3. MATERIALS AND METHODS

3.1. Sample Collection

Ambient air quality assessment was carried out for a period of one year (December 2013–November 2014) in the Townhall, Gandipuram, Thudiyalur, Ganapathy, SITRA, D.B Road, Thekallur, SIDCO/Sundarapuram of Coimbatore city. The air sampling was conducted using Respirable Dust Sampler “Envirotech APM 460 BL”, “Fine particulate sampler Envirotech APM 550”, Carbon monoxide analyser (ECOTECH model Serinus 30) and Ozone analyser (ECOTECH model EC9810).

In the present study the air quality parameters such as Particulate Matters (PM$_{10}$ and PM$_{2.5}$), Gaseous pollutants (SO$_2$, NO$_2$, CO, O$_3$) and meteorological parameters such as temperature, humidity, wind speed and wind direction were collected over a period of twelve months (December 2013–November 2014). The ambient sampling was conducted for 8 Hours period except CO and O$_3$ (One hour duration). Totally 192 samples were measured during the study period as per CPCB guidelines.

The sampling sites I and VI (Townhall and DB Road) located at radius of 5Km (having Townhall is the center point), Ganapathy and Gandipuram (Sites IV and II) located at a distance of 10Km radius, the Sites VIII and Thudiyalur located at a distance of 15Km radius and Thekkallur (Site-VII) remains at a distance of 35Km.
3.2. DETECTION OF CARBON MONOXIDE (CO):

The concentration of Carbon monoxide was detected directly using ECOTECH model Serinus 30 carbon monoxide analyser.

3.3. DETECTION OF OZONE (O₃):

The concentration of Ozone was detected directly using ECOTECH model Ozone analyser EC9810.

3.4. EXTRACTION OF PAH:

One quarter of filter paper samples of all the sampling sites were weighed and cut into small pieces and 15ml of cyclohexane and 3ml of acetonitrile was added. The extraction was carried out by sonication for 30 to 60 minutes at room temperature. The solvent was concentrated in rotary evaporator. About 5ml of acetonitrile was added drop wise in order to prevent the samples drying out. The samples were filtered through 0.45 µm syringe filter and transferred into a tight graduated screw cap vial and the volume was noted down.

3.5. EXTRACTION OF HEAVY METALS:

The filter papers was cut into two halves, one half was taken into the beaker, 5ml of distilled water and 10ml of conc. HNO₃ (69-70% assay) was added to it, covered with watch glass, and kept for heating. The volume of solution was reduced to ~10ml. Addition of conc. HNO₃ was continued till the fumes completely disappeared and then the volume of reaction mixture reduced to ~10ml. About 2-3ml of H₂O₂ was added drop wise till the effervescence completely given out. Now 10ml of conc. HCl was added to it, heated for 10-15 minutes, filtered it, and finally made its volume up to 100ml by using distilled water in a 100ml volumetric flask. Small amount of sample taken from this 100ml acid digested sample to make 10 times diluted sample, it was filtered through 0.22µm syringe filter, and it was used for analysis of trace elements with the help of ICP-MS. The detection and quantification of trace elements was done against a range of standards (5, 10, 20, 50, 100ppb).
3.6. EXTRACTION OF PM$_{10}$ BOUNDED ORGANIC MATTER (OM) FOR COMET ASSAY:

One fourth of the filter paper samples were taken for each sampling sites and weighed. These were cut into small pieces to which 100ml methanol was added and placed in soxhlet apparatus for 30 minutes and cooled. The contents were filtered with sodium sulphate crystals to remove the impurities. The extraction was allowed to evaporate in open air.

After evaporation the content was extracted with 10ml of methanol and transferred to pre-weighed glass tubes and allowed to evaporate and the final weight of glass tubes were noted. To this 2ml of methanol was added which was transferred to pre-weighed centrifuge tubes following it to evaporate which then dissolved to appropriate amounts of Di Methyl sulfoxide (DMSO).

3.7. EXTRACTION OF PM$_{10}$ BOUNDED ORGANIC COMPOUNDS:

One half of the samples collected on PTFE filter paper was placed into Soxhlet extractor. About 300ml of Dichloromethane (DCM) (99.5% assay) was taken into 500ml round bottom flask and the temperature was set to 38°C as the boiling point of DCM is 39-40°C. The extraction with DCM was performed for 16cycles. The obtained extract was concentrated to 10ml using rotary evaporator (temperature = 38°C, rpm = 80-100 and an interval of 20 seconds), and the extract was used for chemical analysis through GC-MS.

3.8. QUANTIFICATION OF PAH:

The HPLC (Shimatzu 10A) was used for detecting the various PAHs present in the sample. The samples were injected manually; the column was 250 mm long; 0.5m diameter; temperature is 200°C placed with 5µm particle size of silica gel. A solvent gradient of acetonitrile (50% acetonitrile) and water was used within 1min, held at 60% acetonitrile for 3mins at a flow rate of 1ml/min; and a pressure psi. Then the mobile phase composition was reset to initial condition. The HPLC system was calibrated using an external standard. A standard reference material (Aqua standard USA) was used at different dilutions to obtain calibration curves for each run. A good agreement between standard and sample chromatogram obtained on a given day. A fluorescence detector was set at 240 and 380nm wavelengths, respectively, for excitation and emission. The detectors were adjusted for maximum selectivity for each PAH. Prior to actual analysis two or three injections of the liquid standard were made to ensure stabilization of the column.
3.9. QUANTIFICATION OF HEAVY METALS USING INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY (ICP-MS):

ICP-MS combines a high temperature ICP (Inductively coupled plasma) source of argon gas with a mass spectrometer. The ICP source is used to convert the atoms present in the sample into ions. These ions are then separated and detected by the mass spectrometer. ICP-MS is an analytical technique used for elemental analysis. It has detection limit as up to ppt (parts per trillion, $10^{-12}$) level. It has superior detection capability, more sensitivity, greater precision and faster as compared to atomic absorption and optical emission spectroscopy techniques, including ICP atomic emission spectroscopy (ICP-AES).

Inductively coupled plasma (ICP) is a type of plasma source in which the energy supplied by the electric currents produced by electromagnetic induction, plasma which carry an enough concentration of ions and electrons to make the gas electrically conductive. The plasma used in spectro chemical analysis must be electrically neutral, with each cation on an ion balanced by a free electron. In the plasma, the cations are almost singly charged and there are few negative ions, so there are approximately equal amounts of cations and electrons per unit volume of plasma. The temperature of plasma is very high, of the order of 1000K.

To maximize the plasma temperature (and hence ionization efficiency) and stability, the sample should be introduced through the central tube with solvent load as much as possible, and with consistent droplet sizes. A nebulizer can be used for liquid samples to remove the larger droplets, or a desolvating nebulizer can be used to evaporate most of the solvent before it reaches the plasma torch. The sample enters the central channel of ICP, evaporates and the molecules break apart, and then the constituent atoms ionize. At the temperature prevailing in the plasma a significant portion of the atoms of many chemical elements are ionized, each atom loses its most loosely bound electron to form cation. The plasma temperature has to be set to maximize ionization efficiency for elements with high first ionization energy, while minimizing second ionization (double charging) for elements that have a low second ionization energy.

Mass spectrometry (MS) is used for coupling and to detect the cations coming from the plasma through a series of cones (i.e. usually a quadrupole). The cations are separated on the basis of their mass-to-charge ratio and a MS detector receives an ion signal which is proportional to their concentration. The concentration of a sample can be determined by calibrating with an authorized reference material such as single or multi-element reference
standards. ICP-MS also furnish itself to quantitative determination through an isotope dilution, a single point method based on an isotopically upgraded standard. Alternative mass analyzers also coupled to ICP systems include double focusing magnetic-electrostatic sector systems with single and multiple collector, as well as time of flight systems (both axial and orthogonal accelerators have been used).

Plate: 13 Inductively coupled plasma mass spectrometry
(PerkinElmer SCIEX ICP-MS(Model ELANs DRC II,Toronto)

3.10. QUANTIFICATION OF PM$_{10}$ BOUNDED ORGANIC COMPOUNDS USING GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GC-MS):

GC-MS is a combined technique of two different analytical techniques, gas chromatography and mass spectrometry. It is a technique for the analysis and quantitation of organic volatile and semi-volatile compounds.

Plate: 14 Gas chromatography mass spectrometry (PerkinElmer)
Gas chromatography (GC) is used to separate mixtures into its individual components present in a sample using a temperature-controlled capillary column. Smaller molecules with lower boiling points are travel down the column more quickly than larger molecules with higher boiling point. The molecules are retained by column and then elute from the column at different times which is known as its retention time (RT). The RT is a unique and an identifying characteristic property for a given molecule.

![Schematic representation gas chromatograph](image)

**Fig:5** Schematic representation gas chromatograph

Mass spectrometry (MS) is used to detect the various components from their mass-to-charge (m/z) ratio and is the detector for GC.

![Schematic representation of a mass spectrometer](image)

**Fig:6** Schematic representation of a mass spectrometer (Peter M. van Galen., 2005)
Working of GC-MS

- Samples to be analyzed (analyte) injected into the GC through auto-sampler.
- Helium (He) gas was used as carrier gas (mobile phase) with the flow rate of 1 ml/min to carry out the analyte through chromatographic column.
- Chromatographic separation of analyte achieved on DB-5 fused-silica capillary column (30m long with 0.25mm internal diameter and 1µm film thickness).
- Column oven temperature programmed as 60°C hold for 1 minute and the temperature increased at the rate of 6°C/min to 280°C hold for 10 minute. The total run time was 47.67 minute.
- Injector and transfer line were maintained at 280°C and 280°C respectively.
- The mass selective detector was operated in electron impact mode at 70eV with an ion source temperature of 270°C.
- Data were acquired in the selected-ion-monitoring (SIM) mode.
- Data were compared with the inbuilt mass spectra library system (i.e. NIST library) of GC-MS.

3.11. MORPHOLOGICAL CHARACTERISATION OF AIRBORNE PARTICULATES BY SCANNING ELECTRON MICROSCOPE (SEM)-EDAX:

The morphological structures of the particulates are characterized using SEM. The elemental concentration of the samples were identified using EDAX.(SEM-ICON ANALYTICAL FEI QUANTA 200)(EDAX GENESIS XM4). The Scanning Electron Microscopy is a technique employed in numerous atmospherically studies (Goodarzi 2006; De La Campa et al., 2007; Fromme et al., 2008; Hiranuma et al., 2008; Campos-Ramos et al., 2009; Wilkinson et al., 2011). SEM-EDAX measurements were performed on an adhesive support. Portions of the substrate were mounted on aluminum support SEM “stubs” with double-sided tape which had a conductive graphite-based. The samples were then coated with a thin layer of gold (coating) film by electric arc high vacuum method and then analyzed by SEM.
3.12. NANO PARTICLE SIZE CHARACTERISATION OF AIRBORNE PARTICUATES BY DYNAMIC LIGHT SCATTERING:

One by fourth of the filter paper was cut into small pieces and about 20ml of the methanol was added and kept under sonication for 30 minutes. The nanosize of the particles were characterized using MALVERN nanosizer nanozs90.

3.12.1 ZETA POTENTIAL:

One by fourth of the filter paper was cut into small pieces and about 20ml of the methanol was added and kept under sonication for 30 minutes. The zeta potential of particulates are identified using MALVERN Zeta sizer Nanosizer nanozs90.
3.13. SINGLE CELL GEL ELECTROPHORESIS (COMET ASSAY):

The genotoxic effect of the organic matter present in the PM$_{10}$ of the collected samples was evaluated using Comet assay. In this study the Lung Cancer Cell lines A549, was exposed to different concentration (1µl and 2µl) of PM$_{10}$ organics for a period of 4 hours.

3.13.1. ALKALINE COMET ASSAY (pH >13):

The comet assay was performed under alkaline conditions following the procedure of Singh et al., (1988) with minor modifications. All the steps of the comet assay were conducted under yellow light to prevent the occurrence of additional DNA damage. The control and treated cells were centrifuged and resuspended in preheated 1% low melting point agarose maintained at 45°C and pipetted onto 1% normal melting point agarose precoated slides, which had been dried overnight, and covered with a coverslip. After keeping the slides on a chilled plate for 10 min for polymerization of agarose the coverslips were removed and the slides were lowered into freshly made ice-cold lysis solution (2.5 M NaCl, 100 mM EDTA-Na, 10 mM Tris, 10% DMSO, 1% Triton X-100, pH 10) and kept at 4°C in the dark for 60 min. After draining the lysis solution, the slides were placed in a home-made horizontal electrophoresis tank, containing freshly made electrophoresis buffer (300 mM NaOH, and 1 mM EDTA-Na, pH >13), for 30 min. Electrophoresis was performed in the same buffer for 25 min by applying an electric field of 25 V (0.8 V) and adjusting the current to 300 mA by slowly changing the buffer level in the tank. After electrophoresis the slides were rinsed gently with 0.4 M Tris-HCl buffer (pH 7.5) for 5 mins. This step was repeated twice. Then the slides were dried at room temperature and kept in a refrigerator in a sealed container until analysis. For staining, the slides were immersed in double distilled water for 30 mins then stained with of Ethidium Bromide and examined at 20X using a fluorescence microscope. All slides were coded and examined blindly. A total of 100 randomly selected cells from two replicate slides (50 cells per slide) were examined per sample. The Comet length, % DNA in Tail, Tail moment, were measured using Comet Score™ Version 1.5 software and used in all comparisons.

3.14. DETERMINATION OF WIND DIRECTION AND WIND SPEED:

Data on local meteorological parameters (Wind speed, Wind direction, Temperature and Humidity) were collected in all the selected sampling sites. The wind speed was measured using Lurtan AM 421 anemometer and wind direction was measured using the Lawrence and nayo wind van. The humidity was measured using the wet and dry thermo hygrometer and digital hygro clock J412TH.
Plate: 17 Respirable Dust Sampler

Plate: 18 Fine Particulate Sampler
Plate: 19 Carbon monoxide analyser

Plate: 20 Ozone analyser

Plate: 21 Trinocular inverted microscope
Plate 22 Glass micro fibre filter papers with Suspended Particulate Matter (SPM)
Plate: 23 Comet Assay