \) and \( D \) is the disintegration rate of the sample.

The efficiency of the detector depend on the gamma ray energy and the detector type, its shape and size. At low energies, the attenuations of the gamma rays in the window and dead layer are predominant. This effect reduces as the energy of the gamma ray increases. For high energy gamma ray for a given size, detector efficiency again falls due to greater chance of escape of Compton scattered gamma ray. Thus depending on the type and size of the detector, efficiency peaks at certain energy and falls on both sides of this value.

**PLANT UPTAKE EXPERIMENTS**

**Hydroponic Experiments**

Hydroponic experiments were performed in clean air conditioned environmental laboratory using standard practices with Hoagland nutrient solution (Hoagland and Amon 1950). Four seedlings per pot (beaker) were carefully transplanted with the support of thermocol. Beakers were covered with dark paper to protect roots from light. Plants were supplied with nutrient solution (half strength) and changed after every 3 days. Aeration was carried out with filtered compressed air every alternate day. Natural light (diurnal cycle of 15 hrs.) was supplemented by the combination of Philips Fluorescent tubes (40W) and Toshiba lamps (15W) giving an irradiance of approximately 600 W/m² at the plant tops with a slow ventilation system. After establishment period of four weeks, plants were shifted to fumehood of radiochemical laboratory for treatments.

**Sand and Soil Experiments**

Quartz sand, used in the experiments was of superior quality obtained from Firozabad (U.P.). Sand was washed thoroughly with tap water to remove the traces of silt and clay and was subjected to cleaning process, using procedure (Hewitt 1966). The process was repeated for 5-6 times until the pH of the incoming and outlet water was similar and neutral. The sand was washed two times with distilled water and left dipped for 24 hours.

Top soil (0-15cm) from the nearby agricultural field of the institute was collected for carrying out pot studies. The soil was mixed properly and cleaned free of the undesirable objects like leaves, stones and pebbles etc. The basal dressing of chemical fertilizer (N:P:K; 60: 20:18 mg/kg) was supplied.
Sand and soil (2.5 kg.) were filled in plastic containers separately. Cotton plug was inserted in the bottom hole of the pot to allow the leaching of excess water to prevent water logging. Although, care was taken not to add water and nutrient in excess. Water holding capacity of the pots was estimated and rate of addition of nutrient and irrigations were adjusted accordingly.

Sand and soil samples were characterized from Nuclear Research Laboratory (NRL), Indian Agricultural Research Institute (IARI), New Delhi and in our department for their various parameters (Table 2:2).

### Table 2:2 Selected physical and chemical characteristics of soil and sand used.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) pH</td>
<td>7.4</td>
<td>6.8</td>
</tr>
<tr>
<td>(b) Texture</td>
<td>Sandy loam (inceptisol)</td>
<td>-</td>
</tr>
<tr>
<td>(c) Organic Carbon (%)</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>(d) Electrical Conductivity* (EC) (dS/m)</td>
<td>0.23</td>
<td>111</td>
</tr>
<tr>
<td>(e) Cation Exchange Capacity (CEC) (cmol (p+)/kg)</td>
<td>25.7</td>
<td>21.6</td>
</tr>
<tr>
<td>(f) Alkality ** (mg/l)</td>
<td>99.00</td>
<td>50.00</td>
</tr>
<tr>
<td>(g) Soluble ions (cmol (p+)/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca**</td>
<td>0.54</td>
<td>-</td>
</tr>
<tr>
<td>Mg**</td>
<td>1.80</td>
<td>-</td>
</tr>
<tr>
<td>Na+</td>
<td>6.10</td>
<td>-</td>
</tr>
<tr>
<td>K+</td>
<td>0.09</td>
<td>-</td>
</tr>
<tr>
<td>Cl−</td>
<td>5.7</td>
<td>-</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>1.3</td>
<td>-</td>
</tr>
<tr>
<td>(h) Soil elemental concentration (µg/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cr</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Total Mn</td>
<td>80.00</td>
<td>-</td>
</tr>
<tr>
<td>DTPA extractable Mn</td>
<td>2.6</td>
<td>-</td>
</tr>
</tbody>
</table>

* Estimated in saturated paste extract.

** Alkality as CaCO₃ by titrating with 0.02 N H₂SO₄.

*** Measured in soil digested in nitric and perchloric acids.
GROWTH OF THE PLANTS

Plant uptake studies (Hydroponic, Sand and Soil culture) were conducted on the following plants viz. Maize (Zea mays), Tomato (Lycopersicum esculentum), Soybean (Glycine max) and Wheat (Triticum vulgare). Seeds of different crops were obtained from IARI, New Delhi and soaked in distilled water for 24 hours. Each pot was seeded with (10) numbers. Pots were placed in plant house and irrigated as and when required. After 10-15 days of sowing, plants were thinned to 4 plants per pot.

NUTRIENT PREPARATION AND SUPPLEMENTATION

Nutrient solution was prepared as per recommendation of (Hoagland and Amon 1950). Iron was supplied as ferric citrate (Hewitt 1966)(Table 2:3).

Stock solution of the nutrient solution was prepared (concentrated solution of 10 times macronutrient, 1000 times micronutrient and 100 times of Fe-citrate solution). Required strength of the nutrient solution was obtained by mixing all the three components in appropriate amount. The final pH of the nutrient solution was kept between 6.5 and 7.0.

Plants were irrigated as per requirement of individual crop, weather and growth of the plants. Equal volume of the nutrient was supplied every alternate day to the plants grown in the sand culture. First two or three weeks of the growth, the half strength and then full strength of nutrient solution was supplied to the plants.

ISOTOPE LABELLING AND TREATMENTS TO THE PLANTS

PREPARATION OF INACTIVE STOCK SOLUTIONS

All the reagents used were of AR (Analytical Reagent) and GR (Guaranteed Reagent) grades. The inactive stock solutions of Cr III and Cr VI were prepared from their respective salts (chromium chloride and potassium di chromate).
PLANTS KEPT IN FUMEHOOD AFTER TREATMENT
### Table 2: Composition of Hoagland nutrient solution

#### Macronutrient Solution

<table>
<thead>
<tr>
<th>Salt</th>
<th>Weight (g)</th>
<th>Conc. (mM)</th>
<th>Volume of stock soln. used to prepare 100 lit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>40.4</td>
<td>4</td>
<td>200 ml.</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>65.6</td>
<td>4</td>
<td>200 ml.</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>36.8</td>
<td>1.5</td>
<td>200 ml.</td>
</tr>
<tr>
<td>NaH₂PO₄.2H₂O</td>
<td>20.8</td>
<td>1.3</td>
<td>100 ml.</td>
</tr>
</tbody>
</table>

#### Micronutrient Solution

<table>
<thead>
<tr>
<th>Salt</th>
<th>Weight (g)</th>
<th>Conc. (μM)</th>
<th>Volume of stock soln. used to prepare 100 lit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnSO₄.4H₂O</td>
<td>0.223</td>
<td>0.01</td>
<td>10 ml.</td>
</tr>
<tr>
<td>CuSO₄.5H₂O</td>
<td>0.025</td>
<td>0.001</td>
<td>10 ml.</td>
</tr>
<tr>
<td>ZnSO₄.7H₂O</td>
<td>0.310</td>
<td>0.002</td>
<td>20 ml.</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.186</td>
<td>0.030</td>
<td>10 ml.</td>
</tr>
<tr>
<td>Na₂MOO₄.2H₂O</td>
<td>0.121</td>
<td>0.005</td>
<td>10 ml.</td>
</tr>
</tbody>
</table>

#### Iron

<table>
<thead>
<tr>
<th>Salt</th>
<th>Weight (g)</th>
<th>Conc. (μM)</th>
<th>Volume of stock soln. used to prepare 100 lit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe. Citrate.3H₂O</td>
<td>2.99</td>
<td>0.1</td>
<td>50 ml.</td>
</tr>
<tr>
<td>Fe. Citrate.5H₂O</td>
<td>3.55</td>
<td>0.1</td>
<td>50 ml.</td>
</tr>
</tbody>
</table>

Actual strength of Cr III solution (hydrolysing nature) was verified using standard colorimetric method (Standard Method 1985). The method is based on the oxidation of trivalent chromium (Cr III) to hexavalent chromium (Cr VI) using potassium permagnate (KMnO₄) as oxidizing agent. The absorbance of pink colour obtained from diphenyl carbazide reagent, was measured at 540 nm (maximum absorption) using UV spectrophotometer (HITACHI U-2000). Based on the exact strength of Cr III solution, the necessary correction in the volume of Cr III stock is considered.
The stock solution of carboxylic acids such as citric acid, malic acid, oxalic acid, malonic acid and amino acids such as aspartic acid, glutamic acid, glycine, asparagine and methionine were prepared by weighing their appropriate amounts and dissolving them in double distilled water (DDW). Mettlor AJI 50 L, single pan electronic balance was used for accurate weighing with accuracy of four digits i.e. 0.1 mg. The pH of the stock solution was maintained between 5.0 - 5.5 in order to keep amino acids, under study, in anionic state.

**SELECTION OF ORGANIC ACIDS**

The predominant organic acids (carboxylic and amino acids) released as root exudates in the plant were considered for the present study. List of organic acids predominantly released by different plants is tabulated:

**Table 2:4 Predominantly released organic acids by different plants.**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Carboxylic Acids</th>
<th>Amino Acids</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato (Lycopersicum esculentum)</td>
<td>*Citric Acid, *Oxalic Acid, Malic Acid, Malonic Acid</td>
<td>*Aspartic Acid, *Glutamic Acid, Serine, Glycine</td>
<td>Vancura and Hovadik (1965)</td>
</tr>
<tr>
<td>Soybean (Glycine max)</td>
<td>*Malic Acid, *Malonic Acid, Oxalic Acid, Citric Acid</td>
<td>*Aspartic Acid, *Asparagine, Glycine, Methionine</td>
<td>Cataldo et. al. (1988)</td>
</tr>
</tbody>
</table>

* Acids selected for study.
PREPARATION OF ACTIVE STOCK SOLUTION $^{51}$Cr

$^{51}$Cr isotope was obtained from Board of Radiation and Isotope Technology (BRIT), Trombay, Mumbai, as CrCl$_3$ in HCl solution. The activity was stored in working stock. Measured activity in working stocks ranged from (±) 10% of nominal activity (provided by BRIT) in 3 of 5 comparisons. Measured activity was used to calculate spike volume for feeding solutions.

$^{51}$Cr activity, supplied, was ensured by the following process: $^{51}$Cr III was oxidised to $^{51}$Cr VI by few drops of KMnO$_4$ (0.01M) and the oxidised $^{51}$Cr was reduced by few drops of Na$_2$SO$_3$ (0.2M) in acidic medium (Standard Method 1985).

$^{51}$Cr VI tracer was obtained by the oxidation of supplied $^{51}$Cr III by few drops of KMnO$_4$ (0.01M) to stock solution to maintain pink colour. The solution was boiled and KMnO$_4$ was added till the pink colour persisted during the boiling. Two- three drops of sodium azide (NaN$_3$) were added to neutralize the excess KMnO$_4$. The pH of both ($^{51}$Cr III and $^{51}$Cr VI) stock solutions were maintained at (5.0 - 5.5).

FEEDING PROCEDURE

After the growth period of 60 days (sand and soil cultures) and 28 days (hydroponic culture), plants were transferred from plant house to the fumehood of radiochemical laboratory.

Carrier solutions of two chromium sources i.e. potassium dichromate (Cr VI) and chromic chloride (Cr III) radiolabelled with their respective oxidation states (2-5 μci per pot) were prepared.

Treatments comprised a single pulse addition of radiolabelled Cr III and Cr VI solutions at 2μg/ml (hydroponic culture) and at 5μg/ml (sand and soil cultures) levels in combination with increasing concentrations (1:1, 1:5, 1:10, 1:50 and 1:100 w/w) of various organic acids. The pH of the solution was finally adjusted to 5.0 - 5.5 with HCl (0.1N). Experiments when no organic acids were supplemented served as control.

Concentration of organic acids in the rhizosphere, of course, are very low, however, in order to highlight the existence of chromium-organic acid interactions and their reflection
on the plant uptake of chromium, experiments have been conducted with higher levels of synthetic amendment of chromium and organic acids.

**RADIOMETRIC ESTIMATION.**

Plants were exposed to the treatments for a period of 10 days in the fume hood of the radiochemical laboratory, well equipped with the facilities required for growth of the plants. After the absorption period, plants were harvested. The roots were rinsed with water, acid water (pH4) and finally with distilled water. pH of the water was tested to ensure that no detectable acidity was left and that there was no detectable external contamination of the plant parts by radiolabelled element. The washing did not eliminate the absorbed element.

After proper washing, plants were separated into various parts viz root and aerial parts (hydroponic culture) and root, shoot and fruit (sand and soil cultures). Plant tissues were kept for drying in oven at 50°C. Dry matter yield of the plants in each case was obtained. Accurately weighted amounts of material were counted over a planar NaI (TI) detector coupled to a 4 K MCA (Multi Channel Analyser) (Canberra Accuspec Card with PC AT 386). The counting geometry was pre-calibrated with $^{51}$Cr from their respective photopeak area. Radioisotope $^{51}$Cr possesses gamma ray energy level 0.320 MeV and half life at 27.7 days.

**DATA ACQUISITION AND STATISTICAL INTERPRETATIONS**

$^{51}$Cr activity was calculated from photopeak area and converted to total amount of element in different plant tissues per gram of dry weight. All the activity was corrected for its decay to arrive at on activity on a common time and date. Samples were counted for varying duration 10 mts. to 120 mts. so as to accumulate at least 8 to 10 thousand counts under photopeak area to keep statistical errors in counting below a few percent.

Plant tissue chromium concentration ($\mu$g/g dry weight) is the ratio of chromium ($\mu$g) transferred into plant tissues (hydroponic, sand and soil experiments) and dry matter yield (DMY) (g dry weight).

Source to plant transfer coefficients (SPT) for chromium with increasing organic acids supplementation in each case were calculated by dividing chromium concentration in
TYPICAL GAMMA RAY SPECTRA
(Recorded on Na (Tl) scintillation detector)

12-Apr-99 02:47:35 SP = ABC/1 OFF CFS 512/ALog CC 5916/662.000

<table>
<thead>
<tr>
<th>4. PEAK DATA</th>
<th>Co$^{57}$ (0.123 MeV)</th>
<th>Cs$^{137}$ (0.662 MeV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chans</td>
<td>8192</td>
<td>8192</td>
</tr>
<tr>
<td>LD=1</td>
<td>RD= 8192</td>
<td>8192</td>
</tr>
<tr>
<td>LM=1</td>
<td>RM= 8192</td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Err</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Cent Ch</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.000 keV</td>
<td></td>
</tr>
<tr>
<td>FWHM</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>FWTM</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>FWTM/FWHM</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Gauss Rat</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>FWHM CAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>
TYPICAL GAMMA RAY SPECTRA
(Recorded on Na (Tl) scintillation detector)

12-Apr-99 04:09:33 SP= ABC/1 OFF CFS 16384/ALog CC 3016/ 320.000

1. ACQUISITION
Chans  8192
LD=1  RD= 8192
Acq Start
12-APR-99
03:17:00

ELAPSED
Live  00:30:00.00
Real  00:31:54.11
Totl

PRESET
Live  00:30:00.00
Real  00:00:00.00
Totl
Start  1579
End    1971

Dead Time  6%

Cr $^{51}$ (0.320 MeV)
the plants (DW) by chromium concentration in feeding solution. Elemental concentration in the whole plant was used as the sum of concentration in various tissues viz. root, shoot, and fruit (Banuelos and Meek, 1990).

STATISTICAL ANALYSIS

The elemental concentration (μg/g) was analysed statistically using the procedure of SPSS/PC* statistical package (SPSS 1983). Experiments were conducted in triplicate and data represents the mean value of four plants per pot. Test for non normal data were computed by Mann Whitney (Independent U test) (Siegel 1956) to compare individual mean. Correlation coefficients were used to relate chromium concentration in root and aerial parts to various treatments of organic acids.

ESTIMATION OF ORGANICALLY BOUND CHROMIUM

Batch and column experiments were conducted in duplicate using standard practices to estimate organically bound chromium.

PREPARATION OF ORGANICALLY BOUND CHROMIUM

Organically bound chromium was obtained by using procedure (James and Bartlett 1983) as follows: A definite amount of Cr III (50μg) was taken in Erlenmeyer flask and radiolabelled with CrIII tracer (0.05 μci). Organic acids (Carboxylic acids: citric, malic, oxalic and malonic acid; amino acids: aspartic acid, glutamic acid, asparagine, methionine and glycine) were added in the concentration range (Cr III: Organic ligands, 1:1, 1:10 and 1:100 w/w) separately. Ionic strength was maintained by adding KCl solution (5ml; IN). Final volume, in each case was maintained at 25 ml with water (pH5) and kept for shaking (72 hrs.). Supematant was removed after centrifuging for 10 min. Supematant solution representing organically bound form was radioassayed.

EXPERIMENTS ON AMBERLITE XAD-2 RESIN

Amberlite XAD-2 (Organo Co. Ltd., USA) was used for experiments and subjected for clean up process (Ishiwatri et.al 1980)
The typical cleanup process for XAD resin involved the washing of resin several times with distilled water and soxhelt extraction for 8 hrs. each with methanol, acetonitrile and diethyl ether. The clean resin was stored under methanol and washed with distilled water before use.

DETERMINATION OF ORGANICALLY BOUND CHROMIUM ON XAD RESIN

Column experiments
Pyrex glass columns of 5 cm. height and 0.5 cm. diameter were used. Packing of columns was carried out using wet method. XAD-2 resin (0.5g) in methanol was carefully transferred in small additions to obtain a well packed column. Interlocking of the air bubbles was avoided. The resin was aclamatized with acid water (pH2). Radiolabelled solution representing organically bound chromium (5ml) was loaded on the column. Elution was carried out with water (pH2) and effluent (eluate) was collected in volumetric flasks till no activity was observed. Eluate (5ml.) was counted in the vials of standard geometry and multiplied for total volume of effluent obtained.

The amount of chromium loaded and collected as eluate (in terms of counts) were converted into μci/ml. and percentage retention was calculated using formula.

\[
\text{Percentage retention} = \frac{\text{Loaded} - \text{Recovered}}{\text{Loaded}} \times 100
\]

Batch experiments
A pre standardized known weight of XAD-2 resin (0.5g) was added to radioassayed solution representing organically bound chromium (25ml) in the Erlenmeyer flask. The solution was maintained at pH2 and equilibrated by shaking for different time intervals (15, 30, 45 and 60 minutes) using automatic shaker. After shaking the solution was allowed to settle and supernatant (5 ml.) was carefully withdrawn and counted in a vial of standard geometry. After assay, the supernatant was readded to the flask and kept for further shaking. Percentage retention of organically bound chromium on XAD-2 was calculated.
EXPERIMENTS ON DOWEX 50 AND DOWEX 1 RESINS

Dowex 50 (H⁺) form and Dowex 1 (Cl⁻) form received from Bio Rad Lab USA., were saturated with KCl and HCl solution of the strength (0.1N) to obtain K⁺ and Cl⁻ form of the resins respectively.

Retention behaviour of (Cr III) and (Cr VI) on cationic and anionic resins

Column Experiments.
Columns were packed (0.5 gm; wet packing) with cationic resin (Dowex 50) and anionic resin (Dowex 1) using water (pH5) separately. Radiolabelled solutions of each Cr III and Cr VI (5ml) having chromium concentration (10µg) were loaded on the cationic and anionic column beds. Both the columns were eluted using water (pH5) at the flow rate 5 sec per drop. The effluent was collected in volumetric flask till no activity was observed. Effluent (5ml) was counted and multiplied for total volume of effluent obtained. Percentage retention Cr III and Cr VI forms for cationic and anionic resins were calculated.

The retention behaviour of Cr III on anionic column and Cr VI on cationic column were also recorded in the similar fashion.

Batch experiments
Batch experiments were conducted in Erlenmeyer flasks. A pre-standardized known weight (0.5g) of cationic and anionic resins were transferred to the flask separately. A known amount 50µg in 25 ml. volume of water pH5 of radiolabelled Cr III and Cr VI were added to the flask containing cationic and anionic resins.

Solution was equilibrated by shaking for different time intervals (15, 30, 45 and 60 minutes) in an automatic shaker. After shaking, it was allowed to settle and supernatant (5ml.) was carefully withdrawn and counted in vial of standard geometry. After counting, supernatant was readded to the flask and kept for next shaking. The percentage retention of chromium was calculated.

The retention behaviour of CrIII on anionic resin and Cr VI on cationic resin was also observed in the same manner.
DETERMINATION OF ORGANICALLY BOUND Cr III BY USING DOWEX 50

The ability of Dowex 50 resin to retain cationic species efficiently and releasing the complexed species has been used as a basis for the determination of organically bound form in the solution of Cr III complexed with organic acids (Schnitzer and Skinner, 1966).

In the batch experiments, a pre-standardized weight of Dowex 50 (0.5g) was allowed to equilibrate with 25 ml. solution representing organically bound from. The solution was shaken for 1 hr. and allowed to settle and supernatant (5ml.) was carefully withdrawn and radioassayed in the vials of standard geometry and magnified for its total volume.