SITE DESCRIPTION AND METHODOLOGY

CHAPTER III
3.1 Study Sites

The present study was conducted in Gurgaon district located in the state of Haryana. The map, showing the boundaries of the state and the location of the district, is given in figure 3.1. The Gurgaon district shares its boundaries with Delhi on one side and is surrounded on the other two sides by Rohtak and Faridabad districts. The land use pattern in Gurgaon district has changed drastically over last ten years. Mainly, agriculture forms the central activity in this district but lately the large part of this district is transforming into urban area. Even with these changes, significant area in this district is still under agriculture.

The state of Punjab and Haryana have been active participant in the green revolution initiated in the early seventies. The pesticide usage, consequently, in both these states, has been, and is high. The states of Haryana and Punjab are the two prominent pesticides user states followed by the western part of Uttar Pradesh. The variation in the amount of pesticides consumption in different years in Haryana is given in Table 1.6 (Director of Agriculture, Haryana, 1997). It is to be noted that the absolute amount of pesticide shows a decrease after 1984-85 but at the same time the area under agriculture has also shown a decrease. Our calculations indicate (figure 3.2) that per 1000 hectare the use of pesticide has shown a linear increase over the years. In this light, the information given in table 1.7 for the district of Gurgaon shows a decreasing trend, contrary to the trend shown in figure 3.2.
Figure 3.1: The location of Gurgaon, green color marked in red, where the sampling was carried out.
However, as indicated earlier the rapid urbanization of Gurgaon district has rapidly decreased the land area under agriculture. In the absence of information concerning the land use pattern trends, it is difficult to ascertain the quantitative amount of pesticide application/hectare in Gurgaon. In view of the state wide trends it can be said that the similar trends will apply in Gurgaon district. The apparent decrease is on account of the changes in the land use pattern (Director of Agriculture, Haryana, 1997).

In view of the past and present pesticide usage trends in Gurgaon district and the suitability as well as feasibility of the present study the agricultural plots at Sohna village of Gurgoan district of Haryana have been selected for the present research work.
3.2 Description of the Site

Sohna village is a tourist place in Gurgaon district of Haryana, a State in India. A hot water spring in the hilly terrain of the village attracts many tourists. Geographical location of the selected study area is presented in figure 3.1. The area selected is located at 28° 15' N latitude and 77° 5' E longitude respectively. Altitude of the area is ~320 m from mean sea level (Geological Survey of India, 1998). This area is located at about 50 km from Delhi, the capital city of India, towards south-southwest. Twelve plots were selected in the different agricultural fields in the Sohna village within the distance of around 5 to 6 km from each other. The information regarding the quality and quantity of the pesticides used in the selected plots has been collected with the help of farmers who own the plots. Each plot represents different levels of pesticide application.

3.3 Collection and Preparation of Samples

Sampling methodology, which is free of bias while collecting samples, is central to the field studies. It is not feasible or practical to collect the samples from all the agricultural fields, therefore, it is necessary to frame a sampling scheme for collecting a representative gross field samples. These field samples should be prepared in such a manner that the sample should become homogeneous (Stevens, 1971).
3.3.1 Soil Sampling

Samples were collected from each selected cultivating plot and also from nearest uncultivated land to see the variation in the concentration of the selected organochlorine pesticide residues in soil and water over a period of cropping season. To prepare a soil-sample, soils have been collected from five places, randomly selected within a plot, from each selected agricultural plot and the collected samples were mixed properly so that the sample became homogeneous (Figure 3.3). First three sampling have been done horizontally and the rest two sampling were done vertically, up to the depth of 40-50 cm from each selected agricultural field. To collect the samples depthwise a special sampler was designed by us with the help of university science instrumentation center (USIC) of Jawaharlal Nehru University. The sampler was made from hardened steel pipes. The outline sketch of the designed sampler is given in figure 3.4. This sampler was inserted inside the ground and then taken out. By opening the soil sampler, soil samples could be retrieved with ease.
Handle used to force the sampler in ground

Hinges to open the sampler to retrieve the samples

Soil Sample

Diameter = 4.4 cm

Figure 3.4: A Special Designed Soil Sampler, the length was kept at 1.25 meters.
3.3.2 Water Sampling

To collect the water samples a approach similar to the one adopted for collecting the soil samples was used. Water samples were collected from the selected agricultural fields within and outside the cultivating land plots according to the availability of water. During summer some of the wells become dry as the ground water level recedes. However, some of the bore wells and hand operated pumps had water available throughout the year. The water samples were collected to quantify the presence of pesticides.

3.4 Extraction and Processing of Samples

The processing of soil and water samples was done differently. The steps involved in this are briefly discussed below.

3.4.1 Extraction of Soil Samples

Collected soil samples were extracted according to the standard method (Mohapatra et al., 1994). About 20 gm of soil was taken in a beaker and to this 4-5 drops of ammonia (NH₃) were added. After stirring soil sample thoroughly it was left for 2-3 hours with intermittent stirring to make it dry. When the soil dried completely it was stirred again to make the sample homogeneous. To this 0.5 gm of activated charcoal and 0.5 gm of flurosil were added and then it was mixed thoroughly. This procedure was followed before using the samples for the elution.
The above sample was packed in a glass column for elution. At the same time 150 ml of 10% acetone in hexane was prepared in a beaker. The packed sample in glass column was eluted with 10% acetone in hexane. After collecting the eluted sample in a conical flask, the elute was concentrated in Danish-Kudrna evaporator, often to the point of dryness. The method of quantification by gas chromatography requires the final volume of cleaned-up solution to be less than 5 ml. This facilitates the ready sample for injecting into the gas chromatograph for estimation of the organochlorine pesticide residues in soil samples. The schematic given in figure 3.5 provides the elution step.

Figure 3.5 : Elution of the Samples.
3.4.2 Extraction of Water Samples

Collected water samples were kept at 4°C and they were extracted according to the standard method (Mohapatra et al., 1994). Water sample, 500 ml, was taken in a beaker and to it 20 gm of sodium chloride (NaCl) was added. The mixture was shaken thoroughly, so that it became a clear mixture. After this it was partitioned three times, each being added with 50 ml of 10 % dichloromethane in hexane. The upper layer formed between water and 10 % dichloromethane in hexane was extracted in a conical flask. This organic phase was passed through a column packed with anhydrous sodium sulphate (Na₂SO₄). It was concentrated to ~5 ml. This sample was subsequently used in gas chromatograph for the estimation of pesticide residues.

3.5 Estimation of Residues

There are several methods to analyze the residues of pesticide, such as volumetric method, spectrophotometric method, chromatographic method, polarographic method and others. Chromatography (gas, thin-layer, paper) is the technique universally available and most used by the residues analyst for the qualitative separation of the complex pesticidal mixtures and their quantification. It is also a powerful aid in the identification of organic compounds. Among these chromatographic methods, the gas-liquid chromatographic (GLC) has become the universally most acceptable and used method of estimation of pesticide residues, specially for chlorinated hydrocarbon pesticides i.e. organochlorine pesticides. A great many pesticides can be detected and determined quantitatively (micro-amount of pesticides) by this procedure. GLC is a rapid, simple, highly specific and...
extremely sensitive technique, which make this technique the obvious method of choice for volatile halogenated grain insecticides, nematocides and soil fumigants such as HCH, DDT, aldrin, dieldrin, endrin, dichropropane, ethylene dibromide, methyl bromide, etc.

Electron-capture detector (ECD) is the most sensitive and useful detector for estimation of various pesticide residues. This detector exhibits a very high sensitivity to chlorinated hydrocarbon pesticides, by detecting up to the level of nanogram and picogram quantities of these compounds. But this detector is not sensitive to many other volatile or organic materials, which are invariably present in the crop extracts. However, several investigators have used the ECD for the detection of certain fungicides, herbicides, and even carbamate insecticides in various environmental constitutes (Barlas, 1999; Caldas, et al., 1999; Waliszewski, et al., 1999; Agarwal, et al., 1997; Adiroubane and Letchoumanae, 1996; Agnihotri, et al., 1996; Gold-Bouchot, et al., 1995; Neelima, et al., 1991).

In the present study the samples have been analyzed by the gas-liquid chromatographic (GLC) method. Nucon model 5740 series gas chromatograph equipped with an electron-capture detector (ECD) and packed with a glass column was used (Plate 4.1 and 4.2).
Plate-I: Photo of Gas Chromatograph

Plate-II: Photo of Gas Chromatograph Showing Capillary Column
Instrument parameters and operating conditions were as follows:

- Capillary column: 30m glass, 0.53mm i.d. X 1.5 μm df
- Oven temperature: 170 °C
- Detector temperature: 280 °C
- Injector temperature: 260 °C
- Carrier gas: Nitrogen (BOC No. 1) with a flow rate of 40 ml/min.

Gas Chromatography was calibrated each time, prior to the analysis of standards and samples. The chromatographic signals were amplified and plotted in the chromatographic sheet by computing integrator (series 3300) attached with a Epson LX-800 printer. The room temperature throughout was kept at 25±2 °C.

3.6 Preparation of Standard Curve

The standard of isomers of HCH, Aldrin and dieldrin were procured from IARI (Pusa, New Delhi). From these standards different concentrations (multiple) were made in hexane. These standards were used in gas chromatograph to get the elution profiles of standards at their respective concentrations. The values of the eluted standard samples in terms of area were noted, at each concentration. The area represents the concentration of the respective injected sample. For a given standard, concentration was plotted against the area of the eluted peak. Regression analysis was used to fit this data to a linear functional form; Area = A x Concentration [ppm]; where ‘A’ correspond to the slope of
the linear fitted line through the plotted points. These fitted standard curves (linear form) with respective fitted parameter ‘A’ were used to quantify the concentrations of respective pesticides in unknown experimental samples, soil and water. The best fitted curves, along with the estimated parameter (A), COD (coefficient of determination), standard deviation of the fit and Corrl. (correlation), are shown in figures 3.6 to 3.11. The obtained peak area of the sample was also matched with the retention time (RT) of standard curves.

3.7 Quantification of Pesticide

The first step in pesticide residues analysis is to separate a very small amount of pesticide quantitatively from a relatively large proportion of soil, water, biological material on or in which it is found. The quantity of pesticide removed by the particular extraction method is not a true measure of the actual original residue.

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\text{Conc. of Pesticide in Soil (mg/g ) = } \frac{\text{Conc. of pesticide in extract } X \text{ Vol. of extract}}{\text{Wt. of soil sample}}
\]
Standard Curves for $\alpha$, $\beta$, $\gamma$, $\delta$ - HCH

**Figure 3.6**
- Area = $A \times$ Concentration [ppm]
- $A = 7671754$
- Std. Dev = 292227
- COD = 0.960
- Corrl. = 0.986

**Figure 3.7**
- Area = $A \times$ Concentration [ppm]
- Std. Dev = 113819
- $A = 7100532$
- COD = 0.994
- Corrl. = 0.995

**Figure 3.8**
- Area = $A \times$ Concentration [ppm]
- $A = 8298038$
- Std. Dev = 171155
- COD = 0.994
- Corrl. = 0.998

**Figure 3.9**
- Area = $A \times$ Concentration [ppm]
- Std. Dev = 246002
- $A = 18435497$
- COD = 0.991
- Corrl. = 0.995
Standard Curves for Aldrin and Dieldrin

**Aldrin**

- Area $A = 17894182$
- Standard Dev. = 200572
- COD = 0.995
- Corr. = 0.999

**Dieldrin**

- Area $A = 15570290$
- Standard Dev. = 253083
- COD = 0.992
- Corr. = 0.997

Figure 3.10

Figure 3.11
3.8 Spectral Finger Printing

Analysis of samples was also confirmed with the spectrophotometer. For this 10g of soil sample was taken in a beaker and a pinch of activated carbon was added to it. Finally it was made 40g by adding n-hexane in this. Then it was stirred by magnetic stirrer and was left for 4 hours at room temperature. After 4 hours it was eluted with a column having anhydrous sodium sulphate.

The SFP method developed was used to study the samples for the presence of pesticides (Bhuwaneshawari, 1999). The samples were scanned between 200 to 700 nm wavelength range, i.e. between UV and visible range, while the hexane was used as a reference blank. For the spectral scans Hitachi - UV 2000 Spectrophotometer was used. The collected data (digitized values) of the absorbance spectra were used for Spectral finger Printing to detect the presence of pesticide. The steps involved in carrying out the spectral fingerprinting of soil samples are given in figure 3.12. The figure shows broadly the 7 steps involved in the processing of the soil samples and getting the spectral fingerprints of a sample (Attri and Bhuvneswari, 1999).
3.9 Measurement of pH, Conductivity, Density and Water Holding Capacity

In addition to the estimation of the concentrations of pesticide residues in soil and water, the pH and conductivity were measured. The water retention capacity of the soil was calculated at different depths, in different seasons. The measurement of pH and conductivity was done by using water analysis kit (Model 161E, SSS Company, India).