DETERMINATION OF GENOTOXIC POTENTIAL OF PARTICULATE MATTER IN HUMAN BLOOD CELLS

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BY

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Certificate

Certified that the contents and form of the thesis entitled " **Determination of Genotoxic Potential of Particulate Matter in Human Blood Cells**" submitted by Ms. Maha Zafar has been found satisfactory for the partial fulfillment of the requirement for the award of degree of Master of Science in Environmental Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The aim of present work was to identify priority polycyclic aromatic hydrocarbons (PAHs) and particulate matter (PM_{10}) concentration in Faisalabad, Pakistan, and finding the potential of PAHs to cause DNA damage. Four sites from the Faisalabad city were selected namely Chenab Chowk (CC), Government Transport Service Chowk (GTS), General Bus Stand (GBS) and Allied Chowk (AC). Average PM_{10} concentrations at these sites were 372, 283, 223 and 150 μg m^{-3}, respectively when measured with high volume air sampler and maximum concentrations were 501, 456, 625 and 271 with Casella Microdust Pro™ sampler. Ten out of 16 priority PAHs were identified using GC/MS technique. These were Naphthalene, Acenaphthylene, Acenaphthene, Flourene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, chrysene and Benzo (e) pyrene. The DNA damage was detected through Comet assay. The significant DNA damage was seen in the cells as compared to the control. The PM_{10} concentrations were higher than the EPA designated limits of 150 μg m^{-3}. So there is urgent need to reduce emissions to meet the standards.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

It has been suspected for a long time that the increased levels of pollution are the cause of ill human health (Brimblecombe, 1987). Abnormally higher concentration of pollution during 1930s-50s caused an increase in the heart diseases, higher rate of illness and death. A lot of work has been done to estimate the bad health impact on humans caused by the air pollution (CEOHA-ATS. 1996)

The health studies in different areas of the world have clearly depicted that an increase in the daily concentration of outdoor pollution due to the particles, increases the chances of mortality from heart and respiratory diseases (Bascom et al., 1996).The studies conducted in America, Canada and Europe have shown that the chances of heart diseases increase with the increased pollution levels. The higher pollution levels cause lung infections, blood clotting or coagulation, hyper viscosity syndrome and other diseases (Liao et al., 1999.)

Firstly the method set to find out the particle levels and standardizing the limits for better health condition was measuring total suspended particulates (TSPs). In 1987, the US EPA changed the standard procedure by measuring the inhalable particles instead of the measurement of TSPs. The inhalable particles include the particles of size ≤ 10 μm (U.S. EPA, 1996).

1.2 POLLUTANT CATEGORIES

The change in the natural atmospheric composition is basically caused by the consumption of fossil fuel that is consumed in different industrial activities and for transportation. A vast variety of chemicals has been introduced up till now. These are different in the composition, physical properties, mode of emissions, distance up to which they can travel and the health effect caused by them.

Table 1: Categories of air pollutants

Type of pollutants	**Components**	**Impacts on environment**	**References**
Gaseous chemicals	sulphur, carbon, nitrogen, ozone and VOCs	Respiratory and Cellular problems	(Katsouyanni, 2003)
Persistent organic	dioxins , by products of products containing	mainly deposit on soil and come in contact	(Schecter et al., 2006)

pollutants	chlorine	with plants and bioaccumulate in them	
Heavy metals	Lead, Mercury, Cadmium, Silver, Nickel, Vanadium, Chromium, Manganese	incomplete burning, wastewater discharge and different production facilities	(Schecter et al., 2006)
Particulate matter	particle having complex mixture and remain suspended in the air	Respiratory diseases, Heart diseases and genetic problems	(Poschl, 2005)

1.3 ROUTE OF EXPOSURE

The pollutants can enter in to the human body through different routes, for example, inhalation of contaminants through polluted air, ingestion through polluted food and water and through dermal contact (Thron, 1996). When these contaminants get into the general circulation they tend to accumulate in different tissues and only a little amount is removed through body by the means of excretion (Madden and Fowler, 2000).

1.4 PARTICULATE MATTER

Particulate matter can be identified as the particles that can be suspended in the air (Mcmurry et al., 2004). It includes the particles with 0.01 – 100 μm. The particulate matter can be distributed into three size modes including nucleation, ultrafine or Aitken mode (<100 nm), coagulation and accumulation mode (100-1000 nm) and coarse mode (>1 μm). The major part of road transportation in UK is mainly due to the particles of smaller size and only 20% is contributed due to larger size particles (AQEG, 2005). Emissions of both fine and ultrafine particles are expected to decrease in near future due to the serious consideration given to the mitigation measures, for example, filters for the diesel soot and substitution of the usage of fossil fuels with alternate sources (AQEG, 2012). The emissions from road transport are expected to reduce by 1.3-2% every year in the upcoming four decades (Yan et al., 2011). Different studies and research papers have presented that the health effect of coarse particles are much significant that they can't be overlooked. The special consideration should be given to each size fraction of the particulate matter and the relevant health problems. Scientists have given consideration to the particulate matter from vehicular emissions due to the suspected health impacts. These particles emitted from vehicles are referred as primary particles.

1.5 CHEMICAL COMPOSITION

The particles from the vehicular emissions are supposed to have elemental carbon along with a little concentration of metals and have a surface coating of organic carbon and sulfate. This composition can be varied with the type of engine, engine efficiency and the quality of fuel used. The overall mass accounts for 75-90% of the total carbon (Williams et al., 1989).The metals included are following with varying abundance Ca, Fe, Mg, Zn, Cr, Ni, Ba, Pb (Mattimaricq, 2007; Sarvi et al., 2011). The elemental carbon is the main component of these particles and accounts for 90% of the carbon in urban environment (Sahan et al. 2008). There are also traces of polycyclic aromatic hydrocarbons and n-alkanes present in the particulate matter (Kendall et al., 2001).

1.6 GENERAL HEALTH IMPACTS

Epidemiological studies have shown the strong linkage between exposure to particulate matter and ill health. Death, heart diseases and asthma are the most common outcomes to be said (Brunekreef and Holgate, 2002; Dockery and Pope III, 1994; Dockery et al., 1993; Le et al., 2002; Pope III et al., 1995). Recent studies from twenty larger cities have shown that the particulate matter increase in the atmosphere by $10\mu g/m^3$ will cause a 0.51 percent increase in mortality and 0.68 percent increase in deaths related with heart and respiratory diseases (Samet et al., 2000). The other ill health impacts include reduced weight of infants (Shah and bulkhari, 2011), Premature birth (Parker et al., 2008; Sapkota et al., 2012; Woodruff et al., 2003), decreased sperm motility (Selevan et al., 2000) and lung cancer mortality (Pope III et al., 2002). The factors that govern the health impacts of particulate matter other than concentration is their size which is directly linked to the penetration of the particles within the body and secondly the components from which these particles are made. Generally, when the particles are made up of components having alkaline nature, they tend to be more harmful than the particles that are made up of constituents having acidic form (Brauer et al., 1995).

1.7 MUTAGENICITY OF PAHS

The present study was designed to find out the mutagenic effects of the PAHs. There are a lot of chemicals in the atmosphere like PAHs, sulphur containing compounds, compounds having nitrogen and halogens derivatives of organic acids and metals. Some of these compounds are able to be attached with dust particles that are respireable due to the small size (Lewtas, 1990). PAHs are the most concerned one because some of them are known and some are suspected human carcinogen (Lewtas, 1993). The studies involving analyzing the impact of PAHs have shown that cytochrome P450 enzymes help binding

the PAHs after activation as electrophilic metabolites and cause DNA damage (Nishioka and Lewtas, 1992 and Donelly et al., 1990).

1.8 NATIONAL NEEDS

Vehicles are one of the most important sources causing the lead, nitrogen oxide, particulate matter with size 10 μm or less, nitrous oxide and carbon mono oxide in Pakistan. The vehicular number in Pakistan is increasing by 10% per year and is more concentrated in urban areas. The average Pakistani vehicle emits much more concentration of pollutants as compared to the USA. They emit about twenty folds more hydrocarbons, 25 folds higher carbon monoxide and 3.6 times higher concentrations of nitrous oxide in gram per kilometer (Brandon, 1995). Levels of all pollutants are higher along roadside as compared to sub urban and even industrial areas. Under the above discussed scenario it is necessary to find the exact situation of air pollution, the pollutants present, their concentrations, what could be the possible health impacts and the regulatory measures. The proposed work would help out in finding the particulate matter concentrations in Faisalabad, one of the largest cities of Pakistan and finding their genotoxic effects on human blood cells.

1.8 OBJECTIVES

Keeping in view the background information presented above, the objectives of present work were;

- Finding the particulate matter (PM_{10}) concentrations in Faisalabad city
- Analysis and quantification of PAHs that may be present in the atmosphere
- Finding the genotoxic effects of the organic extract on human blood cells

CHAPTER 2

LITERATURE REVIEW

2.1 PARTICULATE AIR POLLUTION

The atmospheric particulates are having the variety of solid and liquid particles. These particles can be manufactured by the natural sources as well as the human activities. They can have a diameter ranging from 10 μm to 10^{-3} μm (Kim et al., 2004; Wang et al., 2011). They can affect the balance of incoming and outgoing radiations of the earth as soon as the radiations enter the earth atmosphere. These radiations can be scattered or absorbed by these particles present in the atmosphere that can cause change in the visibility of the atmosphere and the temperature also, thus affecting the growth of different plants (Wang et al., 2009). Many of these particles present in urban environment are the end products of different pollutants present in gaseous state. Due to the smaller size, they have tendency to go to respiratory system along with breathing. Then they get deposited in different parts of the respiratory system and cause damage. If these particles are absorbed by the circulatory system they can be very damaging to human health (Fujii et al., 2001; Kocifaj et al., 2006).Different studies have shown that even a small increase in particulate pollution can cause increase in respiratory and circulatory diseases.

2.2 AMBIENT AIR PARTICLES

Ambient air consist of three types of particles, coarse particles having size 10 μm or less, fine particles having size 2.5 μm or less and ultrafine particles having size 0.1 μm or less. The chemical composition of the particles varies with the variation in geography, weather conditions and the source of emission. Ambient particles include inorganic compounds, black carbon and organic carbon. These particles may be very much potent to evaporation and some are semi-volatile (Harrison and Yin, 2000). These particles when combine with gases, they form aerosol. These particles are originated from both natural and human activities. The anthropogenic sources of particulate matter include dust from road and agricultural sources, tire damages, fuel combustion and constructional activities. Natural sources are wind spreading particles in the atmosphere and the forest fires. Fine particles present in the environment are generated when the gas phase compounds turn to the particles. The industry and fuel combustion is also the cause of their generation. Ultrafine particles are mainly generated from the pipeline emissions of vehicles (Zanobetti et al., 2000).

2.3 GENERAL HEALTH RISKS OF PARTICULATE MATTER

Many public health studies and lab scale experiments have correlated the air pollution with the respiratory and heart diseases that can be fatal (Anenberg et al., 2010; Karmer et al., 2010). US EPA has designated CO, O_3, Pb, NO_2, PM and SO_2 as six common pollutants (Mazzoli et al., 2010; Neher and Koenig, 1994). The particulate matter due to its small respireable size has been considered as an important factor causing morbidity and hence different laboratory scale studies have been done to determine the adverse health impacts of the particulate matter (Saldiva et al., 1995; Schwartz et al., 2001). The most common anthropogenic source through which the PM has been introduced to the urban atmosphere is the vehicular emissions and hence has gained the importance due to occupational exposure for drivers and public exposure for the people using these vehicles for transportation and road sides for daily activities (Patel et al., 2010; Zuurbier et al., 2010). The PM has potential to cause the ill health when a person remains exposed to it for a long period of time (Saldiva et al., 2010). Many epidemiological studies have shown the tenderness, abnormal activity of nervous system that affects the consciousness of a person, disturbance in the procoagulant effect and reductive oxidative stress conditions are related with the exposure to particulate matter (Li et al., 2008; Nel et al., 2006). The experiments done on animals and human volunteers have shown that particulate matter can act as a factor to initiate the respiratory and heart related diseases (Ghio et al., 2000; Nemmar et al., 2002).

2.4 LONG TERM HEALTH IMPACTS

Three American panel studies including the study of six cities by Harvard University (Dockery et al., 1993), study from American cancer society (Pope et al., 1995, 2002) and study of SMOG (Abbey et al., 1999; McDonnell et al., 2000) have shown an increased risk of mortality from the heart diseases, lung diseases and cancer caused by particulate matter. Two European panel studies have shown an increase in the heart, respiratory diseases and cancer depending on the distance of residential location from the main road. The study group includes people between the ages of 55-69 years (Clancy et al., 2002; Hoek et al., 2002). The Dublin interventional studies investigated the infant death rate risk increase with the increase in the particulate matter (Bobak and Leon, 1992; Dejmek et al., 1999). Several studies have shown an increased number of different ailments like cough and bronchitis, lung dysfunction, lymphocytosis and defected lung growth in the children (Braun-Fahrlander et al., 1997; Dockery et al., 1996; Hienrich et al., 1999)

2.5 SHORT TERM HEALTH IMPACTS

Different public health studies have shown the increase in mortality with the short term increase in particulate matter. Most of the deaths were related to the respiratory and heart diseases (Ghio et al., 2000; Nemmar et al., 2002). Most of the patients got admitted that had the history of asthma and bronchitis due to the increase in particulate matter concentration. These effects depend upon the concentration and the composition of the particles (Zanobetti et al., 2000).

2.6 POLYCYCLIC AROMATIC HYDROCARBON

Polycyclic aromatic hydrocarbons are a wide class of compounds having compounds with joined benzene rings and the unsaturated four, five or six members ring. This class of compounds includes chemicals that are semi-volatile and the chemicals having high boiling point depending upon the molecular weight and structural complexity (Harvery, 1997). PAHs are the result of incomplete combustion. Therefore, their emissions and the presence in atmosphere are obvious during past centuries due to the use of fuel for the industrial transport and other purposes. The emissions depend upon the type of fuel properties and the engine efficiency. Vehicles are the most common source of emissions (IARC, 1983).

2.7 PAHS IN PARTICULATE POLLUTION

The polycyclic aromatic hydrocarbons are formed by anthropogenic activities like as a result of incomplete combustion of carbon, oil, gas, tobacco, gasoline and wood. They can also be produced during the forest fire, burning of agricultural land and waste (Barale et al., 1991; Lee et al., 1998). These get attached to the surface of the particulate matter and are introduced to the environment. Some hydrocarbons upon exposure to the sunlight may change into oxidative form while in the presence of ozone and form some nitro-groups. However, this depends upon the chemical composition of the PAHs. When these compounds make covalent bond with DNA in humans, they cause the mutagenicity and carcinogenicity (Brender, 2008; Jeffery et al., 2006). Vehicular emissions are considered the basic source for the production of polycyclic aromatic hydrocarbons in the urban environment and approximately cause 60% of their production (Omar et al., 2002). These are present within the environment in liquid and particulate phase. Most of the PAHs are present in the atmosphere because of higher molecular weights and a little tendency to evaporate. Many of the PAHs are hazardous at genetic level, can cause DNA damage and can have potential to cause cancers in the humans (Liu et al., 2001).

2.8 HEALTH IMPACTS OF PAHS

The IARC has designated the PAHs especially the Benzo (a) pyrene as potent to cause cancer in humans (IARC, 2009). These are not direct mutagenic and cause DNA damage when are given metabolic activation (Binkova and Sram, 2004). Production of reactive oxidative species can cause damage to the human cells (Penning et al., 1999). The PAHs are present in a complex mixture in air and the effect can be enhanced by other chemicals or may be suppressed by other chemicals (Binkova, 2007). The studies analyzing the impact of PAHs have shown that cytochrome P450 enzymes help binding the PAHs after activation as electrophilic metabolite and cause DNA damage (Nishioka and Lewtas 1992; White 2002).

2.9 NON-OXIDATIVE STRESS DNA DAMAGE

The studies on the carcinogenicity of PAHs have designated them as the proven carcinogens in the animal and these are suspected to be the possible human carcinogens (IARC, 1983). It is thought that if the level of PAHs will be reduced, the chances of a person getting cancer will also be reduced but the level up to which the concentration should be decreased is yet not known due to the unavailability of the volunteers and the quantitative exposure data. A biomarker can help us to achieve these concentrations. There is an extensive range of biomarkers. For example, the concentration of PAHs in body can be detected by analyzing the body fluids, e.g. urine but this kind of study can't give the proper idea about the concentration and the related DNA damage (Strickland et al., 1996). DNA adduct formation is the most reliable bio marker as it tells about the dose response relationship, DNA damage, adsorption of the chemical, detoxification process and the DNA recovery metabolism. The metabolic activation is the process mainly through which these PAHs get attached to the DNA and damage it. The PAHs basically affect the growth regulation and the tumor suppression mechanism in the exposed one.

2.10 OXIDATIVE STRESS INDUCED GENOTOXICITY

Oxidative stress can be the root cause of the diseases like heart diseases, loss of vision due to damage on retina, dysfunction of pancreas and cancer (Halliwell and Gutteridge, 1999). Oxidative stress is the condition that promotes the formation of pro-oxidants and suppresses the formation of antioxidants (Sies 1993). Oxidative stress originates from the series of metabolic reactions, blockage of blood supply to tissues reducing oxygen supply, irritation and metabolism of xenobiotics. DNA is the most important target for reactive oxygen species in the consequence of the response to air pollution. The production of the

reactive oxygen species is considered to be the most considerable factor that leads toward the cytotoxicity caused by the diesel exhaust particles. The sources of PM related oxidative stress include creation of reactive species directly through the particles, the metal and organic compounds present in the particulate matter may initiate their production. The aromatic fraction of diesel particles is proved to have the potential to cause the oxidative stress in a dose dependent way.

2.11 RELATED STUDIES

Jung et al. (2012) conducted a study to confirm that the organic extract extracted from the particulate matter that was collected from subway tunnel (KIL-eum station) in Seoul is potentially cytotoxic and genotoxic. Chinese Hamster ovary cells were used to find out the general toxicity and human bronchial cells were used to detect specific toxicity. Two type of experiments Comet assay and micronuclei test were done to analyze the results. Particulate matter (PM10) was collected for whole month. High volume air sampler along with Teflon coated fiber filters was used as apparatus. The organic contents were extracted through soniccation using Di-chloromethane as a solvent the extract was then dissolved in the DMSO for further use. The identification and the quantification of PAHs were done by using GC/MS. When the cells were exposed with the organic extract the bronchial cells did not show any kind of cytotoxicity while the Hamster ovary cells showed a dose dependent response for cytotoxicity. As far as micronuclei test is concerned in the case of Hamster ovary cells the formation of micro nuclei was dose dependent more over the damage was 2.6 folds higher in cells not treated with S9 media as compared to those treated with S9. For human bronchial cells the micro nuclei formation was 2.8 folds higher in treated cells as compared to the controlled (Jung et al., 2012).

Another study was done to measure the concentration of particulate matter in Arafat and Muzdalifa during the pilgrimage season, finding the priority polycyclic aromatic hydrocarbons and finding their genotoxicity. The samples were taken once in a year (study consists of reading from two consecutive years 2004-2006) with the help of high volume sampler and glass fiber filters. The particulate matter was extracted using Soxhlet apparatus and acetone as a solvent the analysis of organic extract was done using GC/MS technique. Ames (salmonella mutagenicity test) and commet assay were done to find out the genotoxicity of the organic extract. DMSO and benzo (a) pyrene were used as negative and positive tests respectively. Both of the experiments showed a dose dependant relationship in the case of Ames test the number of revrants per plate was increased as the concentration of organic extract and benzo (a) pyrene given to them was increased. For

commet assay, no DNA damage was seen for DMSO at any concentration.100µg/ml of OE induced a tail moment of 4.9 and DNA damage was 15.58% while 250µg/ml induced a tail moment of 16.22 and a DNA damage of 27.53%. While the benzo (a) pyrene at the same concentration caused a tail moment of 29.17 and a DNA damage of 34.93% (Elassouli, 2007).

Genotoxic potential of organic extract was determined by analyzing the impact on DNA adduct formation, single strand DNA breakage and p^{53} protein up grading. Three sample sites Prague, Kosice and Sofia. High volume sampler was used to take sample during summers as well as winters the PM 10 concentrations were 30 time higher in winter as compared to summer and PAH concentrations were 10 folds higher. The cells used for the study were human hepatoma cells. DMSO was used as negative control and benzo (a) pyrene as positive control. DMSO showed no effect on adducts formation benzo (a) pyrene showed time dependent response 24 h caused 1189 adducts/10^8 nucleotides. The exposure to extracted organic matter caused 200 adducts/10^8 nucleotides. Damage to DNA was more in winter as compared to the summer. 25-30% tail DNA and decrease in cell viability was seen. No significant impact on p^{53} was seen when exposed to extracted organic matter.

Topinka et al. (2000) preformed studies to find out the genotoxic potential of extracted organic matter by finding the DNA adduct formation in mammalian cells. The locations used for sampling were Czech Republic and Prachatice the sampling was done by high volume sampler using pallflex 20 × 20cm. the sampling was done from October to March during the years 1993-1994 to 1996-1997. The compounds were extracted through Soxhlet procedure using dichloromethane as a solvent. Two types of cells male rat and Chinese hamster ovary cells the procedure used to check the genotoxicity of the PM10 was DNA adduct formation. The adduct formation in winter was much more high than in summer. A linear response was seen in the increase in adduct formation along with the increased concentration of organic extract. 28-40 adducts/ 10^8 nucleotides were seen in winter as compared to the 7/8 adduct/ 10^8 nucleotides in summer.

In Chiang Mai Thailand the particulate matter concentrations were measured using Teflon filters and the sampling periods were March to September 1998 and December 1998 to March 1999. Four different sites having different characteristics were selected for the sampling the filters were weighted and the concentrations were measured gravimetrically. At each site the concentration of particulate matter was higher in the winters as compared to the summer. The organic extract was obtained by sonication using dichloromethane. The extract's genotoxicity was observed using the Ames test. Extracts were directly

genotoxic in the winters and the impacts were enhanced using the S9 liver fractions (Vinitketkumnuen and Kalayanamitra, 2002).

Devi et al. (2009) examined the chances of increased chromosome level damage in the traffic wardens because of the longtime of exposure as compared to the controlled group. The sample population included traffic wardens and controlled group and were then categorized in smokers and nonsmokers. The blood cells were firstly cultured and then were allowed to examine. The genetic damage was higher in the traffic warden as compared to the controlled and even more in the group of people who are smokers.

A study was done in Brazil in order to find out the PM concentrations at 3 different locations and the effects of extract on DNA. The highest concentration was found on site with highest traffic flow. Six PAHs were analyzed in the samples. The mutagenicity was found with Salmonella/ Microsome assay at higher concentration the PAHs showed direct mutagenicity but it was enhanced by the metabolic activation (Rainho et al., 2013).

In Pakistan, a study has reported the PM concentration in Lahore, Rawalpindi, Karachi and Peshawar. These cities are densely populated. The samples were taken for 24 hours a day with the help of Grimm Model 1.109 dust monitor. The concentrations at 4 cities were found to be 198, 448, 461 and 550 $\mu g/m^3$, respectively (Alam et al., 2011).

In Jeddah, 11 sites were selected with different characteristics to find out the concentrations of particulate matter, associated EOM and their genotoxicity in human blood. The air was sampled with a high volume sampler and the EOM was identified using GC/MS technique. The highest concentration was found at the site having high diesel emissions and municipal incinerators. The EOM was found to be carcinogenic when given the S9 metabolic activations the toxicity was determined using comet assay (Elassouli et al., 2007).

MATERIALS AND METHODS

3.1 Site selection

Faisalabad city is present in the north east of the Punjab (31°25′4.8″N, 73°4′44.4″E), Pakistan. Four sites based on traffic intensity were selected for the sampling. These include Chenab Chowk, Allied Chowk, General bus stand and the Government Transport Service Chowk. The site map is given below (Figure 3.1).

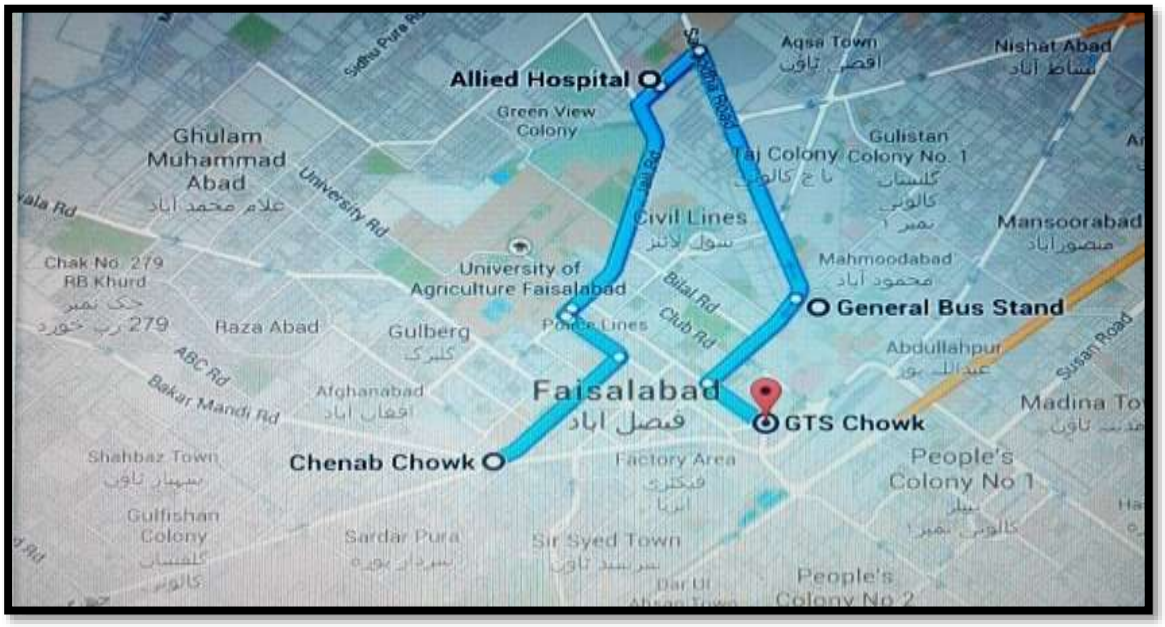

Figure 3.1: Sampling sites at Faisalabad

3.2 Air sampling equipments

The sampling was done with the help of two types of samplers, Casella Microdust Pro™ sampler (manufactured by Casella, Canada) and the high volume air sampler (Wedding and Association incorporation critical high volume sampler US patent#4,649,760). The micro pro dust sampler having the size selective adapter using poly-urethane foam filters for particulate matter (PM10). This is a portable sampler and gives the instant concentration of target particulate matter. The high volume sampler is recommended by Pak-EPA or (US-EPA) for the measurement of the particulate matter. Glass fiber filters were used to find out the concentration using gravimetric method.

3.3 Sampling with Microdust Pro™ sampler

Microdust Pro™ sampler is a portable sampler having a sampling probe, size selective adapters and suction pumps according to the desired air volume. The size selective adapter includes a cyclone type inlet and the polyurethane foam filters of desired size (10 μm and

2.5 μm). The sampler is given power through charged batteries. Once the batteries are fully charged, the sampler can operate for 24 hours. While sampling, the sampler is faced in the direction of air flow and the suction pump start sucking the set air volume. In our case, the volume rate was 1 L/min (Figure 3.2). The particles move through the inlet filtered through polyurethane foam filters go to the detector and the immediate concentrations are identified. The samples were collected three times a day in the morning, noon and evening at 8-9 am, 12-1 pm and 4-5pm.

Figure 3.2: Sampling with Microdust Pro™ sampler

3.4 Sampling with high volume sampler

High volume air sampler is designed to find out the concentration of total suspended particles and when attached with the cyclone it can measure the particulate matter of size 10μm or less. For the measurement of the PM10, Glass fiber filters were used. The filters were weighed before sampling and after the sampling on a top loaded balance (Figure 3.3). The sampler was installed at different sites for 8 hours from 8.00 in morning till 5.00 in the evening. Then the concentrations were measured by gravimetric method.

Total concentration = $\frac{W2-W1\times1000000}{Total\ volume}$

Whereas total volume is measured using the following formula;

Total volume = volume of air per minute × time of sampling

Figure 3.3: Sampling with High-volume sampler

The high volume sampler installed at GTS Chowk to take the air sample. The installation was done at 8 o'clock in morning and the sampler was stopped at 5.00 in evening.

3.5 Sonication

The extraction of the organic matter from the particulate matter was done through soniccation. The chemicals used for soniccation are described as under.

1. Dichloromethane : 70 mL/ extraction
2. Acetonitrile : 0.1mL
3. Dimethyl sulfoxide: 1 mL

The filters were sonicated in a water bath using a glass beaker. Filters were placed in a glass beaker and the extraction solvent 70 mL of dichloromethane was added to the beaker. Then the filters were sonicated for thirty minutes with a break of 5 minutes to avoid overheating and process was started again (figure 3.4). After thirty minutes the beaker was placed in open air, avoiding the sunlight. Acetonitrile was added to the beaker while the extraction solvent was about to evaporate, the rest of the extract was mixed with dimethyl sulfoxide and then were drained into the ependorff with help of a syringe. The samples were sent to the ALS Canada for the analysis. The analysis was done using Agilent 5975 GC/LRMS (Gas Chromatography coupled to Low Resolution Mass Spectrometry). The

analytical method used was as per California Air Resources Board method 429, isotope dilution technique.

Figure 3.4: Sonication of sampled filters

Sonication of the sample filter was performed using dichloromethane as an extraction solvent to extract polycyclic aromatic hydrocarbons.

3.6 Comet assay protocol

For assessment of mutagenic activity, the comet assay was used. Reagents used are as under.

Lysing solution

1. NaCl : 14.6 g
2. EDTA : 3.72 g
3. Trizma : 0.12 g
4. Distilled water : 70 mL
5. NaOH : 0.88 g
6. Ph > 10
7. 1% Triton
8. 10% DMSO

Total 100 mL of the lysing solution was made and then was stored at 4 degrees.

Electrophoresis buffer

1. NaOH : 12 g

2. EDTA : 0.75 g
3. Distilled water
4. Ph > 13

The buffer was then chilled at 4 degrees.

Neutralization buffer

1. NaOH : 6.064 g
2. Trizma Base : 12.125 g
3. Distilled water
4. Ph > 7.5

The solution was placed at 4 degrees.

LMPA

1. Low melting phosphate agarose : 10 mg
2. Distilled water : 1 mL

Slide preparation

- Frosted microscopic slides were used.
- Cover slips to cover the slides were used.
- Single layer slides were made to avoid any background disturbance.
- 0.6 mL of LMPA and 0.2 ml of blood cells were mixed and placed on slides.
- The slides were then covered and were allowed to settle down.

Processing of slides

- The slides were then placed in the lysing solution.
- It was carefully taken in view that the slides should be placed in the glass plate first and then the solution was poured.
- The slides were given overnight lysing period.
- After taking them out from the lysing solution the slides were placed in the electrophoresis buffer for 20 minutes.
- The slides were then given electrophoresis in electrophoresis tank for about 20 minutes at 15V and 155mA.
- After that the slides were taken out and were placed in the neutralization buffer for 5 minutes.
- Then the slides were stained with Ethidium Bromide.
- Were analyzed under electron microscope soon after the electrophoresis was done.

Protocol 1

- Five people exposed to the heavy traffic were selected from the sides of busy roads (figure 3.5).
- 5 mL peripheral was taken from each person.
- Soon after the collection the blood was centrifuged at 3500 rpm with the addition of histopaque.
- The final volume of white blood cells was 1 mL.
- DMSO with no reported damage was used as control.
- Naphthalene and PAHs extract were used as experimental chemicals.
- 250, 350, 450, 650 and 750 µg/ml doses were given to the cells.
- Exposure time was 18 hours.

Figure 3.5: Blood sampling

The blood sample was taken from a hawker working since 5 years at the road side.

Protocol 2

- Five healthy persons were selected from the residential area of University of Agriculture, Faisalabad, Pakistan.
- 5 mL peripheral blood was taken from each person.

- Soon after collection, the blood was centrifuged at 3500 rpm with the addition of histopaque.
- The final volume of white blood cells was 1 mL.
- The blood cells were then placed in RPMI: 1640 media.
- DMSO with no reported damage was used as control.
- Naphthalene and PAHs extract were used as experimental substances for the exposure.
- 250, 350, 450, 650 and 750µg/mL were the doses for exposure to the cells.
- Exposure time was 18 hours in an incubator.

Protocol 3

- Five healthy persons were selected from the residential area of University of Agriculture, Faisalabad, Pakistan.
- 5 mL of peripheral blood were taken from each person.
- Metabolic activation S9 was introduced to the blood sample.
- DMSO with no reported damage was used as control.
- Naphthalene and PAHs extract were used as experimental chemicals.
- Exposure time was 18 hours in an incubator.
- 100, 150, 250 and 350µg/mL were the doses for exposure to the cell.
- Then were analyzed under an electron microscope (figure 3.6).

Figure 3.6: Analysis under fluorescent microscope

Prepared slides were analyzed under electron microscope to detect the DNA damage.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Particulate Matter Levels Determined with Microdust Pro™ sampler

The concentration of the particulate matter taken from the pro dust sampler showed the same trend at each site. The concentrations were higher in the morning as compared to the concentration in noon and the evening and were 588, 319, 565 µg/m^3 at Chenab Chowk, Allied Chowk and GTS respectively. In the noon, the concentrations were lower than the morning and the evening at Chenab Chowk and government transport service Chowk and were 450 and 350 µg/m^3 in the noon and 467 and 452 µg/m^3 in the evening respectively. As compared to the general bus stand where the concentrations were higher at noon as compared to the morning and evening in noon concentrations were 755 while in evening 580 µg/m^3. At general bus stand the concentrations in the evening are a little higher than the morning. At Allied Chowk the concentrations are almost same in noon and evening and were 251 and 242 µg/m^3. The highest per day average concentrations were seen at the general bus stand that were 625µg/m^3, concentrations at Chenab Chowk were found to be the 501 µg/m^3, the GTS Chowk concentrations were detected to be 456µg/m^3, and the lowest were at allied Chowk and were found to be 271µg/m^3 (Table 2).

The differences in the concentrations are related to the traffic loads, the capacity of the roads, roads surrounded by the shops, burning of kilns, weekend or week days, precipitation and the direction of the air. These all conditions are the factors that usually control the level of particulate matter.

This is clearly depicted through the graphs that the concentration is approximately same at 3 sites excepting the allied Chowk having lowest. At general bus stand the concentrations were higher at some points due to the passing of heavy traffic. The fluctuations of the graph are because of the reason that whenever heavy fleets or traffic flow is higher the concentrations keep on increasing and a decreasing trend can be seen in other way (Figure 4.1, 4.2 and 4.3).

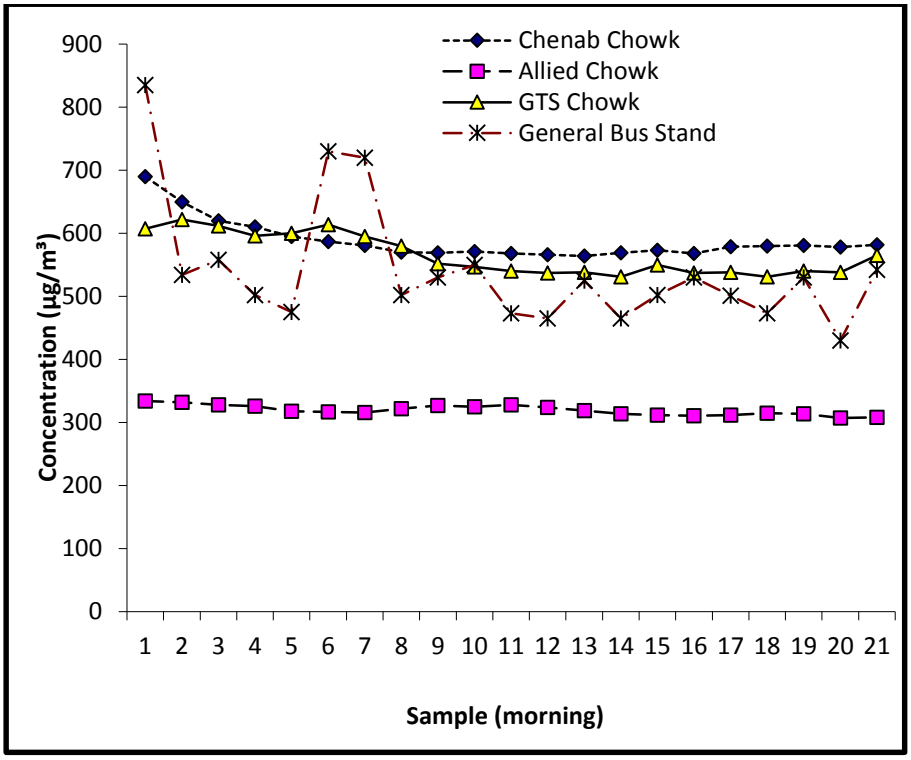

Figure 4.1: Concentrations in morning with Microdust Pro™ sampler

The concentrations in the morning were higher at each site as compared to the noon and evening the concentrations. The highest concentrations were at Chenab Chowk and the lowest were at Allied Chowk.

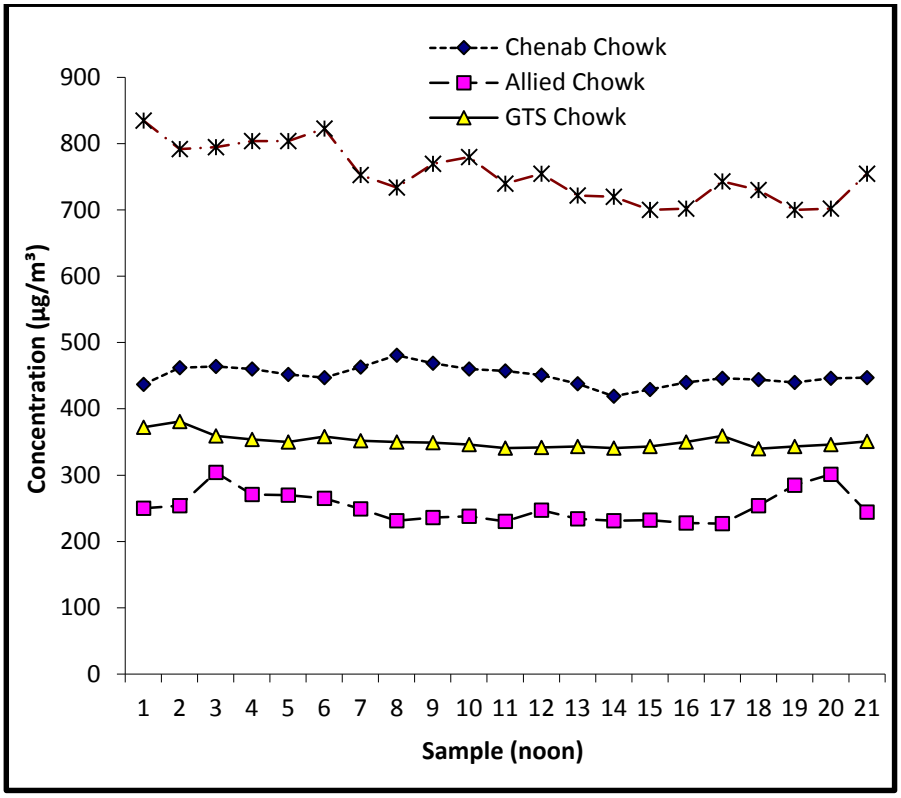

Figure 4.2: Concentrations in noon with Microdust Pro™ sampler

The concentration on each site has been decreased sharply in the noon excepting the general bus stand due to the flow of heavy traffic. The fluctuations of the graph are because of the reason that whenever heavy fleets or traffic flow is higher the concentrations keep on increasing and a decreasing trend can be seen in other way.

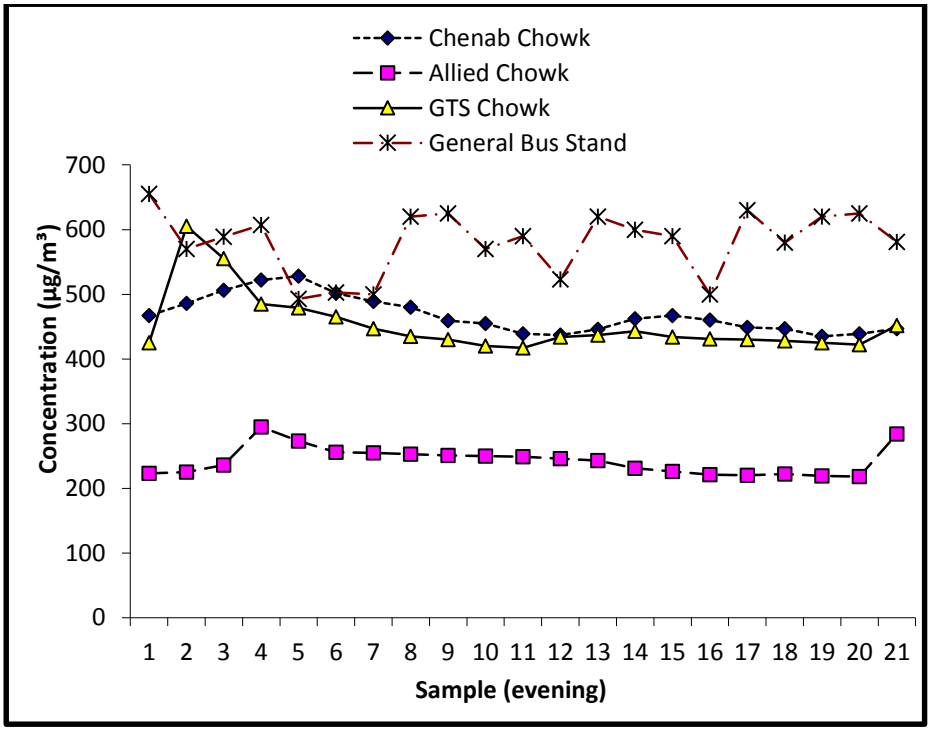

Figure 4.3: Concentrations in evening with Microdust Pro^{TM} sampler

In the evening the concentration got higher as compared to that at noon and the other trends were the same as was in the morning at Allied Chowk with lowest concentration. The fluctuations of the graph are because of the reason that whenever heavy fleets or traffic flow is higher the concentrations keep on increasing and a decreasing trend can be seen in other way.

Table 2-: Means and standard errors of the measurements taken with *Microdust Pro^{TM} sampler*

Location	Morning	Noon	Evening	Mean
Chenab chowk	588.14±6.83 b	450.10±3.12 d	467.71±6.14d	501.98±8.42B
Allied chowk	319.48±1.72 f	251.48±5.09 g	242.67±4.81g	271.21±4.96D
GTS chowk	565.24±7.07bc	350.95±2.28 e	452.33±10.22d	456.17±11.86C
GBS	541.52±21.76 c	755.19±9.14 a	580.52±10.70 b	625.7±14.54A
Mean	**503.60±13.19 A**	**451.93±20.89 B**	**435.81±14.01C**	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

4.2 Particulate Matter Levels Determined with High-Volume Air Sampler

The concentrations measured by the high volume sampler shows highest concentration at Chenab Chowk that was 372 µg/m^3, then the general bus stand that was about 283 µg/m^3,

the GTS Chowk concentrations was 233µg/m^3 and the lowest concentration at allied Chowk 150 µg/m^3. When we compared the results taken from the two different samplers we found that the difference between the measurements from two samplers 125 µg/m^3 is same at Chenab Chowk and allied Chowk having less heavy traffic as compare to the GTS and GBS and the difference between the measurements 340 µg/m^3 were same for these two sites also (Table 3).

Table 3: Concentrations taken from high volume sampler

LOCATION	WEIGHT OF FILTER		DIFFERENCE (g)	TOTAL VOLUME m^3	µg/m^3
	BEFORE (g)	AFTER (g)			
Chenab Chowk	2.81	3.10	0.25	672	372.02
Allied Chowk	2.79	2.89	0.10	672	150
GTS Chowk	2.81	2.96	0.15	672	223.21
GBS	2.83	3.02	0.19	672	283

4.3 PAHs Concentrations in Particulate Matter

The analysis of the extracts for PAHs showed the detection of almost 10 PAHsout of 16 priority pollutants designated by EPA named as Acenaphthylene, Acenaphthene, Fluorine, Phenanthrene, Anthracene, Fluoranthene, Naphthalene, Pyrene, benzo (A) anthracene, Chrysene, benzo (e) pyrene (Figure 4.4). The total amount per day detected was 2272 ng and the total amount of the PAHs per meter cube was 37ng/m^3 .The most dangerous pollutant benzo (a) pyrene was not detected in the samples. The permissible limits for the PAHs are 0.001mg/m^3. Our concentration is 0.0001 these are coming within the permissible limits as set by the EPA. The highest concentration was of naphthalene 62.34 ng that becomes 55% and the lowest were of chrysene with 1.0695 ng that becomes 0.95% of the total concentration 111.7 ng (Table 4).

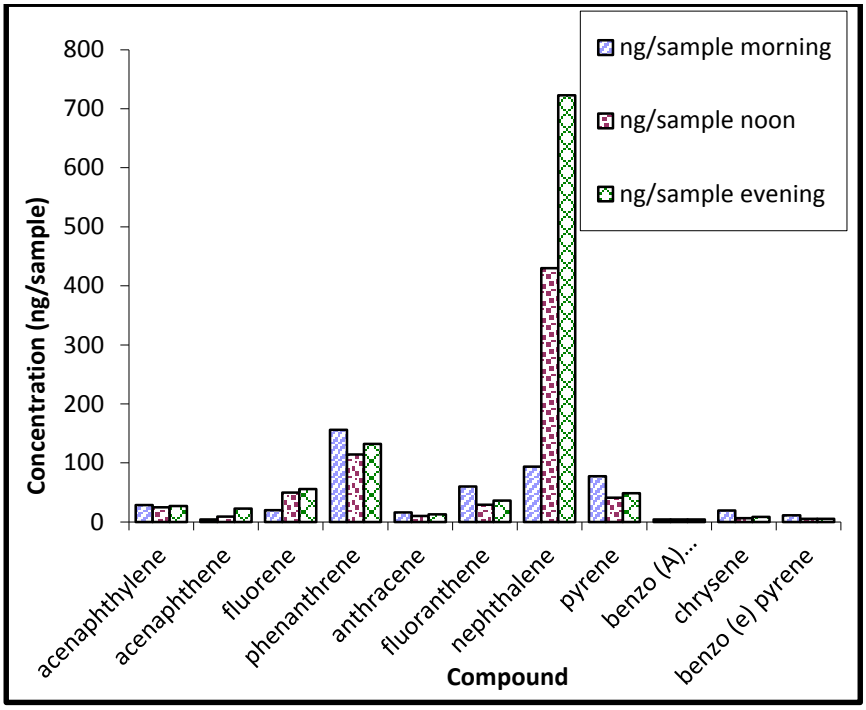

Figure 4.4: Graphical representation of the concentration of PAHs

The naphthalene was found to have the highest concentration in the extracted samples while the benzo (a) pyrene could not be detected in samples.

Table 4: Concentrations of polycyclic aromatic hydrocarbons

Compound	Concentration in morning ng/sample	Concentrations in noon ng/sample	Concentration in evening ng/sample
Acenaphthylene	28.8	26.7	24.7
Acenaphthene	< 4	22.6	9.06
Flourene	19.7	55.8	49.7
Phenanthrene	156	132	114
Anthracene	16.2	12.8	9.94
Fluoranthene	60	35.9	29.3
Naphthalene	93.8	723	430
Pyrene	77.4	48.8	41
Chrysene	19.1	8.3	6.5
Benzo (e) pyrene	11	5.4	4.98

4.4 Chromatograms for detected substances

Below given are the chromatograms for the detected substances and their concentrations in the morning, noon and evening. The higher concentrations were found for the naphthalene and the lowest for benzo (e) pyrene.

4.4.1 Naphthalene

The molar mass of naphthalene is 127.18g/mole. Below are the structural diagram (Figure 4.5) for naphthalene and the chromatograms for the concentrations at different times in a day (Figure 4.6, 4.7 and 4.8).

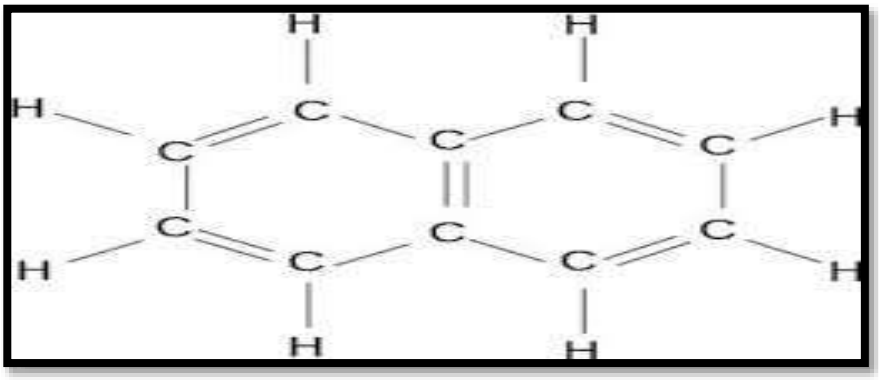

Figure 4.5: Structural diagram for naphthalene

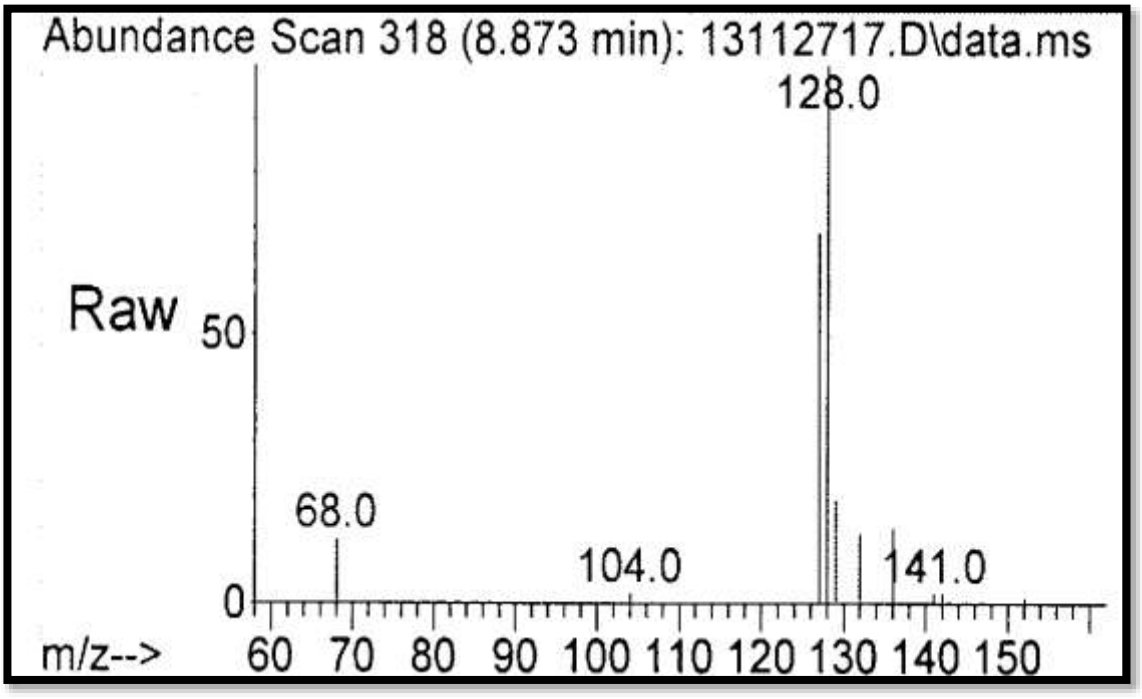

Figure 4.6: Chromatogram for naphthalene concentration in the morning

The naphthalene concentrations in the morning were detected to be 93.8 ng/sample. The peak should be at 127.18 but we can see three peaks between127 to 130. This may be due to the substitution of substituted hydrogen with the additive hydrogen. The other peaks are

may be due to the noise interruption. The peak at 128 is bigger than the 127 and 129 this shows that mostly on substituted hydrogen in the naphthalene has been substituted with the additive hydrogen. As the extract consisted mixture of polycyclic hydrocarbons there may be the reaction and side by side conversions or chemical reaction take place within the sample so in order to seize these reactions the samples were kept at -4 degrees.

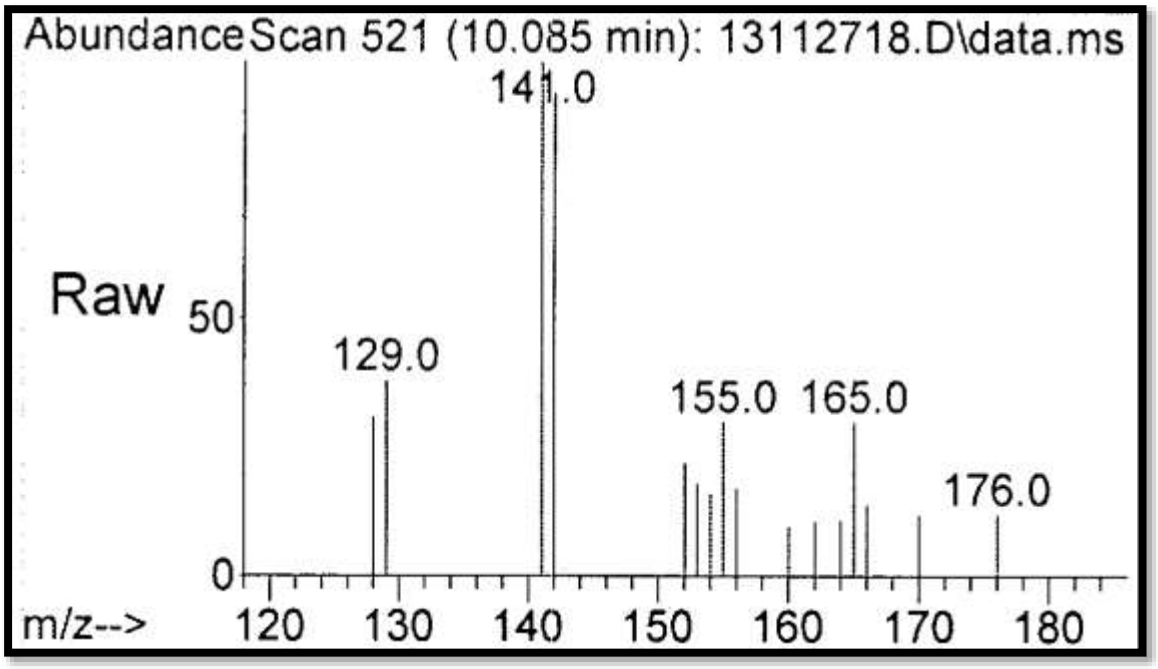

Figure 4.7: Chromatogram for naphthalene concentration at noon

The concentrations in the noon were detected to be 723ng/sample. Here we got two peaks one at 127 and the other at 129. The peak at 129 is higher than the one at 127. It depicts that may be two substitutive hydrogen atoms are exchanged with the additive ones.

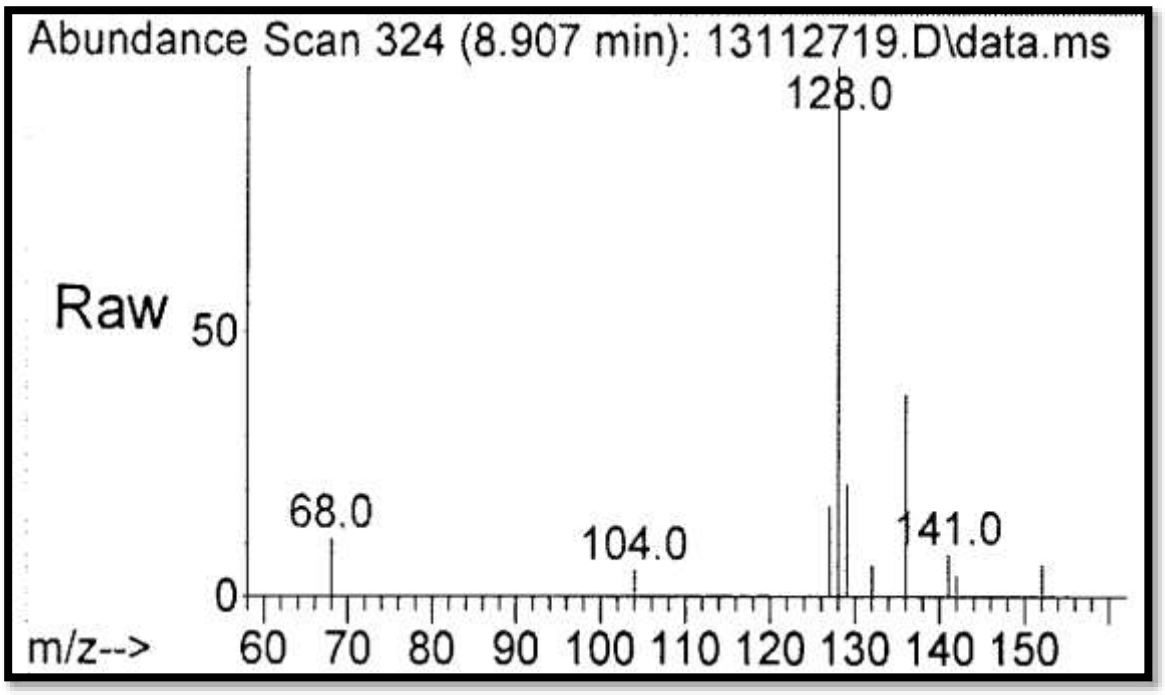

Figure 4.8: Chromatogram for naphthalene concentration in the evening

The concentrations in the evening samples were detected to be 430ng/sample. Here we found the three peaks and again the higher peak was found at 128.

4.4.2 Acenaphthylene

It has the molar mass of 152.19g/mole. Below given are the structural diagram of Acenaphthylene (figure 4.9) and chromatograms for the concentrations at different day times (Figure 4.10, 4.11 and 4.12).

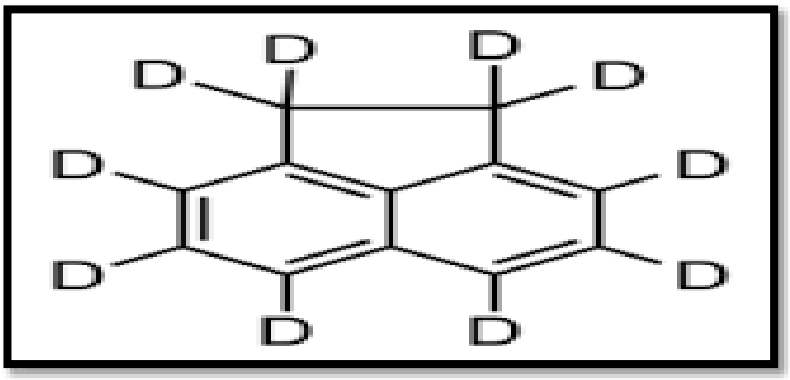

Figure 4.9: Structural diagram for Acenaphthylene

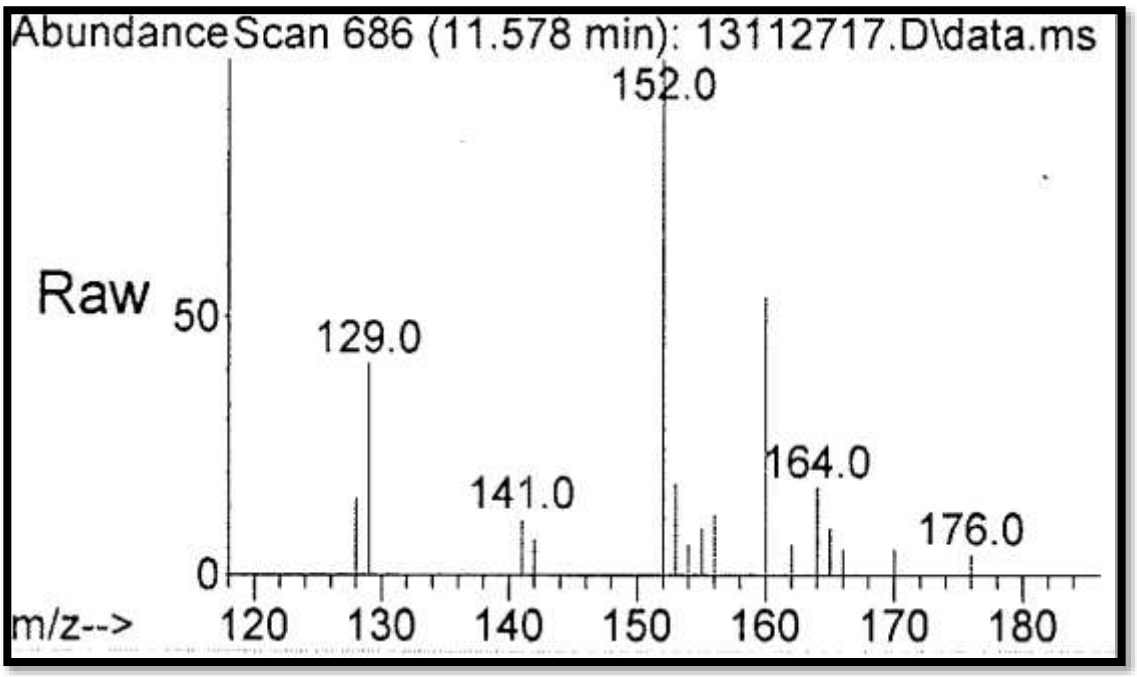

Figure 4.10: Concentrations of Acenaphthylene in the morning

The concentrations for the Acenaphthylene were found to be the 28.8ng/sample in morning the highest peak obtained is exactly at 152.

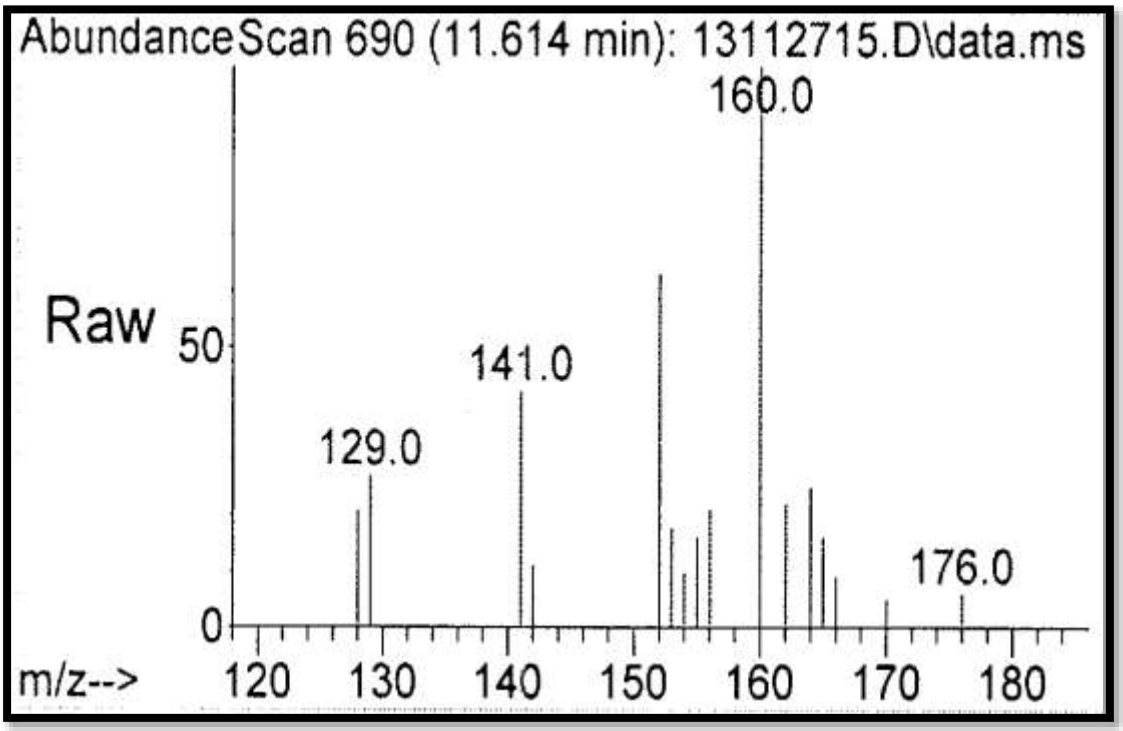

Figure 4.11: Concentrations of Acenaphthylene in noon

The concentrations in the noon were 26.7ng/sample in the noon the peak can be seen at 152. The extra peaks in chromatograms are due to the reason that the samples contained mixture of PAHs.

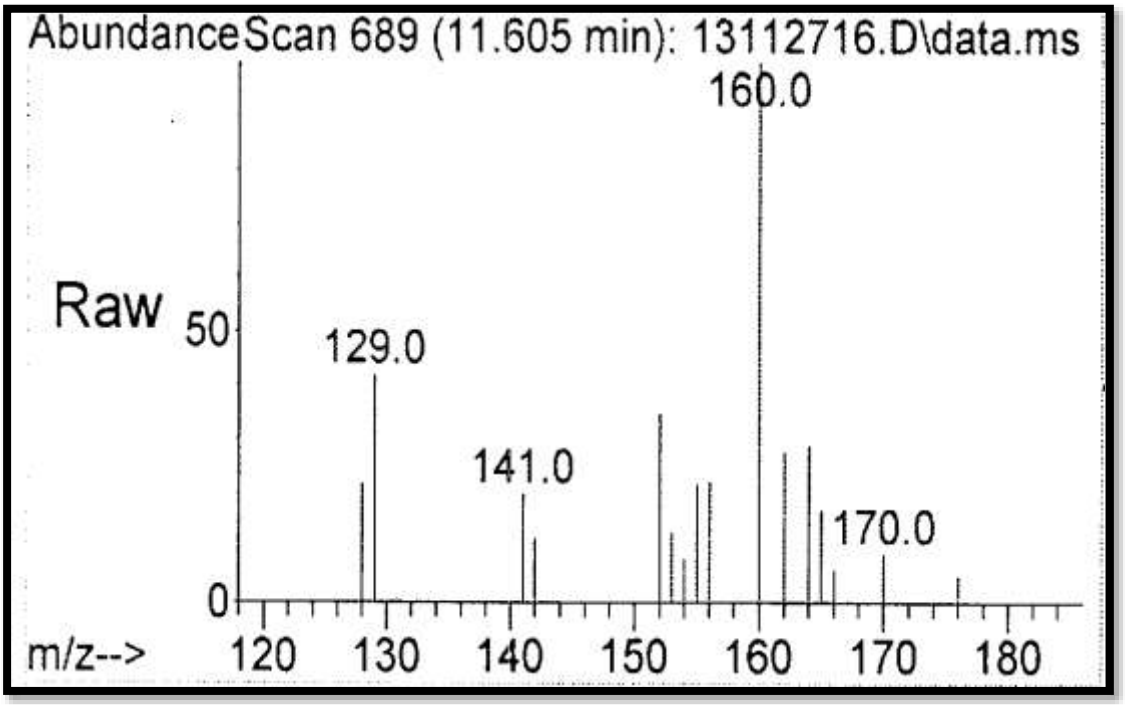

Figure 4.12: Concentrations of Acenaphthylene in the evening

The concentrations of Acenaphthylene in the evening were detected to be 24.7ng/sample we can see the peaks between 152 to 156, this may be due to the addition of hydrogen or the conversion by reacting with other substances in the mixture.

4.4.3 Acenaphthene

The molar mass is 154.21g/mole. The below given are the structural diagram (Figure 4.13) and the chromatograms for the concentrations at different times of day have been given (Figure 4.14 and 4.15).

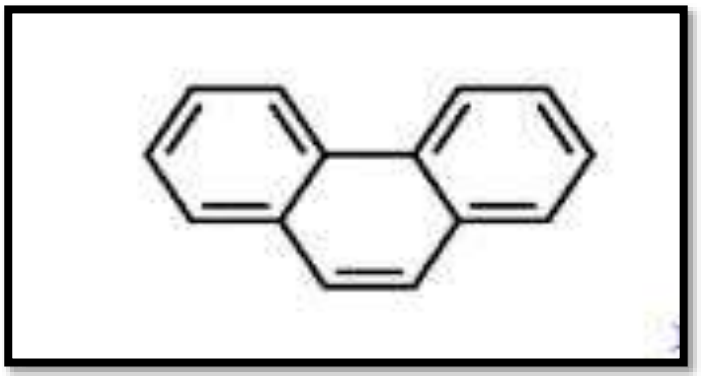

Figure 4.13: Structural diagram for Acenaphthene

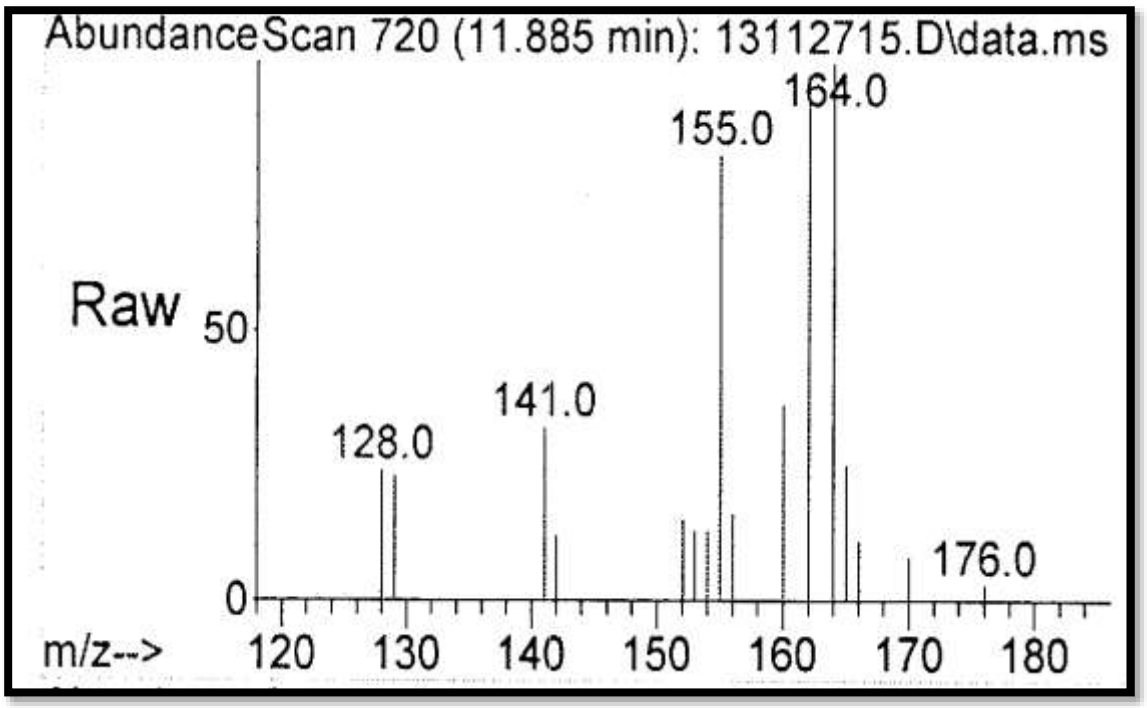

Figure 4.14: Concentration of Acenaphthene at noon

The concentration of Acenaphthene in the noon was found to be 24.6ng/sample. The peak at 154 is the peak for Acenaphthene we can see the highest peak at 155 this is may be due to the reason that the substituted hydrogen is exchanged with the additive one.

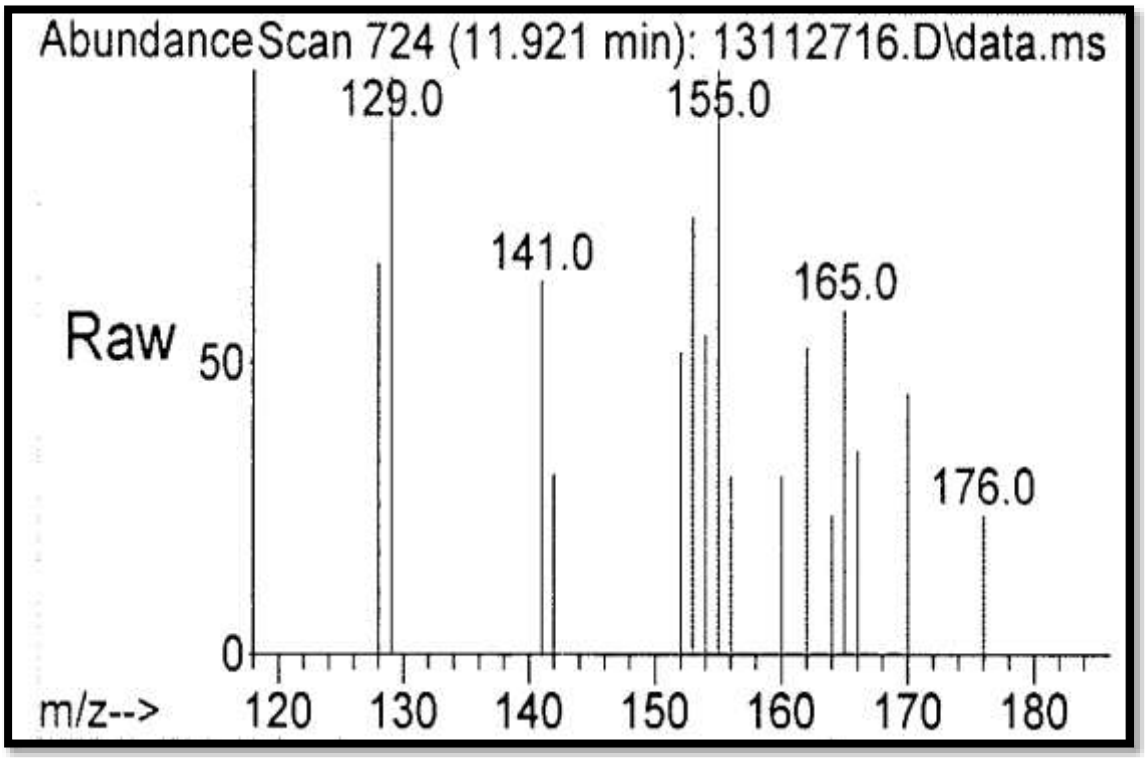

Figure 4.15: Concentration of Acenaphthene in the evening

The concentration of Acenaphthene in the evening was detected to be 9.06 ng/sample. The different peaks show that some kind of reactions may be taking place because of the reason that different PAHs are present in the samples.

4.4.4 Fluorine

The atomic mass of fluorine is 166.2g/mole. The structural diagram (Figure 4.16) and the chromatograms for the concentrations at different day times are given below (Figure 4.17, 4.18 and 4.19).

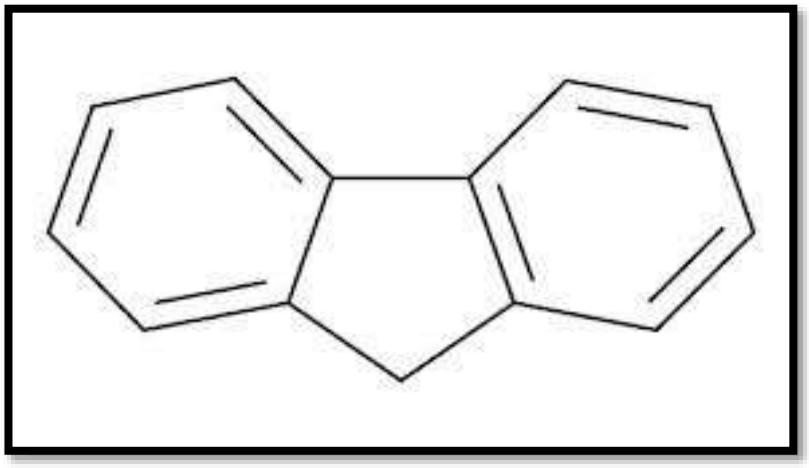

Figure 4.16: Structural diagram for flourene

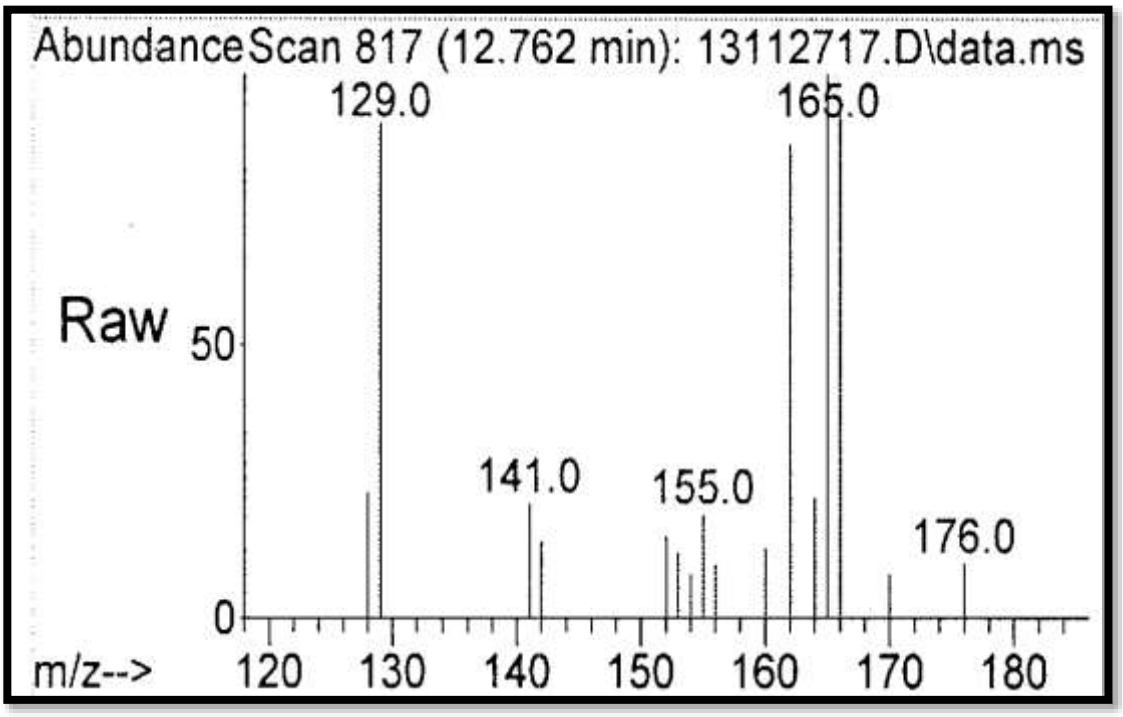

Figure 4.17: Concentration of flourene in morning

The concentrations of flourene detected in the morning were 19.7ng/sample. The peaks from 162 to 166 can be taken as the peaks for the flourene. Basically the peak should be at 166 but the peaks below 166 shows the changes in the molecule like the loss of hydrogen is the most common way to this change

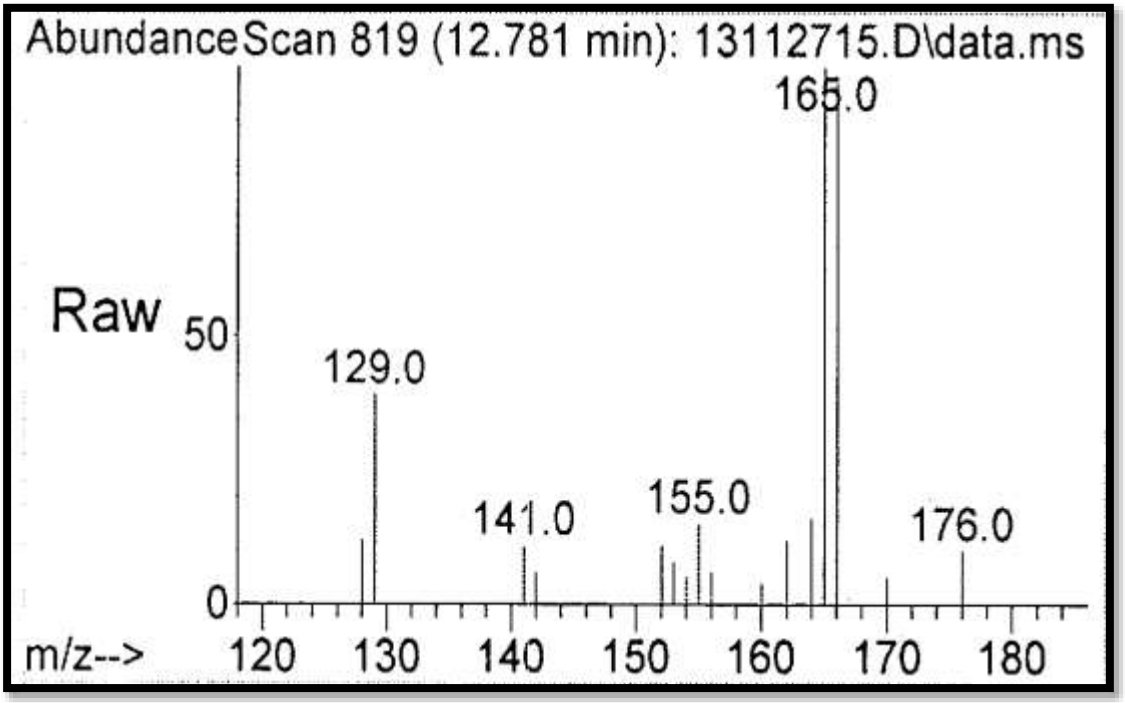

Figure 4.18: Concentration of flourene at noon

In the noon the concentrations for the flourene were detected to be 55.8ng/sample. The peaks at 165 and 166 are for the flourene and the other peaks are may be due to the noise disturbance or interference of the other compounds present in the sample. But here the degradation is less than the one in the morning sample the peak at 162 and 164 are very small.

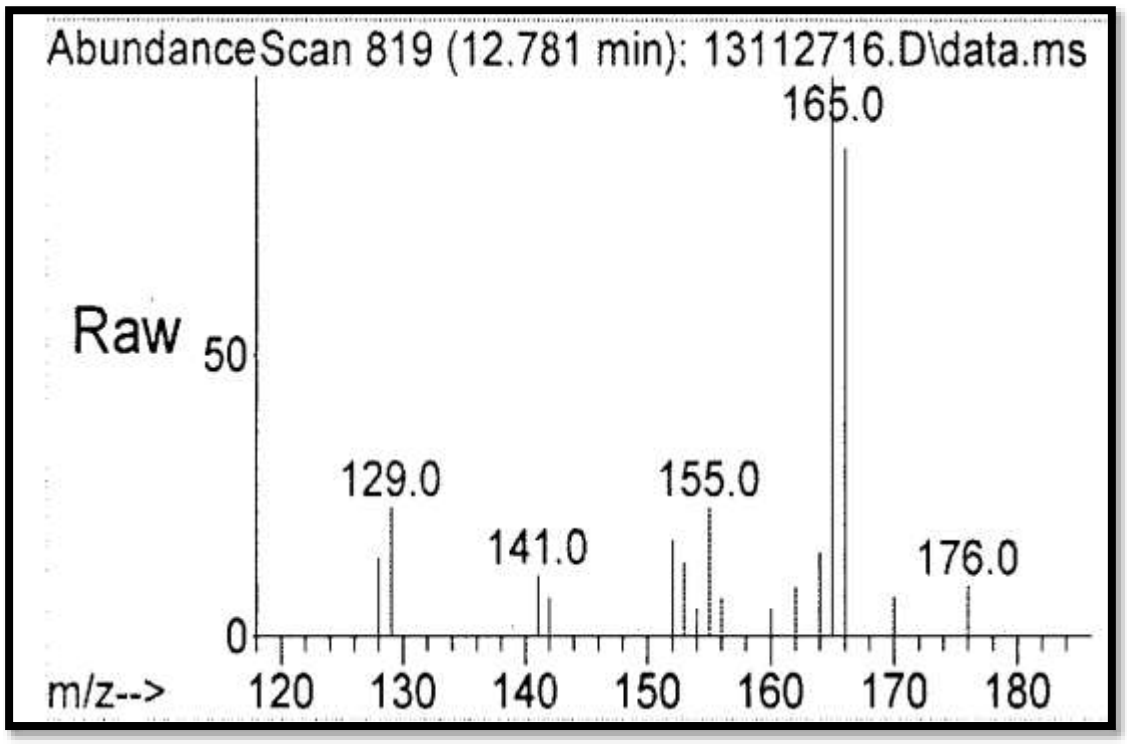

Figure 4.19: Concentration of flourene in the evening

The concentrations of the flourene in the evening samples were detected to be 49.7ng/sample. The same trend can be seen as in the noon sample the highest peak can be seen at 165 and 166 and other peaks are neglect able.

4.4.5 Phenanthrene

The molar mass of Phenanthrene is 178.23g/mole. The structural diagram (Figure 4.20) and the chromatograms for different concentrations at different times of the day are shown below (Figure 4.21, 4.22 and 4.23).

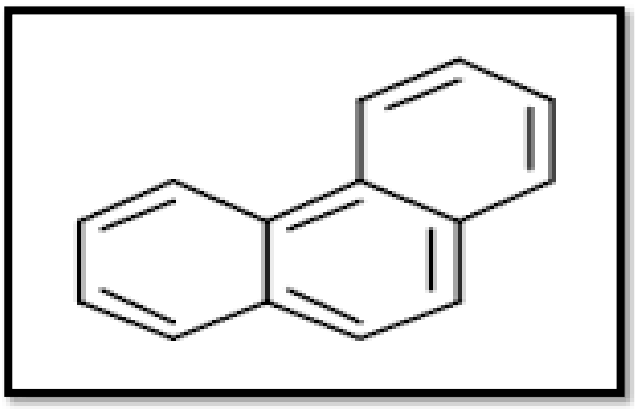

Figure 4.20: Structural diagram for Phenanthrene

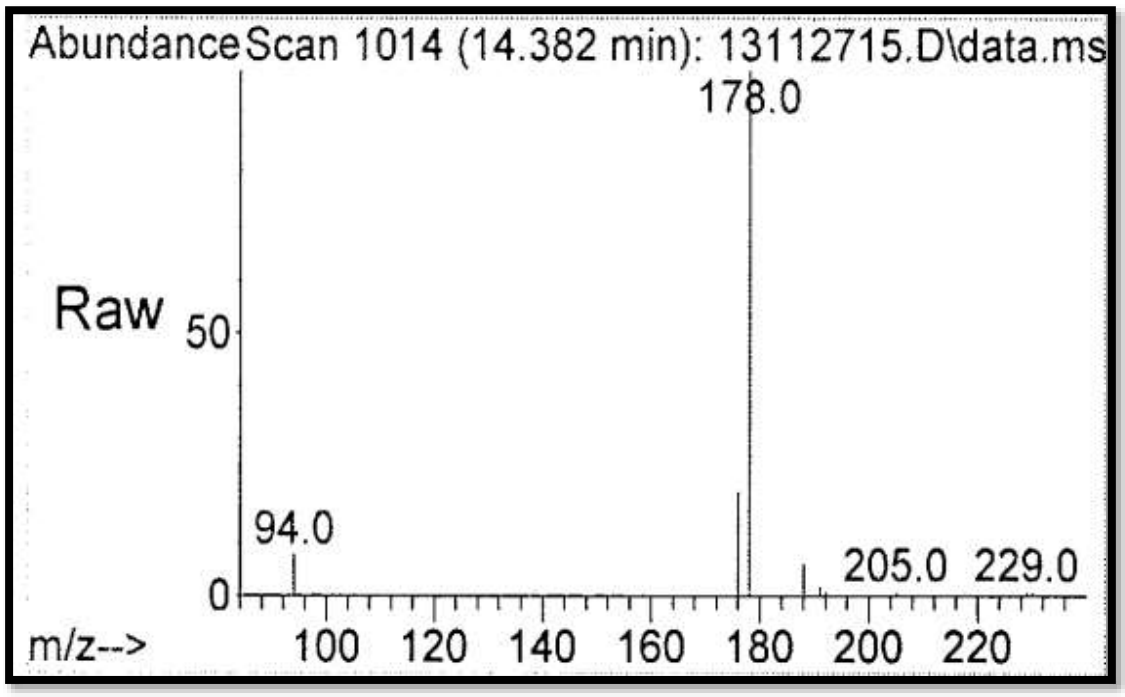

Figure 4.21: Concentration of Phenanthrene in the morning

The concentration of Phenanthrene in the morning was detected to be 156ng/sample. The highest peak can be seen at 178 only two very small peaks can be seen at 176 and 184 that are insignificant.

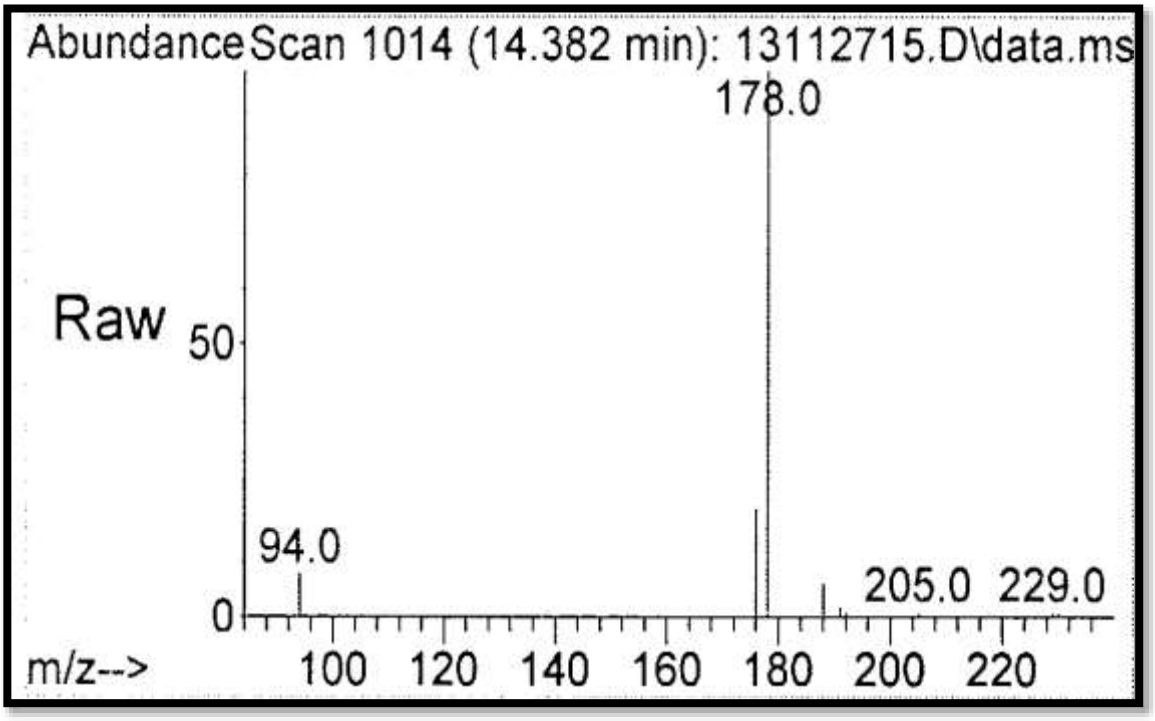

Figure 4.22: Concentration of Phenanthrene at noon

The concentrations for phenanthrene were 132ng/sample the same trend can be seen in noon sample as in morning sample.

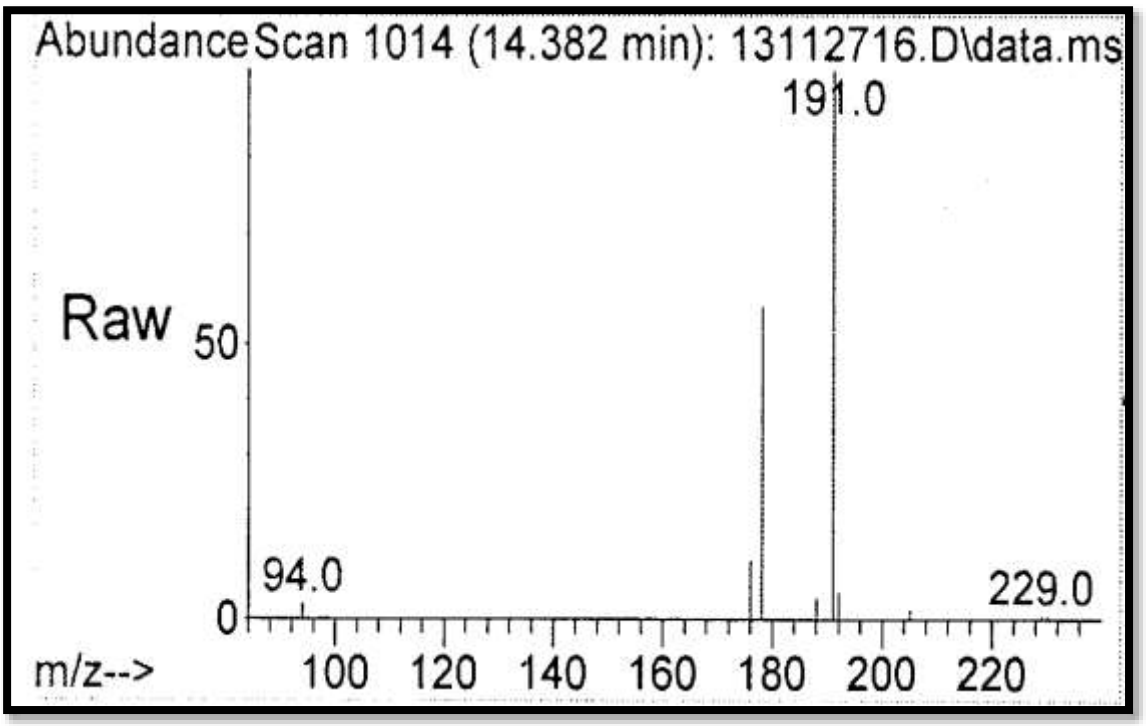

Figure 4.23: Concentration of Phenanthrene in the evening

The concentration of Phenanthrene in the evening was found to be 114ng/sample. Here the highest peak is at 191 may be due to impurity or presence of other substance.

4.4.6 Anthracene

The molar mass of Anthracene is 178.23g/mole. The structural diagram (Figure 4.24) and the chromatograms are given below (Figure 4.25, 4.26 and 4.27).

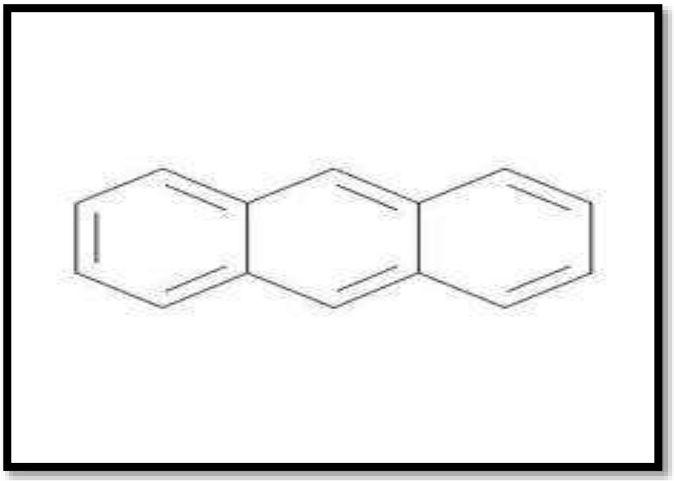

Figure 4.24: Structural formula for Anthracene

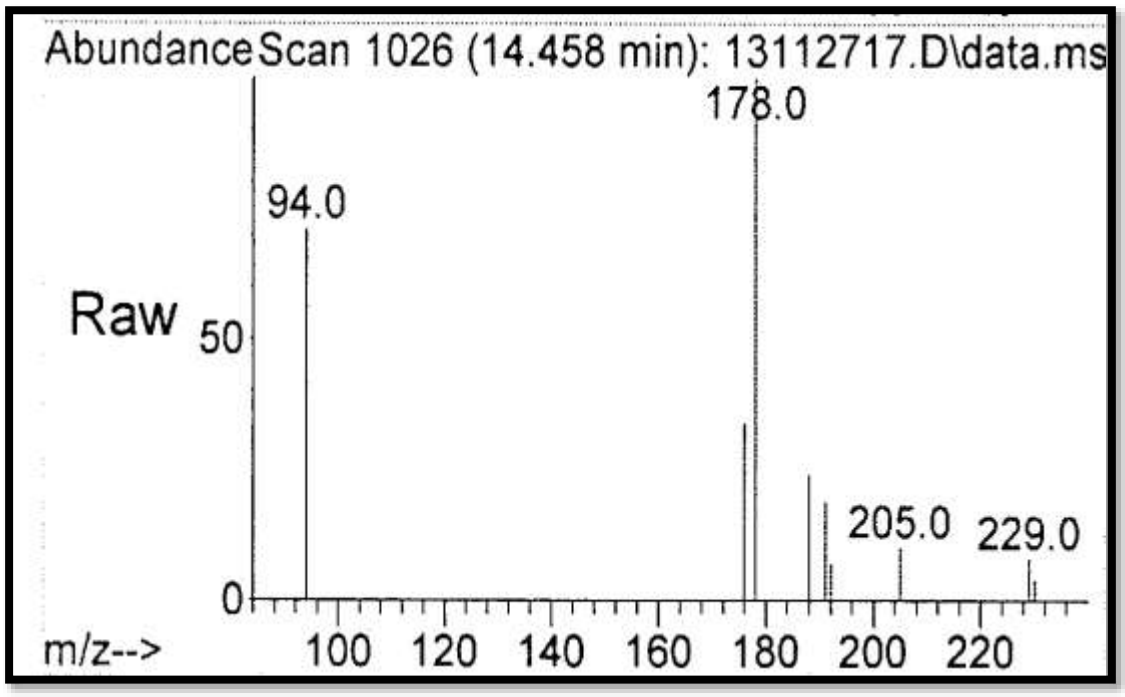

Figure 4.25: Concentration of anthracene in the morning

The concentrations of anthracene in the morning were 16.2ng/sample. The highest peak can be seen at 178 the other peaks are very small and can be neglected.

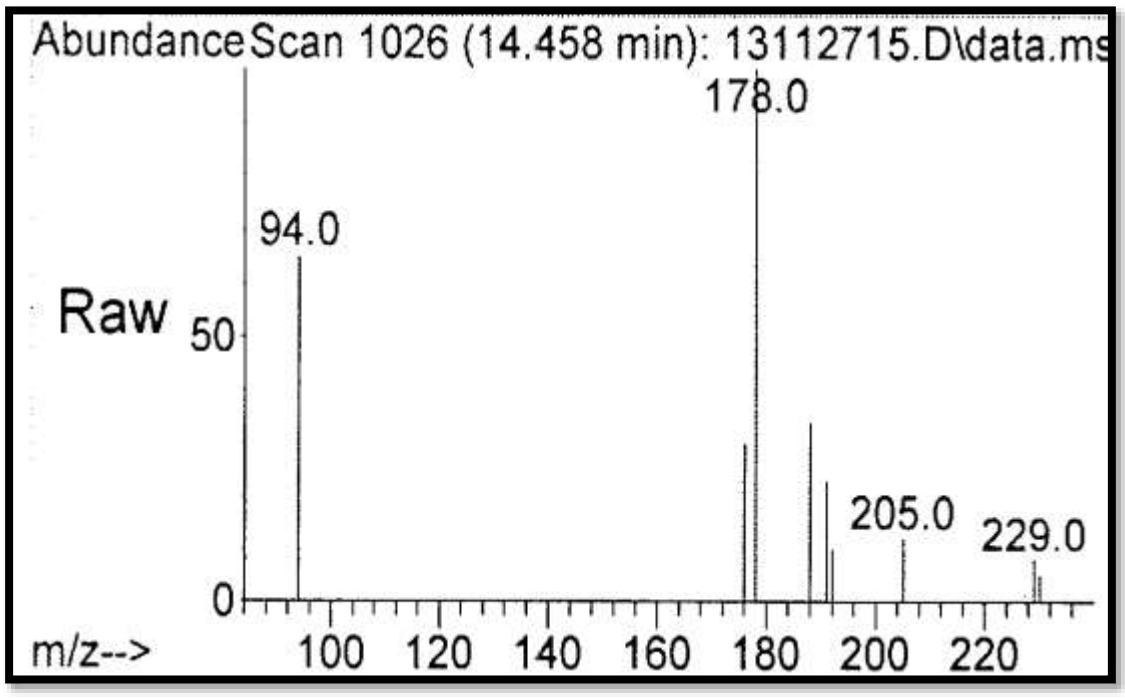

Figure 4.26: Concentration of Anthracene at noon

The concentrations of anthracene in the noon were found to be 12.8ng/sample. The highest peak at 178 is for the anthracene the other small peaks can be neglected. The peak at 94 is may be due to the noise disturbance or interference by any other compound.

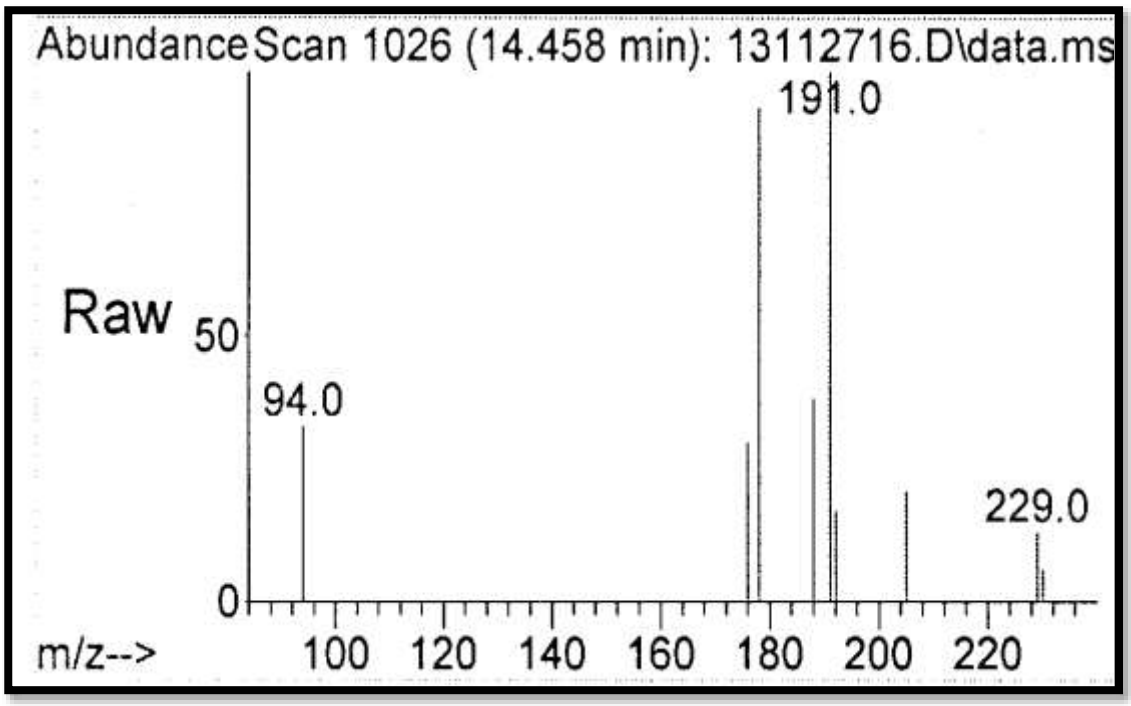

Figure 4.27: Concentration of Anthracene in the evening

The concentrations for anthracene detected in evening sample were 9.94ng/sample. Here the peak at 178 and 179 are for anthracene. Some small peaks can be neglected and are may be due to the noise disturbance but the highest peak at 191 can be due to the interference from other compounds.

4.4.7 FLUORANTHENE

The molar mass of fluoranthene is 202.6g/mole. The structural diagram (Figure 4.28) and the chromatograms are given below (Figure 4.29, 4.30 and 4.31).

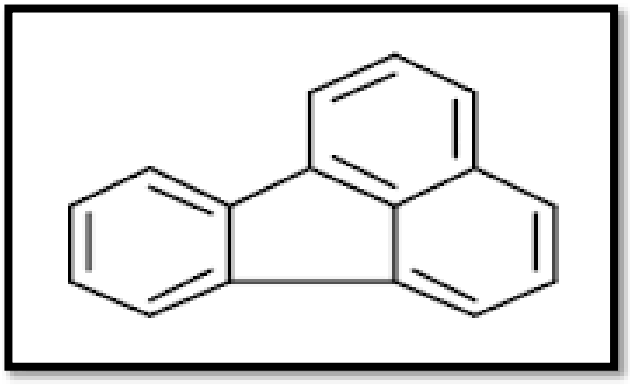

Figure 4.28: Structural diagram for Fluoranthene

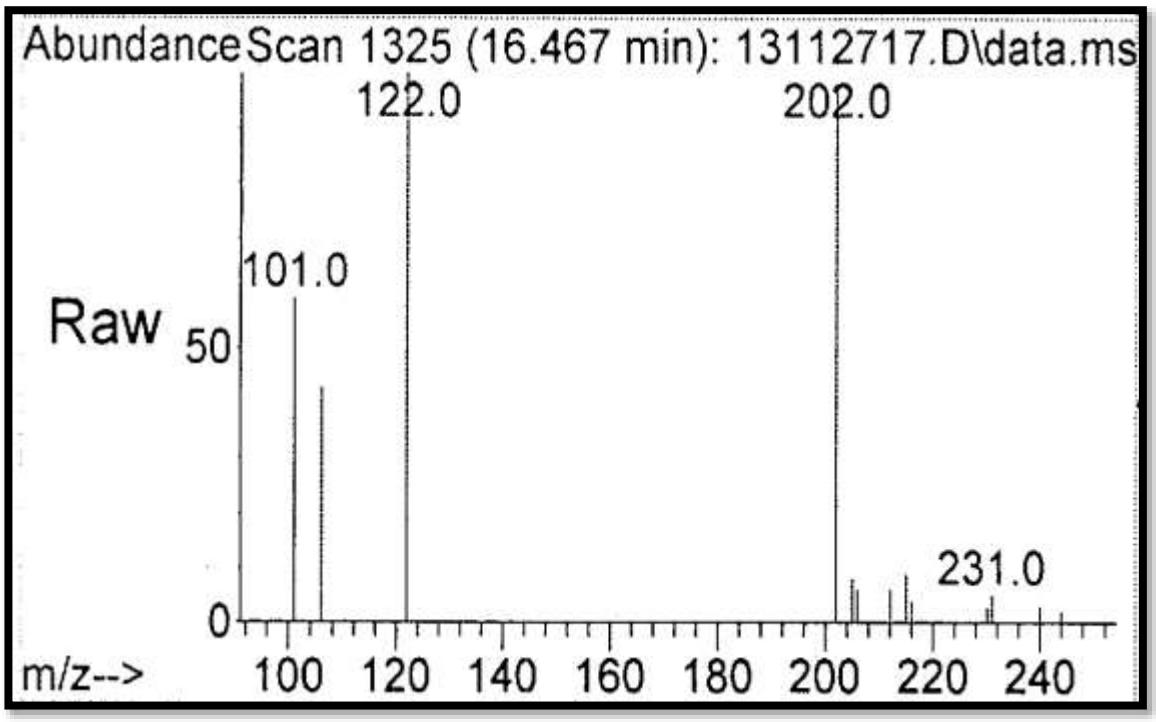

Figure 4.29: Concentration of Fluoranthene in the morning

The concentrations of fluoranthene were found to be 60ng/sample the peak at 202 is showing the presence of fluoranthene. The other high peaks at 122 and 101 are due to the interference by other compounds.

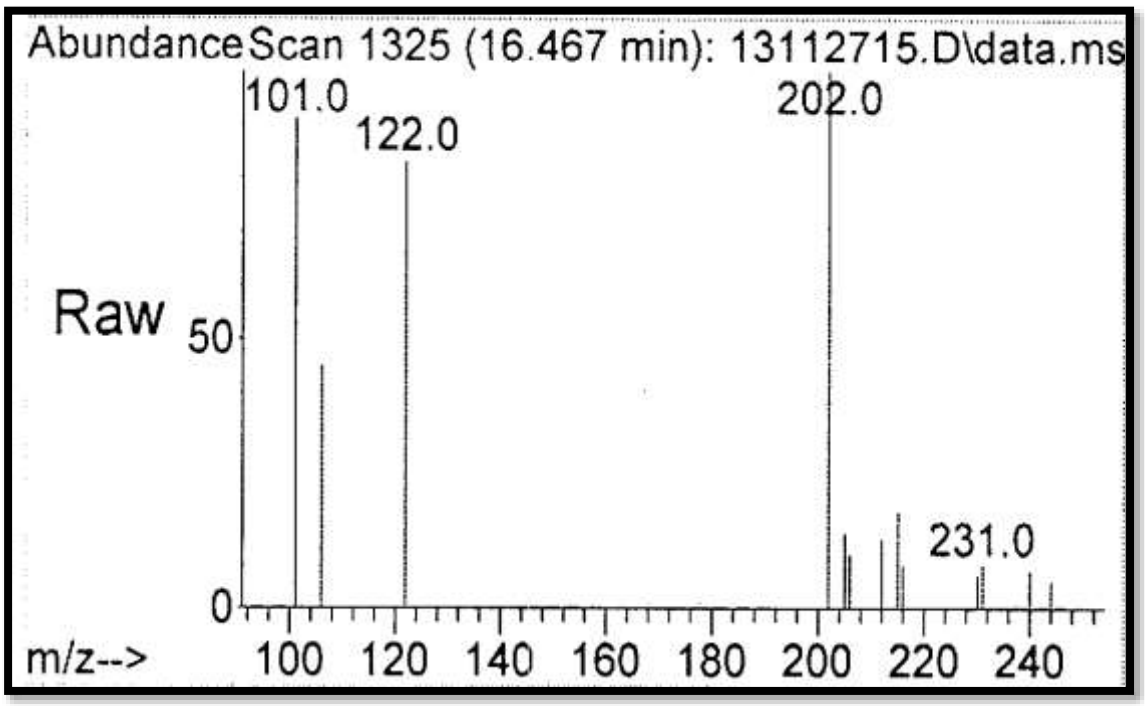

Figure 4.30: Concentration of Fluoranthene at noon

The concentrations in noon were 35.9 ng/sample. The peak at 202 is for fluoranthene the peaks at 101 and 122 are due to the interference by other compound already present in compound or form with interaction among different compound within the sample.

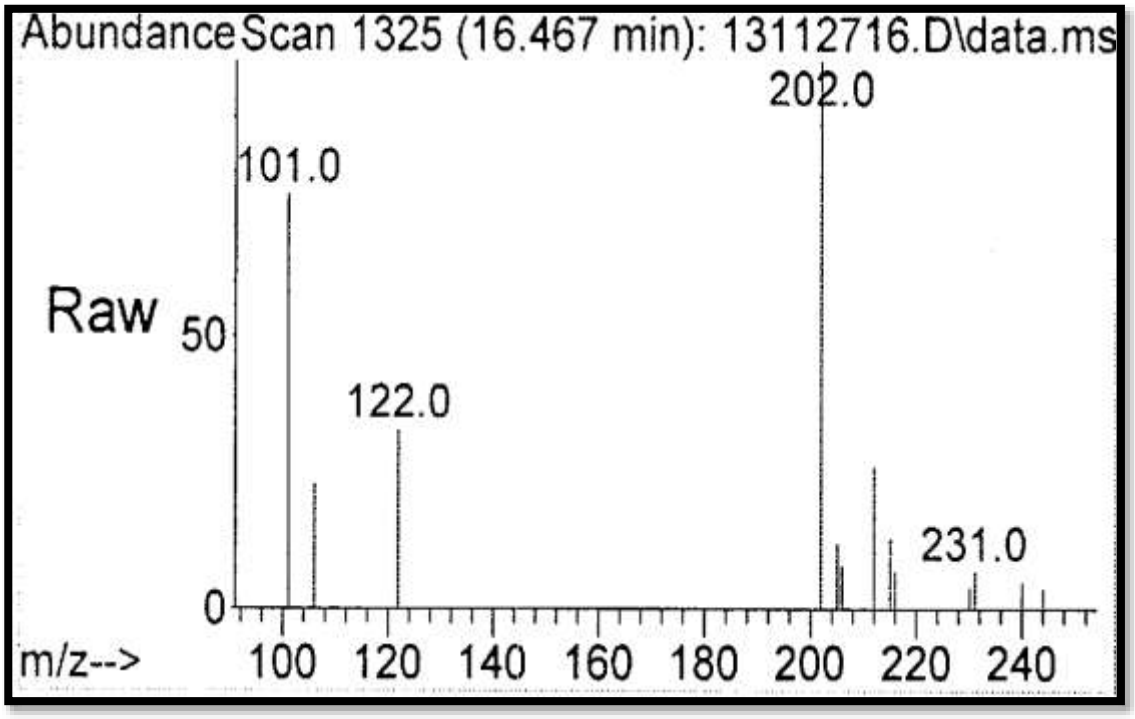

Figure 4.31: Concentration of Fluoranthene in the evening

The concentrations in evening samples were found to be 29.3ng/sample. The peak at 202 is for fluoranthene.

4.4.8 Pyrene

The molar mass of pyrene is 202.25g/mole. The structural diagram (Figure 4.32) and chromatograms are given below (Figure 4.33, 4.34 and 4.35)

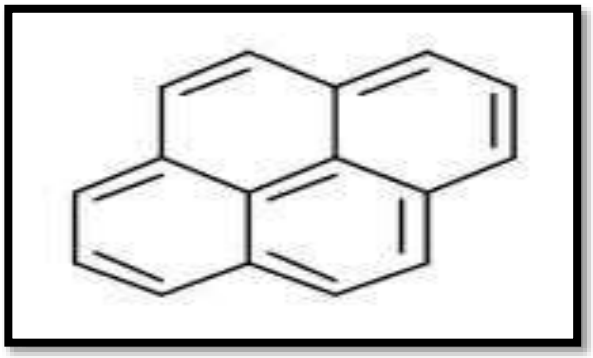

Figure 4.32: Structural diagram for pyrene

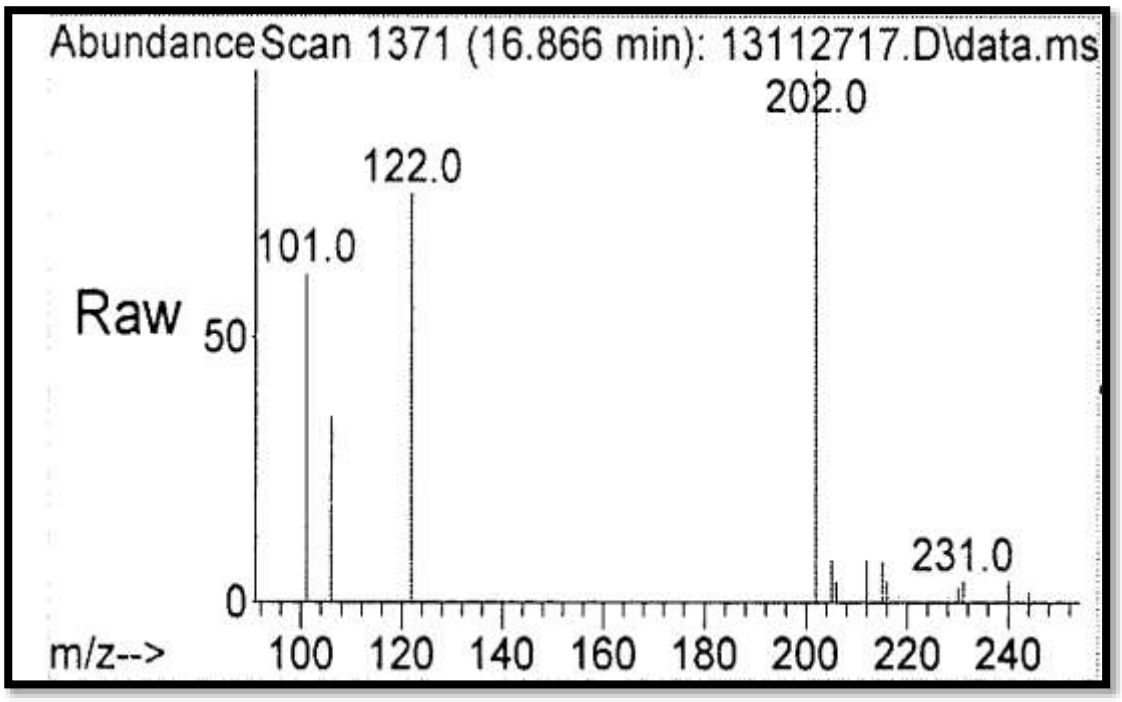

Figure 4.33: Concentration of pyrene in the morning

The concentration of the pyrene in morning was 78.3ng/sample. The peak at 202.25 is the peak for pyrene. The other high peaks are due to the disturbance by other compounds.

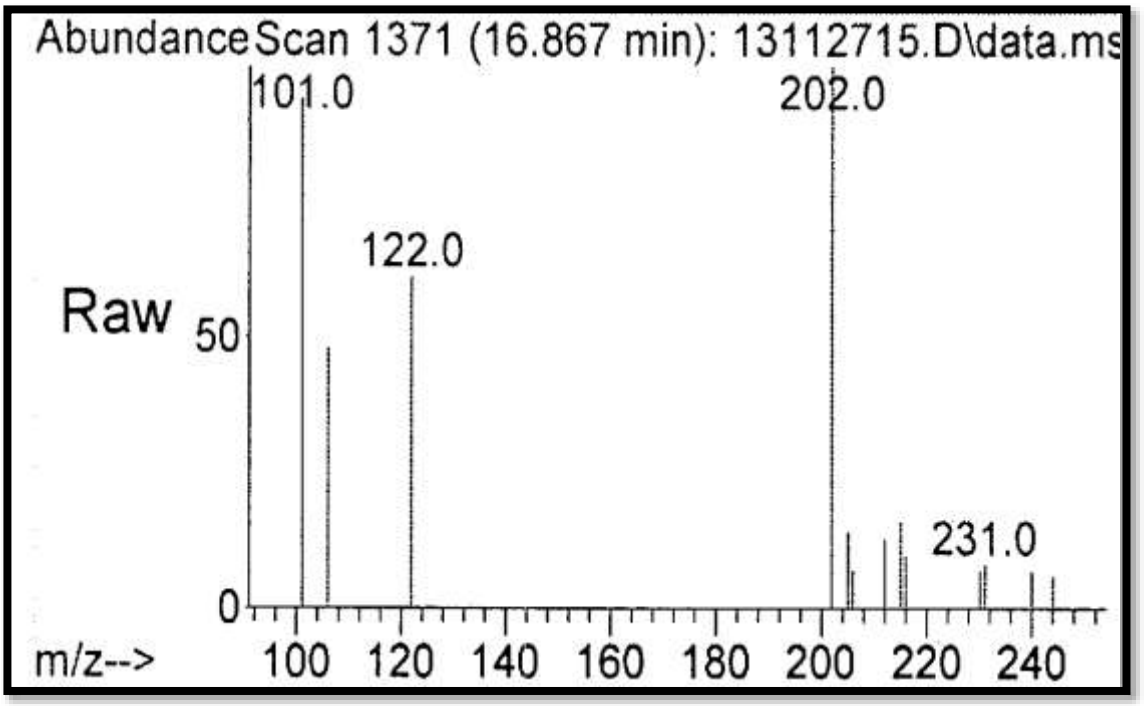

Figure 4.34: Concentration of Pyrene at noon

The concentrations of pyrene in noon were found to be 48.8ng/sample. The peak at 202.25 is for pyrene while the other peaks are due to the disturbance by other compounds.

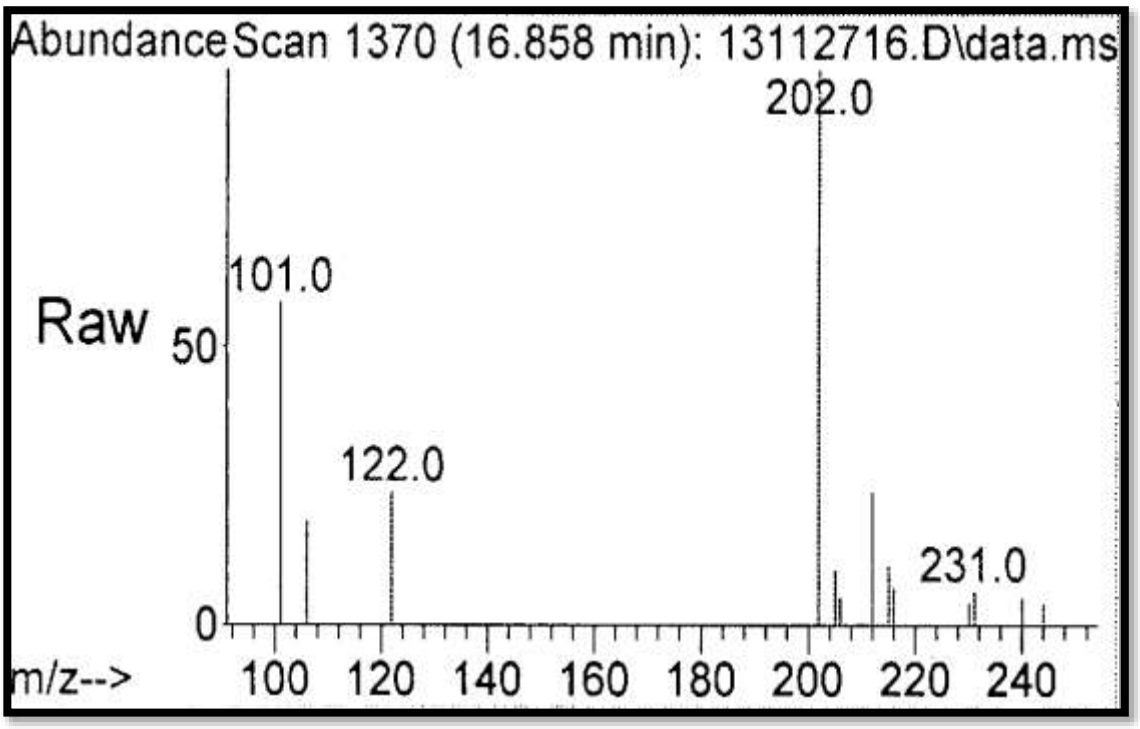

Figure 4.35: Concentration of pyrene in the evening

The concentrations in the evening were 41ng/sample. The highest peak at 202.25 is for pyrene.

4.5 Comet assay

The naphthalene started to cause damage in the cell at the concentration of 250µg/ml the doses lower than this did not cause any damage to the cell viability (Figure 4.38). In case of extracted PAHs the damage was seen at a concentration of 350µg/ml the extract caused DNA damage to 22% of the blood cells (Figure 4.37).

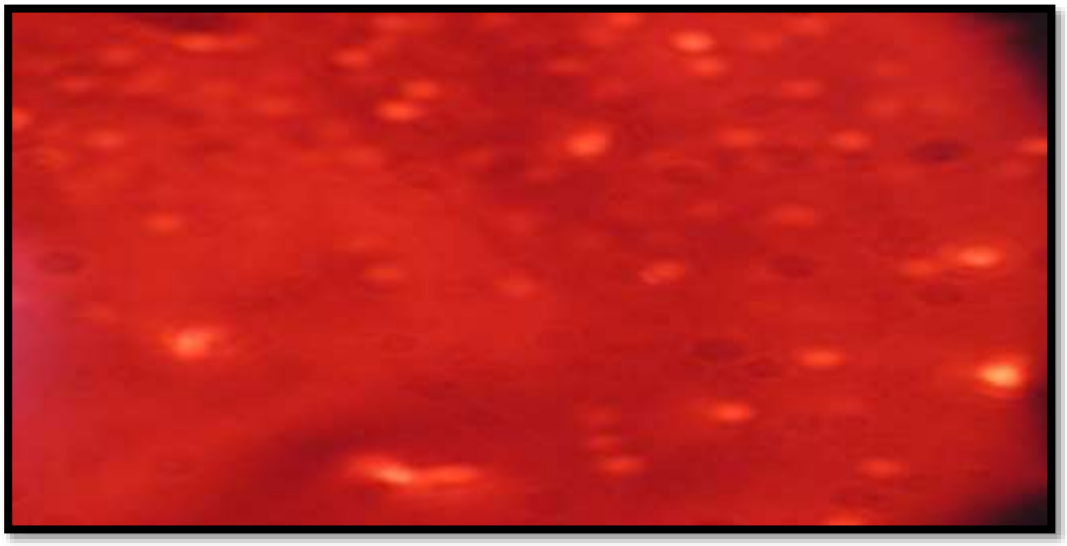

Figure 4.36: Cells without DNA damage

The figure is showing the normal blood cells without any treatment or exposure. The round shape is showing that these are un- damaged cells.

Figure 4.37: Cells with DNA damage caused by the PAHs

The comets like cells are showing the DNA damage caused by the exposure of PAHs to the normal blood cells.

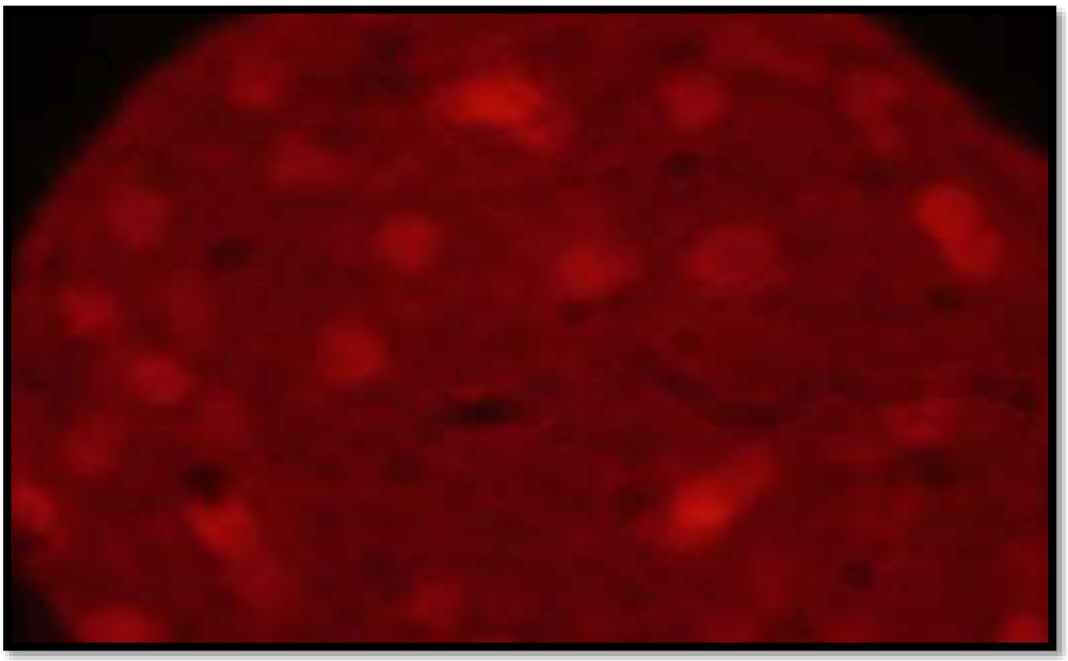

Figure 4.38: Cell damage caused by the naphthalene

The cells without the distinct boundary and having haziness are due to the damage by naphthalene.

DISCUSSION

4.6 Particulate Matter (PM_{10}) Concentrations

The results show that the concentrations taken at different location varied from each other. The reason for the differences can be the spatial variations and the type of activities other than vehicular circulations and the day of the week. Generally, these factors determine the concentration of particulate matter (*Alam et al., 2011, Bashyal et al., 2008 and Elassouli et al., 2007).* The highest mean concentration of PM_{10} was found at the general bus stand. This could be due to heavy traffic as it is a bus stand, emissions from the generators during the load shedding, and auto rickshaws. The concentrations in the noon are considerably high due to the higher number of working generators at the time of sampling and in the evening it was again close to the levels in the morning. The second highest concentrations taken from the micro pro dust sampler were at the Chenab Chowk. This point is close to the university gate where pick and drop activity for the students, heavy traffic load and emissions from the generators from nearby shops. At GTS the traffic is heavy but the sampling site did not had any kind of shops and the roads are comparatively wide and an underpass has separately made for the buses to leave for different and the area is not congested. The concentrations at Allied Chowk are the lowest because of the reason that the sampling day was Sunday and due to the weekend the traffic was low at the site as compared to the week-days. The results taken from the pro dust sampler can be related to the study of (Bashyal et al., 2008). They reported higher concentrations of PM_{10} citing the heavy traffic and re-suspension of the PM_{10} in the air that were settled down on the roads due to the circulation of traffic and sweeping on the roads in the morning as possible reasons. In noon the traffic flow is less as compared to the morning and evening and the concentrations are lower in weekends as compared to the week days. When measured with the high volume sampler, the 8 hour average showed the highest concentrations at Chenab chowk. The concentrations at the GBS are lower than Chenab Chowk because there was a slight rain fall resulting into a little decrease in previous suspended particles before the placement of sampler and the sampler gathered only those particles that were generated after that period. The concentration at Chenab Chowk, G, GBS and Allied Chowk were 372, 282, 223 and 150$\mu g/m^3$, respectively. The average concentration from four sites was 256.75$\mu g/m^3$. The results can be compared with the study by Alam et al. (2011) in which the concentration of PM_{10} in Karachi, Lahore, Rawalpindi and Peshawar were reported as 270, 198, 448 and 550 $\mu g/m^3$ respectively. So the concentrations in Faisalabad are higher

than Karachi and Lahore and less than Rawalpindi and Peshawar. The result showed that the PM_{10} concentrations mainly depend upon the traffic flow as reported in different studies (Elassouli et al., 2011; Fransen et al., 2013, Zuurbier et al., 2010). The difference in the concentrations taken by both samplers is obvious because the pro dust sampler gives the immediate concentration of the desired size of the particulate matter on sampling site. The values were higher as compared to the high volume sampler that first intake flow separate the desired size fraction through cyclone and then collect them on the filter paper so there might be chances of loss. The concentrations of PM are higher than designated by the EPA.

4.7 Contents of Priority Pollutants

The extracts that were analyzed showed the presence of 10 EPA designated priority pollutants that are potential carcinogens. The concentrations are higher than the studies that were consulted as reference (Elassouli. 2011, Elassouli et al. 2007). The method used for the extraction was different. The method followed in these studies used Soxhlet apparatus for the extraction. The method used in this study was sonication using the dichloromethane as the solvent. It showed better result than the Soxhlet method and also the method 429 designed by Canadian standards for extraction that was used by the analytical lab to extract the PAHs in lab from the filter paper used for sampling. The sampling sites had similar characteristics for the PM sources so any of the sites can be selected to have extract for the analysis. If the sites have different sources of PM emissions, then the extract should be taken from each sampled site to show the differences as described in literature (Elassouli et al. 2007).

4.8 DNA damage

The results show that the PAHs produce the dose dependent response as the damage started at the maximum concentration. The damage can be much more significant then that reported in the study as the samples are taken in summer and are less damaging than the extracts taken in winters as reported by the (Buschini et al., 2001).moreover no comet was seen without S9 fraction and the damage was seen when the metabolic activation was introduced so PAHs are not direct mutagens and require activation as reported by the (Elassouli. 2011).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The work has been done for the first time to find out the concentrations of particulate matter with two different kinds of samplers; the portable sampler and the high volume sampler recommended by EPA. The analysis of 16 priority pollutants in the selected area's environment has been done for the very first time. Particulate matter (PM_{10}) concentrations in Faisalabad, Pakistan at four sites i.e. Chenab Chowk (CC), Government Transport Service Chowk (GTS), General Bus Stand (GBS) and Allied Chowk (AC) were 372, 283, 223 and 150 μg m^{-3}, respectively when measured with high volume sampler. The maximum concentrations were 501, 456, 625 and 271 with Casella Microdust Pro™ sampler. Ten out of 16 priority PAHs were detected in PM_{10} sample, determined by GC/MS technique. The significant DNA damage (22%) was seen in the cells as compared to the control. The PM_{10} concentrations were higher than the EPA designated limits of 150 μg m^{-3}.

5.2 Recommendations

Further work is required to find out $PM_{2.5}$ in the area which is more toxic as reported in literature. Source apportionment can help to control the emissions. Identification of different contributors to PM can be useful to take actions in order to reduce the pollution. In addition to human being, animals and plants may also get affected by the particulate matter. So there is need to check the effects on these living organisms in the area. DNA damage assessments upon exposure to PAHs extracted from the particulate matter are of critical importance for environmental monitoring. Continuous monitoring can help the policy makers and environment agency to take stringent actions for making the environment healthy.

REFERENCES

Abbey, D. E., Nishino, N., McDonnell, W. F., Burchette, R. J., Knutsen, S.F., Beeson,W. L., Yang, J. X.1999. Long term inhalable particles and other air pollutants related to mortality in nonsmokers. American Journal of Respiratory and Critical Care Medicine, 159: 373-382.

Alam, K., T. Blaschke, P. Madl, A. Mukhtar, M. Hussain, T. Trautmann and S. Rahman. 2011. Aerosol size distribution and mass concentration measurements in various cities of Pakistan. Journal of Environmental Monitoring, 13(7): 1944–52.

Anenberg, S.C., Horowitz, L.W., Tong, D.Q., West, J.J. 2010. An estimate of the global burden of anthropogenic ozone and fine particulate matter on premature human mortality using atmospheric modeling. Environmental Health Perspectives, 118: 1189–1195.

AQEG: Fine Particulate Matter (PM2.5) in the United Kingdom. London: Defra; 2012.

AQEG: Particulate Matter in the UK. London: Defra; 2005.

Halliwell, B., Gutteridge, J.M. 1999. Free Radicals in Biology and Medicine, Oxford University Press, 1999.

Barale, R.,Giromini, L., Ghelardini, G., Scapoli, C.,Loprieno, N., Pala, M., Valerio, F., Barrai, I.1991.Correlations between 15 Polycyclic Aromatic Hydrocarbons (PAH) and the mutagenicity of the total PAH fraction in ambient air particles in la Spezia (Italy). Mutation Research, 249: 227-241.

Bascom R, Bromberg PA, Costa DA, et al.1996. Health effects of outdoor air pollution. American Journal of Respiratory and Critical Care Medicine, 153: 3–50.

Binkova, B., Sram, R.J., 2004. The genotoxic effect of carcinogenic PAHs, their artificial and environmental mixtures (EOM) on human diploid lung fibroblasts. Mutation Research, 547: 109–121.

Binkova, B., Topinka, J., Sram, R.J., Sevastyanova, O., Novakova, Z., Schmuczerova, J., Kalina, I., Popov, T., Farmer, P.B., 2007. In vitro genotoxicity of PAH mixtures and organic extract from urban air particles: Part I: Acellular assay. Mutation Research, 620: 114–122.

Bobak, M., Leon, D. A.1992. Air pollution and infant mortality in the Czech republic 1986 ± 88. Lancet, 340: 1010-1014.

Brandon C. Valuing environmental costs in Pakistan: the economy wide impacts of environmental degradation. Background paper for Pakistan 2010 report; 1995.

Brauer M, Dumyahn TS, Spengler JD, Gutschmidt K, Heinrich J, Wichmann HE.1995.Measurement of acidic aerosol species in eastern Europe: implications for air pollution epidemiology. Environmental Health Perspectives, 103(5):482–8.

Braun-Fahrlander, C., Vuille, J. C., Sennhauser, F. H., Neu, U., Kunzle, T., Grize, L., Gassner, M., Minder,C., Schindler, C., Varonier, H. S., W¸thrich, B.1997.Respiratory health and long-term exposure to air pollutants in Swiss schoolchildren. American Journal of Respiratory and Critical Care Medicine, 155: 1042 - 1049.

Brender, J. D.2008. Residential proximity to waste sites and industrial facilities and chromosomal anomalies in offspring. Journal of Hygene and Environmental Health, *211:* 50-58.

Brimblecombe P. The Big Smoke. London: Methuen, 1987.

Brunekreef, B., Holgate, S.T., 2002. Air pollution and health. Lancet, 360 (9341): 1233–1242.

Clancy, L., Goodman, P., Sinclair, H., Dockery, D. W.2002. Effect of air-pollution control on death rates in Dublin, Ireland: An intervention study. Lancet 360:1210-1214.

Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society (CEOHA-ATS).1996.Health effects of outdoor air pollution. American Journal of Respiratory and Critical Care Medicine, 153:3-50

Dejmek, J., Selevan, S. G., Benes, I., Solansky, I., Sram, R. J.1999. Fetal growth and maternal exposure to particulate matter during pregnancy. Environmental Health Perspectives , 107: 475 - 480.

Dockery, D. W., Cunningham, J., Damokosh, A. I.,Neas, L. M., Spengler, J. D., Koutrakis P., Ware, J. H., Raizenne, M. E., Speizer, F. E. 1996.Health effects of acid aerosols on North American children: Respiratory symptoms. Environmental Health Perspectives, 104: 500-505.

Dockery, D. W., Pope, C. A., Xu, X. P., Spengler, J. D.,Ware, J. H., Fay, M. E., Ferris, B. G., Speizer, F. E.1993. An association between air pollution and mortality in six U. S. cities. The New England Journal of Medicine, 329: 1753 ± 1759.

Dockery, D.W., Pope III, C.A., 1994. Acute respiratory effects of particulate air pollution. Annual Review of Public Health, 15: 107–132.

Donelly, K.C., Brown, K.W., Anderson, C.S., Barbee, G.C., Safe, S.H., 1990. Metabolism and bacterial mutagenicity of binary mixtures of benzo(a)pyrene and polychlorinated aromatic hydrocarbons, Environmental and Molecular Mutagenesis . 16, 238–245.

Elassouli, S. M. (2011). Airborne particulate matter (PM10) composition and its genotoxicity at two pilgrimage sites in Makkah , Saudi Arabia, *3*(April): 93–102.

Elassouli, S. M., M.H. Alqahtani and W. Milaat. 2007. Genotoxicity of air borne particulates assessed by comet and the Salmonella mutagenicity test in Jeddah, Saudi Arabia. International Journal of Environmental Research and Public Health, 4(3): 216–33.

Fujii, T., Hayashi, S., Hogg, J.C., Vincent, R., Eeden, S.F.V. 2001. Particulate matter induces cytokine expression in human bronchial epithelial cells. American Journal of Respiratory Cell and Molecular Biology, 25: 265–271.

Ghio, A.J., Kim, C., Devlin, R.B., 2000. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. American Journal of Respiratory and Critical Care Medicine, 162: 981–988.

Harrison RM, Yin J. 2000. Particulate matter in the atmosphere: which particle properties are important for its effects on health? Science of the Total Environment, 249:85–101.

Harvey RG. 1991. Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity. Cambridge, UK: Cambridge University Press.1997

Heinrich, J., Hlscher, B., Jacob, B., Wjst, M., Wichmann, H. E. 1999. Trends in allergies among children in a region of former East Germany between 1992, 1993 and 1995, 1996. European Journal of Medical Research, 4: 107-113.

Hoek, G., Brunekreef, B., Gold bohm, S., Fischer, P., van den Brandt, P. A.2002. Association between mortality and indicators of traffic-related air pollution in the Netherlands: A cohort study. Lancet, 360: 1203-1209.

IARC, 2009. Monographs on the Evaluation of Carcinogenic Risks to Humans.

IARC. 1983. Polynuclear Aromatic Compounds. Part 1. Chemicals, Environmental and Experimental Data. IARC Monogr Eval Carcinog Risks Hum 32. Lyon, France: International Agency for Research on Cancer

International Agency for research on Cancer (1983) Poly nuclear Aromatic Compounds. Part I. Chemical, Environment and Experimental Data, IARC Monographs, Vol. 32, Lyon, France, International Agency for Research on cancer.

Jeffrey, S. Ch., Selvin, S., Metayer, C., Crouse, V.,Golembesky, Buffler, P. A.2006.Parental Smoking and the Risk of Childhood Leukemia. American Journal of Epidemiology, 163(12)*:* 1091-1100.

Jung, M. H., Kim, H. R., Park, Y. J., Park, D. S., Chung, K. H., & Oh, S. M. (2012). Genotoxic effects and oxidative stress induced by organic extracts of particulate matter (PM10) collected from a subway tunnel in Seoul, Korea. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, *749*(1-2): 39–47.

Katsouyanni, K., 2003. Ambient air pollution and health. Br. Med. Bull. 68,143.

Kendall M, Hamilton RS, Watt J, Williams ID, 2001. Characterisation of selected speciated organic compounds associated with particulate matter in London. Atmospheric Environment, 35(14):2483–2495.

Kim, D.-H., Sohn, B.-J., Nakajima, T., Takamura, T., Takemura, T., Choi, B.-C., Yoon, S.-C., 2004. Aerosol optical properties over East Asia determined from ground-based sky radiation measurements. J. Geophys. Res. 109.

Kocifaj, M., Horvath, H., Jovanović, O., Gangl, M., 2006. Optical properties of urban aerosols in the region Bratislava—Vienna I. Methods and tests. Atmospheric Environment, 40 (11): 1922–1934.

Kramer, U., Behrendt, H., Dolgner, R., Ranft, U., Ring, J.,Willer, H., Schlipkˆter, H. W.1999. Airway diseases and allergies in East and West German children during the first 5 years after reunification: time trends and the impact of sulphur dioxide and total suspended particles. International Journal of Epidemiology, 28: 865-873.

Kramer, U., Herder, C., Sugiri, D., Strassburger, K., Schikowski, T., Ranft, U., Rathmann, W., 2010. Traffic-related air pollution and incident type 2 diabetes: results from the SALIA Cohort Study. Environmental Health Perspective, 118: 1273-9

Lee, R., Hung, H., Mackay, D., Jones, K.1998. Measurement and Modeling of the Diurnal Atmospheric PCBs and PAHs. Environmental Science and Technology, *32*, 2172-2179.

Lewtas, J. 1990. Experimental evidence for the carcinogenicity of air pollutants, in: Air Pollution and Human Cancer, Springer-Verlag, Berlin, pp. 49–61.

Lewtas, J. 1993. Complex mixture of air pollutants: characterizing the cancer risk of polycyclic organic matter, Environmental Health Perspective, 100: 211–218.

Li, N., Xia, T., Nel, A.E., 2008. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. Free Radical Biology and Medicine. 44, 1689–1699.

Liao D, Creason J, Shy CM, et al.1999. Daily variation of particulate air pollution and poor cardiac autonomic control in the elderly. Environmental Health Perspective. 107, 521–525.

Liu, Y., Zhu, L., Shen, X., 2001. Polycyclic aromatic hydrocarbons (PAHs) in indoor and outdoor air of Hangzhou, China. Environmental Science and Technology, 35: 840–844.

Madden, E.F., Fowler, B.A., 2000. Mechanisms of nephrotoxicity from metal combinations: a review. Drug Chemical Toxicology, 23: 1.

Mattimaricq M, 2007. Chemical characterization of particulate emissions from diesel engines: A review. Journal of Aerosol Science, 38(11): 1079–1118.

Mazzoli-Rocha, F., Fernandes, S., Einicker-Lamas, M., Zin, W.A., 2010. Roles of oxidative stress in signaling and inflammation induced by particulate matter. Cell Biology.

McDonnell, W. F., Nishino-Ishikawa, N., Petersen, F. F., Chen, L. H., Abbey, D. E.2000. Relationships of mortality with the fine and coarse fractions of long-term ambient PM10 concentrations in nonsmokers. Journal of Exposure Analysis and Environmental Epidemiology, 10: 427- 436.

McMurry P, Shepherd M, Vickery J, 2004. *Particulate Matter Science for Policy Makers: a NARSTO Assessment*: EPRI Report 1007735, Cambridge: Cambridge University Press.

Miller, C.A., Hidy, G., Hales, J., Kolb, C.E., Werner, A.S., Haneke, B., Parrish, D., Frey, H.C., Rojas-Bracho, L., Deslauriers, M., Pennell, B., Mobley, J.D., 2006. Air emission inventories in North America: a critical assessment. Journal of the Air & Waste Management Association, 56: 1115–1129.

Neher, J.O., Koenig, J.Q., 1994. Health effects of outdoor air pollution. American Family Physician ,49: 1397–1404.

Nel, A., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nano level. Science. Journal of Drug Delivery, 311: 622–627.

Nemmar, A., Hoylaerts, M.F., Hoet, P.H., Dinsdale, D., Smith, T., Xu, H., Vermylen,J., Nemery, B., 2002. Ultrafine particles affect experimental thrombosis in an in-vivo hamster model. American Journal of Respiratory and Critical Care Medicine, 166: 998–1004.

Nishioka, M.G., Lewtas, J. 1992. Quantification of nitro- and hydroxylated nitro-aromatic polycyclic aromatic hydrocarbons in selected ambient air daytime winter samples. Atmospheric Environment, 26A: 2077–2087.

Omar, N.Y.M.J., Abas, M.R.B., Ketuly, K.A., Tahir, N.M., 2002. Concentrations of PAHs in atmospheric particles (PM10) and roadside soil particles collected in Kuala Lumpur, Malaysia. Atmospheric Environment, 36: 247–254.

Parker, J.D., Mendola, P., Woodruff, T.J., 2008. Pre-term birth after the Utah Valley Steel Mill closure: a natural experiment. Epidemiology19 (6), 820–823.

Patel, H., Kwon, S., 2009. Interplay between cytokine-induced and cyclic equibiaxial deformation-induced nitric oxide production and metalloproteases expression in human alveolar epithelial cells. Cellular and Molecular Bioengineering, 2: 615–624.

Patel, M.M., Chillrud, S.N., Correa, J.C., Hazi, Y., Feinberg, M., Kc, D., Prakash, S., Ross,J.M., Levy, D., Kinney, P.L., 2010. Traffic-related particulate matter and acute respiratory symptoms among New York City area adolescents. Environmental Health Perspectives. 118: 1338-43.

Penning, T.M., Burczynski, M.E., Hung, C.F., McCoull, K.D., Palackal, N.T., Tsuruda, L.S.,1999. Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones. Chemical Research in Toxicology,12: 1–18.

Pope III, C.A., Burnett, R.T., Thun, M.J., Calle, E.E., Krewski, D., Ito, K., Thurston, G.D., 2002. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. Journal of American Medical Association,287 (9): 1132–1141.

Pope, C. A., Burnett, R. T., Thun, M. J., Calle, E. E., Krewski, D., Ito, K., Thurston, G. D.2002. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. Journal of American Medical Association, 287: 1132-1141.

Pope, C. A., Thun, M. J., Namboodiri, M. M., Dockery,D. W., Evans, J. S., Speizer, F. E., Heath, C. W.1995. Particulate air pollution as a predictor of mortality in a prospective study of US adults. American Journal of Respiratory and Critical Care Medicine, 151: 669-674.

Poschl, U., 2005, Atmospheric aerosols: composition, transformation, climate and health effects, Angew, 44: 7520.

Ramos de Rainho, C., Machado Corrêa, S., Luiz Mazzei, J., Alessandra Fortes Aiub, C., & Felzenszwalb, I. (2013). Genotoxicity of polycyclic aromatic hydrocarbons and nitro-derived in respirable airborne particulate matter collected from urban areas of Rio de Janeiro (Brazil). BioMed Research International,765352.

Sahan E, Brink H, Weijers E. 2008. Carbon in atmospheric particulate matter.Tech. rep. ECN Energy Research Centre of the Netherlands.

Saldiva, P.H., Clarke, R.W., Coull, B.A., Stearns, R.C., Lawrence, J., Murthy, G.G., Diaz, E.,Koutrakis, P., Suh, H., Tsuda, A., Godleski, J.J., 2002. Lung inflammation induced by concentrated ambient air particles is related to particle composition. American Journal of Respiratory and Critical Care Medicine, 165: 1610–1617.

Saldiva, P.H., Pope, C.A., 3rd, Schwartz, J., Dockery, D.W., Lichtenfels, A.J., Salge, J.M., Barone, I., Bohm, G.M., 1995. Air pollution and mortality in elderly people: a time-series study in Sao Paulo, Brazil. Archives of Environmental Health, 50: 159–163.

Samet, J.M., Dominici, F., Curriero, F.C., Coursac, I., Zeger, S.L., 2000. Fine particulate air pollution and mortality in 20 U.S. cities, 1987–1994. The New England Journal of Medicine, 343 (24): 1742–1749.

Sapkota, A., Chelikowsky, A.P., Nachman, K.E., Cohen, A.J., Ritz, B., 2012. Exposure to particulate matter and adverse birth outcomes: a comprehensive review and meta-analysis. Air Quality Atmosphere and Health, 5(4):369–381.

Sarvi A, Lyyranen J, Jokiniemi J, Zevenhoven R: Particulate emissions from large-scale Medium-speed diesel engines: 2. Chemical composition. Fuel Process Technology, 92(10): 2116–2122.

Schecter, A., Birnbaum, L., Ryan, J.J., Constable, J.D., 2006. Dioxins: an overview. Environmental Research,101: 419.

Schwartz, J., Ballester, F., Saez, M., Perez-Hoyos, S., Bellido, J., Cambra, K., Arribas, F., Canada, A., Perez-Boillos, M.J., Sunyer, J., 2001. The concentration–response relation between air pollution and daily deaths. Environmental Health Perspective, 109: 1001–1006.

Selevan, S.G., Borkovec, L., Slott, V.L., Zudova, Z., Rubes, J., Evenson, D.P., Perreault, S.D., 2000. Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environmental Health Perspectives, 108: 887-94.

Shah, P.S., Balkhair, T., 2011.Air pollution and birth outcomes: a systematic review. Environment International, 37 (2), 498–516.

Sies, H. 1993. Strategies of antioxidant defense, Eur. J. Biochem. 215: 213–219.

Sree Devi, V., Durga Rao, V., Hara Gopal, V. V, Siva Prasad, B., Sandhya Devi, G., Jyothy, a, Hema Prasad, M. (2009). Cytogenetic evaluation of traffic policemen occupationally exposed to vehicular exhaust. The Indian Journal of Medical Research, 130(5), 520–5.

Strickland, P., Kang, D. and Sithisarankul, P. (1996) Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. Environmental Health Perspective, 104:927-932.

Thron, R.W., 1996. Direct and indirect exposure to air pollution. Otolaryngol. Head Neck Surg. 114, 281.

Topinka, J., Schwarz, L. R., Wiebel, F. J., Cerná, M., & Wolff, T. (2000). Genotoxicity of urban air pollutants in the Czech Republic. Part II. DNA adduct formation in mammalian cells by extractable organic matter. Mutation Research, 469(1), 83–93.

Vinitketkumnuen, U., & Kalayanamitra, K. (2002). Particulate matter , PM 10 & PM 2.5 levels , and airborne mutagenicity in Chiang Mai , Thailand, *519*, 121–131.

Wang, K., Dickinson, R.E., Liang, S., 2009. Clear sky visibility has decreased over land globally from 1973 to 2007. Science 323 (5920), 1468–1470.

Wang, Y., Xin, J., Li, Z., Wang, S., Wang, P., Hao, W.M., Nordgren, B.L., Chen, H., Wang, L., Sun, Y., 2011. Seasonal variations in aerosol optical properties over China. J. Geophys. Res. 116 (D18).

White, P.A. 2002. The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures, Mutat. Res.515. 85–98.

Williams D, Milne J, Roberts D, Kimberlee M, 1989. Particulate emissions from in-use motor vehicles: Spark and ignition vehicles. Atmospheric environment, 23(12):2639–2645.

Woodruff, T.J., Parker, J.D., Kyle, A.D.,Schoendorf, K.C., 2003. Disparities in exposure to air pollution during pregnancy. Environmental Health Perspective. 111(7), 942–946.

Yan F, Winijkul E, Jung S, Bond TC, Streets DG, 2011. Global emission projections of particulate matter (PM): I. Exhaust emissions from on-road vehicles. Atmospheric Environment, 45(28):4830–4844.

Zanobetti A, Schwartz J, Dockery DW. 2000. Airborne particles are a risk factor for hospital admissions for heart and lung disease. Environmental Health Perspective; 108: 1071–7.

Zuurbier, M., Hoek, G., Oldenwening, M., Lenters, V., Meliefste, K., van den Hazel, P., Brunekreef, B., 2010. Commuters' exposure to particulate matter air pollution is affected by mode of transport, fuel type, and route. Environmental Health Perspective, 118: 783–789.