Methodology

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Sampling sites were selected in different parts of the Delhi region. Site selection was based on the type of land use in a particular area. However, it must be considered that distinctions between the various land-use types may sometimes not be sharp. For a fast growing city like Delhi the distinctions between urban, sub-urban and rural areas is rapidly diminishing. Global positioning system (GPS) was used for the site location. Location of the sampling sites is displayed in Figure 3.1.

3.1. Description of the sampling sites

Sampling sites were selected under following six broad categories-

i. Background sites

Two background sites namely BG01 and BG02 were selected as the background sites in the remote areas of Delhi. Site BG01 was located in the Palla village which is situated in extreme north of Delhi. Site BG02 was located in AsalatPur village of Najafgarh sub-district which is located in the south-west of Delhi. Samples were taken from the uncultivated land of these two background sites. There was no direct source of emission in the surroundings, i.e. no residential, industrial or vehicular emissions in the close vicinity.

ii. Agricultural sites

Four agricultural sites namely AG01, AG02, AG03 and AG04 were taken. Three agricultural sites were chosen in the sub-urban areas of the city and one site in the rural area of Delhi region. Site AF01 and AF02 were located in the Kadipur and Kushak villages respectively. The third agricultural site AF03 was located in Palam village. Kadipur and Kushak villages are present in the north while Palam village is located in the south of the city. Site AF04 was taken away from Delhi city in the south east direction. Wheat, paddy, jowar and vegetables were the
principal crops of these areas. Flori-culture was also practiced in these sites. These sites were primarily irrigated with tube wells and canal waters.

iii. Flood plain sites

Yamuna flood plains of Delhi region are being used for a variety of purposes, which include, dry season agriculture and temporary makeshift human settlements etc. The local people predominantly use major portion of this floodplains for practicing dry season agriculture. Major portion of the floodplain area is used for practicing seasonal agriculture of crops like wheat, cabbage, cauliflower, spinach, radish, beet root etc. in the winters and cucurbits, tomatoes, water melons etc. are grown in summers. Soils of the flood plain areas can sustain and support growth of large variety of seasonal vegetables due to their enriched nutrient status and increased soil moisture. Cucurbits like watermelon, sweet melon, and cucumber are grown exclusively on the floodplains in the Delhi. Cucurbit cultivation is practiced in about 64 ha area of the floodplains from November to June. Cucurbits require characteristic soil properties for their growth, which are present only in the floodplain areas.

To assess the PAH contamination level of flood plain soil five sites were selected spreading over 22 km river stretch in Delhi. These are: (1) Najafgarh upstream (FP01), upstream to the point, where Najafgarh drain joins Yamuna; (2) Najafgarh downstream (FP02), this sampling site is after the Najafgarh drain opens into Yamuna. Among 22 major drains carrying total quantity of city sewage to the river, Najafgarh drain is the largest one, having average discharge of about 25.65 m$^3$/sec (http://www.cpcb.delhi.nic.in/annual report2000–01). Here the river flow is slow and it is not as deep as it is at upstream of drain; (3) Income tax office (FP03), which is closely located to the centre of the city. This site was taken
near ITO Bridge; (4) Okhla (FP04); and (5) Kalindi kunj (FP05) where Yamuna leaves Delhi i.e. downstream to the city.

Figure 3.1 Map of Delhi region showing location of sampling sites
iv. Power plant site

One site PP01 was selected near Indraprastha power plant (IPP). IPP is located on the western side flood plain of the River Yamuna. It has a capacity of 285 MW comprising of three 63, one 60 and one 36 MW units. It burns about 4000 tonnes daily or 1.46 Mt/Y, of bituminous coal.

v. Traffic sites

Four traffic sites namely TR01, TR02, TR03 and TR04 were selected. Site TR01 was selected at the traffic intersection near All India Institute of Medical Sciences (AIIMS). This site has high traffic load which is shared by many flyovers at the intersection. This site holds immense importance due to its proximity to the AIIMS hospital and Safdarjung hospital. TR01 lies close to residential areas, Kidwai Nagar, Laxmi Bai Nagar and South Extension. Site TR02 was selected near Kalindi Kunj, having moderate traffic load. Site TR03 was chosen at the Income Tax Office (ITO) traffic intersection which is located close to the city center. This site had highest traffic load and congestion among the selected traffic sites. There were tall commercial buildings in the surrounding area of the site. Site TR04 was chosen near Sarai Kale Khan Inter State Bus Terminus (ISBT). This site mainly receives pollution load from heavy duty vehicles like buses and trucks coming from different states.

vi. Industrial sites

Industrial sites IA01 and IA02 were selected in two major industrial areas Okhla and Wazirpur respectively. The site IA01 was located in Okhla Industrial Estate or Phase III. Phase III is a very planned and organized industrial area. Most of the industries at IA01 concentrate on electronics, telecommunications, biotech and IT sectors. Site IA02 was selected in wazirpur industrial area. Wazirpur industrial
area consists of a huge number of open unorganized industrial units spread over the entire area. The main polluting industries in IA02 are rolling, pickling, electroplating, textiles, rubber, plastic, candle etc. It is surrounded by residential areas such as Shalimar Bagh, Ashok Vihar Azadpur Sabzi Mandi and Model Town.

3.2. Soil sampling

3.2.1. Sampling strategy

The sampling was planned to collect the soil samples from 0-5 cm depth to represent the surface soil PAH contamination. All the samples were collected over a span of one year i.e. December 2005 to December 2006. Except the traffic and industrial category, the samples from each site were collected in three seasons i.e. during winter (December), summer (April) and monsoon (August) seasons. For traffic and industrial category, seasonal variation was studied only for one site. Seasonal variation was studied only for one traffic (TR03) and one industrial (IA01) site. For the remaining traffic sites (TR01, TR02 and TR04), only winter sampling (December, 2006) was conducted.

3.2.2. Sample collection

Soil sampling was done using a stainless steel soil auger. The samples were collected at a depth of 0-5 cm. The uppermost vegetative material was carefully removed before collecting the sample. Ten to twelve samples were collected over an area of several hundred square meters. Proper care was taken to avoid the internal mixing of soil samples during collection.

3.2.3. Sample processing

In the laboratory samples were dried in the dark at room temperature. Samples were pooled and homogenized to provide a composite sample. The pebbles, plant
leaves if any were removed by hand picking as well as coarse sieving. The soil samples were gently rolled to break up large soil aggregates. Soil samples were sieved through 2 mm sieve and representative samples were obtained after quartering and coning. For quartering and coning, the soil sample was thoroughly mixed and was shaped into a cone that was then leveled into a flat circular heap. The heap was then divided diametrically into four equal quarters. Two opposite quarters were discarded and the remaining two opposite quarters were retained and formed into a second cone. The process was repeated until the four equal quarters contain the desired amount (100 gms) of sub sample.

3.3. Physico-chemical analysis

3.3.1. pH

The pH meter was calibrated with buffer solutions of pH 4.0, 7.0 and 9.2. pH of all the samples was measured by taking 1:2.5 soil and water ratio. The mixture was allowed to stand for 20 min. to get saturation. The samples were stirred during the measurement to get a representative pH of the homogenous solution.

3.3.2. Electrical Conductivity (EC)

For measuring the electrical conductivity, the conductivity meter was calibrated with 0.1N KCl solution. Conductivity of all the samples was also measured by taking 1:2.5 soil and water ratio.

3.3.3. Total organic carbon (TOC)

For total organic carbon determination, the Walkley Black method (1947) was adopted.

3.4. PAH determination

3.4.1. Chemicals
Standard mixture containing sixteen PAHs (16 compounds specified in EPA method 610) was procured from Supelco (Bellefonte, PA, USA). All solvents (toluene, n-hexane, acetonitrile etc.) used for sample processing and analysis, were of HPLC grade. High purity deionised water from Milli-Q system was used as one of the mobile phase.

3.4.2. Sample Extraction and clean-up

Soil samples were extracted by ultra-sonication, a method developed and recommended by various authors (Chen et al., 2005; Agarwal et al., 2006; Ray et al., 2007). Soil samples (10 gms) were extracted twice in 50 ml of toluene for 15 min by ultrasonic agitation (Misonix Ultrasonic Processor-XL) with a frequency of 20 KHz in a water bath (10–15°C). Both the extracts were subsequently mixed and centrifuged at 4000RPM for 20 min. The extracts were concentrated to 0.5–1.0 ml by rotary evaporator.

PAHs in the extracts were fractionated by a silica gel column (4mm i.d.). Three grams of silica gel (Silica gel 60, particle size 0.0630–0.200 mm, 70–230 mesh ASTM purchased from Merck KgaA, Darmstadt, Germany) was activated at 180°C for 24 h and kept overnight in dessicator. Before use, silica gel was deactivated with 1% water. Then 40 ml of n-hexane was added to make slurry, and was kept overnight for degassing. Concentrated sample extract was poured over the packed column. The column was first eluted with 10 ml of n-hexane and the eluate was discarded. Further elution was carried out with a 20ml mixture of hexane and toluene in 1:1 ratio to obtain PAHs fraction (Caricchia et al., 1999). The PAHs containing fraction was concentrated to 0.5–1.0 ml by using a rotary evaporator and solvent was exchanged with acetonitrile for further chromatographic analysis.
3.4.3. Analysis: Qualification and Quantification

The Waters HPLC system consisted of: Waters 510 HPLC Pump, Waters 484 Tunable absorbance detector, Waters Automated gradient controller, Waters 746 data module, Injection valve (20 μL). The PAH residue were separated by HPLC, and the individual PAHs were quantified using reference PAH standard (Supelco Inc. USA) using Waters PAH C18 column. Gradient elution was executed with acetonitrile-water mixture. The PAH detection was made on a UV detector at wavelength 254 nm.

Operating Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Waters PAH C18 (4.6 x 250 mm, 5μm-particle size)</td>
</tr>
<tr>
<td>Temperature</td>
<td>28°C ± 0.3°C</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>5 min 50%/50% acetonitrile/water, 20 min 100% acetonitrile, 28 min 100% acetonitrile, 32 min 50%/50% acetonitrile/water</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.5 ml/min</td>
</tr>
<tr>
<td>Detector</td>
<td>UV-254 nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 μl</td>
</tr>
</tbody>
</table>

The column was equilibrated for 30 min before the first analysis. All the analysis was made in duplicates.

3.4.4. External standard calibration

External standard calibration is one of the most common approaches to calibrations. It involves a simple comparison of instrument responses from the sample to the responses from the target compounds in the calibration standards. Sample peak areas (or peak heights) are compared to peak areas (or heights) of the standards. The ratio of the detector response to the amount (mass) of analyte in the
calibration standard is defined as the calibration factor (USEPA, 2003). Five point 
calibration was employed. For calibration, external standards of five concentration 
levels viz. 0.125 ppm, 0.25 ppm, 0.50 ppm, 1.0 ppm and 2.0 ppm were prepared.

Calibration factors (CF) for each compound was calculated by (USEPA, 2003):

\[
CF = \frac{\text{peak area of the compound in the standard}}{\text{mass of the compound injected (in nanograms)}}
\]

The working calibration curve, calibration factor were verified on each 
working day by preparing at least one calibration standard and assured that the 
variation was not more than ±15%. If the variation was more than 15% the test 
was repeated using a fresh calibration standard.

3.4.5. Calculations

Concentration in the sample was calculated by using the formula (USEPA, 2003):

\[
\text{Concentration (µg/kg)} = \frac{(As)(Vt)}{(CF)(Vi)(Ws)}
\]

where:

\[
\begin{align*}
\text{As} & = \text{Peak area of the analyte in the sample} \\
\text{CF} & = \text{Average calibration factor (ng}^{-1}\text{)} \\
\text{Vt} & = \text{Total volume of the concentrated extract (in µL).} \\
\text{Vi} & = \text{Volume of the extract injected (in µL).} \\
\text{Ws} & = \text{Weight of sample extracted (in grams).}
\end{align*}
\]
Using the units listed above for these terms will result in a concentration in units of ng g⁻¹, which is equivalent to μg kg⁻¹.

3.4.6. Quality control

Analytical methods were checked for the precision and accuracy. All the samples were analyzed in duplicate. Replicate analyses gave an error between ±10% to ±15%. The recovery efficiency was checked by analyzing soil samples spiked with known amount of PAH standard. Recoveries ranged from 79 to 95% for the reported PAHs in soil samples. Procedural blanks were performed periodically to prevent contamination.

3.5. Statistical analysis

Graphs of detailed statistics were plotted by using the SigmaPlot 2001 software. The Statistical Package for the Social Sciences (SPSS), version 10.0 was used to execute the Correlation exercise and Principal Component Analysis (PCA). Other statistical tests were performed by using the software GraphPad Prism 5. Map was prepared by using Golden Software Surfer 8.
Picture 1: Yamuna Flood Plain site upstream of Najafgarh drain

Picture 2: Yamuna Flood Plain site downstream of Najafgarh drain

Picture 3: Yamuna Flood Plain site near ITO bridge
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Picture 4: Power plant site during winter season

Picture 5: Power plant site during summer season

Picture 6: Yamuna Flood Plain site near Okhla
Picture 7: Yamuna Flood Plain site near Kalindi Kunj

Picture 8: Agricultural site in Kushak village

Picture 9: Traffic site near AIIMS