Materials & Methods

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3.1. Sampling Sites

Transects have been used successfully to assess the influence of urbanisation as a diffuse anthropogenic source on surrounding environment. The wind direction in Delhi is north-west to south east in summer and winter and south east to north-west in monsoon. The transect was selected such that it crossed north to south of the city. Sampling sites are shown in Fig.3.1. The sampling sites were divided into five categories on the basis of land use patterns, namely:

1. Site I: Background (Bawana City Forest)
2. Site II: Industrial (Wazirpur Industrial Area)
4. Site IV: Suburban (Vasant Kunj)
5. Site V: Rural (Mahipalpur)

3.2. Site Description

3.2.1. Site I: Background (Bawana City Forest)

Site I is a background site. It is situated in the extreme north of Delhi. Undisturbed soil samples were collected from Bawana City Forest. The vegetation in this forest was of arid nature, mainly consisting of Babool (Acacia nilotica) and Cactus (Opuntia ficus-indica). Sampling sites were located 100 metres from Bawana Road. There was no direct source of emission in the surroundings, i.e. no residential, industrial or vehicular emission in the close vicinity.

3.2.2. Site II: Industrial (Wazirpur Industrial Area)

Site II, Wazirpur Industrial Area is an industrial site. Samples were collected from Block A Wazirpur Industrial Area. This site consists of a huge number of open unorganized industrial units spread over the entire area. The sampling sites were located in the midst of small scale factories, mainly steel processing, steel polishing units and factories recycling scrap metals. The main polluting industries of Site II were rolling, pickling, electroplating, textiles, rubber plastic, candle, scrap recycling etc.
3.2.3. Site III: Urban (R. K. Puram)

Fig. 3.1. Map of India showing Delhi and location map of the sampling sites in Delhi
Site III, R. K. Puram is an urban site. It is located in the southern part of Delhi. Samples were collected from Sector 12 of R. K. Puram. Samples were taken from a small park inside a residential complex of R. K. Puram. The site could receive pollution from adjacent heavy traffic roads.

3.2.4. Site IV: Suburban (Vasant Kunj)

Site IV, Vasant Kunj (VK) is a sub-urban site. It is located in south Delhi. Sampling was done from the residential area of Sector B-10. Soil samples were collected from a vacant plot inside the Sector B-10 complex. The site might be polluted through domestic or household emissions.

3.2.5. Site V: Rural (Mahipalpur)

Site V, Mahipalpur (MHP) is a rural site. It is located in south Delhi. It was primarily a village site. The main sources of pollution could be household and agricultural activities. There was no direct source of emission in the surroundings, i.e. no industrial or vehicular emissions in the close vicinity.

3.3. Soil Sampling

3.3.1. Sample Collection

Soil samples were collected using a stainless steel auger up to a depth of 0-5 cm of the surface soil. Samples were collected for three seasons, monsoon, winter and summer from August, 2007 to June 2008. The upper most vegetative material was carefully removed before collecting the sample. Ten to twelve samples were collected over an area of several hundred square metres.

3.3.2. Sample processing

In the laboratory, the samples were dried in dark at room temperature. Samples were pooled and homogenized to provide a composite sample. Pebbles, plant debris and leaves were removed by hand picking as well as coarse sieving. The soil samples were gently rolled to break the large soil aggregates. Soil samples were sieved through 2 mm sieve and representative samples were obtained after quartering and coning.
3.3.3. Fractionation of soil samples

Soil samples were size fractionated by sieving using sequential sieve sizes from 2000 μm down to 53 μm mounted on a sieve shaker, Fritsch Analysette (Germany), Type-03.502, No. 6635. The following five fractions corresponding to five aggregates were obtained:

- 0 - 2000 μm (bulk sample) – Fraction B
- 500 μm - 2000 μm (coarse sand) – Fraction 1
- 250 μm - 500 μm (fine sand) – Fraction 2
- 53 μm - 250 μm (coarse silt) – Fraction 3
- < 53 μm (clay and silt) – Fraction 4

3.4. PAH Determination

3.4.1. Extraction of samples

Recent works (Song et al., 2002; Sun et al., 1998; Marvin et al., 1992) report that ultrasonic methods are best suited for the extraction of PAHs from sediments and soils as compared to Soxhlet extraction where long time is consumed. Therefore, soil samples were extracted by ultra-sonication, a method used by various authors (Agarwal et al., 2009; Ray et al., 2008; Zhou et al., 2000). Soil samples (approximately 10g dry weight) were extracted in 50 ml of Toluene for 15 minutes by ultrasonic agitation (Misonix 3000) with a frequency of 20 KHz in a water bath (10-15°C). After 15 minutes, the extract was decanted and another 50ml of Toluene was added freshly and then kept for sonication for another 15 minutes. These two extracts were mixed and were centrifuged at 4000 RPM for 20 minutes. The samples were filtered and then concentrated with the help of rotary evaporator (Buchi R110, Switzerland) to 0.5-2 ml.

3.4.2. Clean up of samples

PAHs in the samples 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 (procured from Merck KgaA, Darmstadt, Germany) was activated at 180°C for 24 hours, and then deactivated with 1% Milli Q water. Then 40 ml of n-Hexane was added to make slurry, and was kept overnight for degassing. After degassing the
slurry was packed in a column. Precaution was taken to avoid cracking of column. Hexane was eluted and the previously concentrated extract was transferred to the column. Further elution was done by 20 ml of 1:1 Hexane: Toluene to obtain PAHs fractions (Caricchia et al., 1999). The fraction containing PAHs were concentrated to 2ml by rotary evaporator. The sample was filtered by 0.20 µm nylon filter and then transferred to a clean vial and stored at 4°C till further analysis.

3.4.3. PAH Analysis

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), and 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.

3.4.4. Operating Condition

Column: Waters PAH C18 (4.6 x 250 mm, 5µm-particle size)
Temperature: 28°C ± 0.3°C
Mobile phase: 5 min 50%/50% Acetonitrile/water
20 min 100% Acetonitrile
28 min 100% Acetonitrile
32 min 50%/50% Acetonitrile/water
Flow rate: 1.5 ml/min
Detector: UV detector- Detection at 254 nm
Injection volume: 20 µl
The column was equilibrated for 30 min before the first analysis. All the analyses were made in duplicates and their average is reported.

3.4.5. External standard calibration

External standard method was used for the calibration. It 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 are compared to peak areas 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 (CF).

\[
CF = \frac{\text{Peak Area (or height) of the compound in the standard}}{\text{Mass of the compound injected (in nanograms)}}
\]

Standard mixture of sixteen PAHs (16 compounds specified in USEPA Method 610) in a mixture were obtained from Supelco (USA). Calibration standards of five concentration levels viz, 0.1 ppm, 0.5 ppm, 1 ppm, 2 ppm and 3 ppm of external standard were prepared by diluting to 1000 µl of volume with Acetonitrile.

The working calibration curve and 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 (USEPA, Method 8000B, 1996). Concentration (C) in the sample using the calibration factor was calculated by,

\[
C (\mu g/g) = \frac{(A_s \times V_t)}{(V_i \times CF \times W_s \times 1000)}
\]

where,

- \(A_s\) = Response for the parameter to be measured.
- \(V_t\) = Total volume of the concentrated extract (in µL).
- \(CF\) = Calibration Factor (in nanogram⁻¹)
- \(W_s\) = Amount of the sample extracted (gms).
- \(V_i\) = Volume of the extract injected (µL)
The calibration of the instrument was checked after each analytical run by analyzing 3 standard concentrations and a control standard.

3.5. Black Carbon (BC) Determination

In the present study, Black Carbon (BC) was determined by Chemothermal Oxidation method at 375°C (CTO 375) (Gelinas et al., 2001). The flowchart presented in Fig. 3.2. summarizes the major steps included in this method.

3.5.1. Mineralization

All the samples were finely ground with mortar and pestle. The samples were gently mineralized with HCl and HF. The samples were first dissolved in 1 N HCl for 30 minutes at 20°C. The residue was separated from supernatant by centrifugation and rinsed with MilliQ water. Silicate minerals were removed using two successive 12 hour treatments with a mixture of 1N HCl and 10% HF at 20°C. The solid residue was separated from supernatant by centrifugation and rinsed with MilliQ water.

3.5.2. Removal of Hydrolyzable Organic Matter (HOM)

HOM was then removed from demineralized residues using hydrolytic method. First polysaccharides were released by trifluoroacetic acid (TFA) hydrolysis. Two 2(N) TFA hydrolyses were carried out at 100°C for 3 hours in glass vials, followed by two additional ones [4(N) and 6(N) TFA respectively] at 100°C for 18 hours. Finally, the residual hydrolysable organic matter was removed with 6(N) HCl at 110°C for 24 hours. Following each hydrolysis, the supernatants were separated from the residues by centrifugation as described above. The samples were rinsed with MilliQ water after the last TFA treatment and HCl hydrolysis. The selective removal of sugars and other forms of HOM with increasing TFA concentration and reaction time was found to be an efficient approach to reduce the potential for the formation of melanoidin like material during the 6(N) HCl hydrolysis.

3.5.3. Thermal Oxidation of Non-HOM

The demineralized and HOM free samples were thermally oxidized in a muffle furnace at 375°C for 24 hours.
3.5.4. Black Carbon Analysis

Finally, BC was analyzed on CHNSO Analyzer (Euro Elemental Analyzer, Euro Vector EA3000) by combusting the samples at 1050°C.

3.5.5. Operating conditions

Column: PTFE packed GC column, 2 metre length, 8mm outer diameter

Detector: Thermal Conductivity Detector (TCD)

Software: Callidus

Carrier Gas: Helium

Carrier Gas Flow: 100 ml/min
Carrier Pressure: 89 KPa

Purge Flow: 80 ml/min

Furnace Temperature: 1050°C

GC Oven Temperature: 114°C

3.6. Total Organic Carbon (TOC) Determination

Total Organic Carbon (TOC) was determined by Walkey Black method (1947) and the results were verified on CHNSO Analyzer (Euro Elemental Analyzer, Euro Vector EA3000). Walkey Black method involves the rapid dichromate oxidation of organic matter in soil and the determination of excess dichromate using ferrous ammonium sulphate.

3.7. Quality Control

Analytical methods were checked for the precision and accuracy. All the samples were analyzed in duplicate for PAH, BC and TOC analyses. Replicate analyses gave an error between ±10% to ±15%. The recovery efficiency of PAHs 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 for PAH, BC and TOC periodically to prevent contamination.

3.8. Statistical Analyses

Box plots of detailed statistics were plotted by using the SigmaPlot 2001 software. The Statistical Package for the Social Sciences (SPSS), version 11.5 was used to execute Pearson Correlation and Principal Component Analysis (PCA). Map was prepared using Golden Software Surfer 8.