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Characterization of cytotoxicity of airborne particulates from urban areas of Lahore

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Abstract

A number of species (organic and inorganic) in airborne particulates cause the toxicity to living being. The potential of *in vitro* test methods were explored for toxicity assessment of trace toxic elements (inorganic species) present in ambient air on human being (lungs). A year long sampling of airborne particles ($PM_{2.5}$) was carried (April 2008 to March 2009) in Lahore, Pakistan. A total of thirty nine samples were collected on 47 mm Zefluor Teflon filter membranes and each was analysed to characterize for the elements: Sb, As, Be, Cd, Cr, Co, Pb, Mn, Hg using ICP-MS in water extract and total acid digestate. The samples cytotoxicity was also established using lung derived cells and MTS colorimetric assays. This generated dose response curves and IC_{50} values for the elemental mixtures identified on the Teflon filter membrane. The results indicated that even at low concentrations airborne elemental mixtures displayed an additive toxic effect.

Key words: particulate matter; trace elements; *in vitro* toxicity
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Introduction

Exposures to ambient particulate matter (PM), especially to fine PM with an aerodynamic radius of 2.5 μm or less ($PM_{2.5}$), have been consistently related to increases in mortality and morbidity (Dockery et al., 1993). PM exposures appear to induce oxidative stress, pulmonary inflammation and modulate the immune responses of the lungs. Respiratory infections such as pneumonia, for example, have been found to increase significantly among individuals following exposures to higher levels of PM, especially in the elderly persons (Schwartz, 1994; Winder and Stacey, 2004; Pereira et al., 2010; Bloutsos and Yannopoulos, 2011). According to their physicochemical characteristics airborne particles may enter different regions of the respiratory tract. Breathing heavy metal particles, even at levels well below those considered nontoxic, can have serious health effects. Toxicological studies have frequently implicated the metal contents (particularly water soluble) as possible harmful component of particulate matter. Although other toxic materials such as methylene chloride, isopropyl alcohol (2-propanol), acetone, acetaldehyde etc are often used in the workplace and are known to have potential heath. Transition metals have been reported showing to generate reactive oxygen species (ROS) which in turn cause cellular pro-inflammatory response pathways *in vitro* and *in vivo*. Using particles of size less than 10 microns (PM_{10}), Frampton et al. (1999) indicated the importance of soluble metal content for toxicity and pro-inflammatory potential in a range of human, animal *in vitro* studies. In addition, toxic metals can increase allergic reactions, cause genetic mutation, compete with "good" trace metals for biochemical bond sites, and act as antibiotics, killing beneficial bacteria. Their general effects include significant decrease in ventilatory function, congestion, edema, and hemorrhage of the lung.

In vitro toxicology has now become one of the most rapidly expanding areas of alternative methods to animal testing with many practical applications (Balls and Fentem, 1992; Barile, 2008; Faustman and Omenn, 2001; Holme and Dybing, 2002; Valentine and Kennedy, 2001, Bakand et al., 2009; Amanda and Shahnaz, 2010; Bakand and Hayes, 2010). *In vitro* methods can be used for screening, ranking and risk assessments of chemicals and new products. These methods can be implemented to determine the adverse effects of industrial chemicals, pharmaceuticals and consumer products that are being manufactured and marketed at rapid and extraordinary rates (Barile, 2008). The heavy metals contained in such PM have been suspected to have toxic effects on epithelial lung cells. The main technical challenge of *in vitro* testing of airborne chemicals is to replicate inhalation exposure in cultured cells or tissues. While in many studies cells are exposed to airborne chemicals in such a way that differ significantly from *in vivo* condition, the necessity of

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the direct exposure of target cells to airborne chemicals which resemble *in vivo* fashion as close as possible have been recognised by researchers (Hayes et al., 2007; Khalil, 2006; Valentine and Kennedy, 2001). Most of the indirect exposure methods, especially studies conducted on PM is limited to exposure of cells to test chemicals solubilised or suspended in culture medium.

The major objective of the current study was to explore the potential of *in vitro* test methods for toxicity assessment of trace toxic elements present in ambient air on human being (lungs). A toxic profile of elemental particulates in the airborne phase was established using *in vitro* cytotoxicity screening technique.

Lahore, the second largest city of the country with a population of 7 millions is experiencing very high ambient particulate concentrations posing a heavy health risk to congested populated areas of the city. A bad ambient air quality in terms of higher TSP, PM_{10}, and $PM_{2.5}$ has been reported in Lahore as compared to those in the other major cities of Pakistan (Ghauri et al., 2007). Therefore, an in-depth study of toxic air pollutants and their health impact on human being is required to be conducted to develop appropriate policies and controls.

1 Materials and methods

1.1 Study area

$PM_{2.5}$ samples were collected using $PM_{2.5}$ reference ambient air sampler (RAAS) (Thermo Electron Corporation, USA) at an urban site Lahore (31.57˚N, 74.31˚E), the second largest city of Pakistan bordering India. The sampler was placed near University of Punjab. Local emissions from many sources dominate the contributions to the fine particle mass.

1.2 $PM_{2.5}$ sampling

Most of sampling duration was 24 hr, however, some short duration samples were also collected to see the short term exposure effects. The samples were collected on 47 mm pre-weighed Zefluor^{TM} filter papers (Pall Cooperation, USA) at constant air flow rate of 16.67 L/min. The sampling was carried out 4 times in a week through out the sampling period. Weighing was done on a Saritorius MC5 six place balance. The filter papers were kept in controlled environmental conditions at temperature of 20–23°C and relative humidity of 30%–40% for 24 hr, as per USEPA standard, prior to and after the collection of $PM_{2.5}$ mass. $PM_{2.5}$ mass was determined gravimetrically by the difference of weight of filter paper before and after collecting $PM_{2.5}$. A set of weigh blank filters were taken in between the pre and post-weighing sessions of each batch of sample filters and the final filter mass was corrected using any change in weight of filter blank during weighing sessions.

1.3 Preparation of particle extracts for toxicity experiment

Thirty nine $PM_{2.5}$ particle samples collected on Zefluor^{TM} filters at Lahore, Pakistan were brought to Chemical Safety and Toxicology (CSAT) Laboratory of UNSW, Sydney. The filters were stored at room temperature before extraction. Because intact particles could not be physically separated from the filters, we obtained aqueous extracts of the particle. Precisely cut half of each filter was ultra-sonicated in a 10-mL conical polypropylene tube containing 5 mL of Milli Q deionized water for 90 min at 70°C. The extract was used for toxicity experiment after thorough mixing of the sample on a vortex mixer. Filters were removed, and the aqueous extract was centrifuged for 30 min to pellet insoluble matter. Between analyses the samples were stored sealed at 4°C. The solubility of an air borne trace element depends on the origin, particle size and the sample pH. The factors influencing aerosol solubility in cloud processes in atmosphere. Elements of highly soluble can be more readily available in lungs and thus may be potentially more harmful.

1.4 Metal analysis

To determine the water soluble metal, precisely cut half of the filters were placed in 10 mL of Milli Q deionized 18 MΩ water in a polypropylene tube and ultra-sonicated at 70°C for 90 min. It was made sure that the exposed side of the filter faces the water for complete extraction. After uniform mixing off the prepared sample in a vortex mixer, the supernatant was acidified to 0.5% HNO_3 to stabilize against any trace metal loss. The total digestion was carried out using a micro-wave oven by addition of 0.2 mL of HCl, 6 mL of HNO_3 concentrated acid and 2 mL of H_2O_2 to the filters. The digested samples after several washing were shifted to 30 mL polypropylene tubes, and in each sample, volume was made up to 30 mL. Since low detection limits were chosen, therefore these samples were again diluted four times before running on ICP-MS. Samples along with the field blanks were digested in batches of 10 samples in addition to two blank filters and two QC samples. The analytical blanks were Zeflour filters taken directly from the manufacturer's package to the extraction vessels in the Elemental Analysis Chemistry laboratory of Chemical Sciences at UNSW, Sydney. These blanks were in addition to the field blanks that were accessioned along with regular filter paper samples. The samples collected were also analysed for trace metals using ICP-MS (Perkin Elmer, Dynamic reaction cell DRC2) at Elemental Analysis Chemistry Lab. of UNSW, Sydney was used to determine concentrations of As, Be, Cd, Cr, Co, Hg, Mn, Ni, Pb Se, Sb, Sr and V in water soluble and digested fraction of the collected particulate matter. The basic operating procedure followed was that of USEPA Method 2008. Basically each result was based on three integrations. The final corrected sample concentration was obtained by subtracting from each sample the median concentration of that element in the weigh-blank filters taken through the same analytical protocol with each batch of sample filters. The sum of the mass of metal from both extractions gave the total metal content. The mean concentrations of selected heavy trace metals in soluble and digested fraction of these samples appear in Table 1.

1.5 Culture conditions

Human cells, namely, pulmonary type II-like epithelial cell lines (A549) (Cytogenetics Department, Westmead Hospital, Sydney, Australia), were used. Cells were cultured in DMEM/F12 (Dulbecco's modified Eagle's medium: Ham's F-12 nutrient mixture; Gibco, USA) supplemented with 5% (*V*/*V*) fetal calf serum (FCS; JS Bioscience, Australia), and L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (0.1 mg/mL) solution (Sigma, USA). Cultured cells were kept at 37°C in a humidified 5% CO_2 incubator. For cytotoxicity experiments, newly confluent cell layers were enzymatically removed, using trypsin/ethylenedimaine tetra-acetic acid (EDTA; Gibco, USA), and resuspended in culture medium. Cell viability was assessed by vital staining with trypan blue (0.4%, *W*/*V*; Sigma, USA), and cell number was determined using a light microscope (Leitz Wetzlar, Germany). Optimum cell numbers were determined in preliminary studies for each cell type and *in vitro* assay, based on the linearity range of cell concentration and absorbance data.

Cell cultures were incubated at 37°C in a humidified incubator for 24 hr. Before exposure, cell confluence (90%–95%) and attachment were observed using the light microscope. The medium was removed from newly confluent cells and membranes were washed with Hanks balanced salt solution (HBSS; Gibco, USA). Cells were exposed to water extracts of airborne particulate matter. The respiratory tract, skin and liver are considered target organs that are more likely to be exposed or affected by the elements. The A549 cells originate from lung epithelial type II cells. These cells are thought to be progenitor cells for damaged type II epithelial cells in the alveoli. They are involved in the alveolar epithelial proliferative response observed by long-term inhalation of high doses of particulate matter and carbon black (Janssen et al., 1997; Nikula et al., 1995).

To optimise the colorimetric *in vitro* assays, the effects of cell number on absorbance level were investigated by using varying number of human lung cells selected for *in vitro* assays. The appropriate cell concentration required for *in vitro* assays was then determined based on the linearity range of the cell number and cell viability. This means that with the increase in cells density number per 100 μL, the viability of the subject cells remains same. According to this optimum cell density of 70,000 cells/100 μL were used in *in vitro* assays. This has been experimentally has been demonstrated in Fig. 1.

1.6 Viability and cell counting

The most reliable method for determining the number of viable cells in cytotoxicity assays is treatment of cell culture with a vital dye such as trypan blue. This viability test relies on a breakdown in membrane integrity that is determined by the uptake of trypan blue dye to which the cells are normally impermeable. To obtain an accurate cell count, a uniform suspension containing single cells was necessary. Equal amounts (100 μL) of cell suspension and trypan blue (0.4%, *W*/*V*; Sigma, USA) were mixed. The uniform mixture was introduced into the counting chamber covered by a coverslip using a micropipette and tip. While the viable cells appeared clear, non-viable cells (if present) appeared blue and thus viable and non-viable cells were easily distinguished under the light microscope (Leitz Wetzlar, Germany).

This viability test relies on a breakdown in membrane integrity that is determined by the uptake of trypan blue dye to which the cells are normally impermeable. To obtain an accurate cell count, a uniform suspension containing single cells was necessary. Equal amounts (50 μL) of cell suspension and trypan blue (0.4% *W*/*V*; Sigma, USA) were mixed. The uniform mixture was introduced into the counting chambers A and B of the countess slide. Cell number was determined using a cell counter COUNTESS. Appropriate cell numbers were determined and *in vitro* assay, based on the linearity range of cell concentration and absorbance data. Therefore in this confluent stage, cells are subcultured to maintain active and health growing cells.

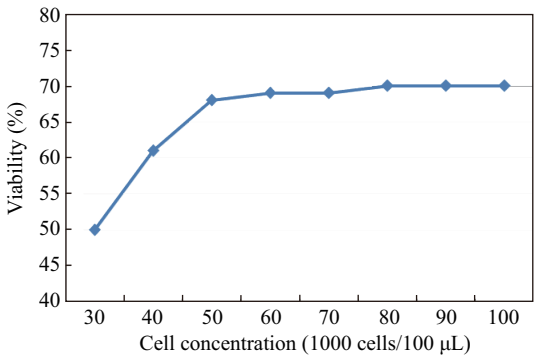

Fig. 1 Optimization of cell density for *in vitro* assays.

Table 1 Mean concentrations of selected toxic elements in $PM_{2.5}$ samples (unit: ng/L)

Component	Water extract	Serial dilution in columns									
		#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
As	3.56 ± 3.04	1.51	0.64	0.27	0.12	0.05	0.02	0.01	0.00	0.00	0.00
Cd	6.30 ± 11.68	2.68	1.14	0.48	0.21	0.09	0.04	0.02	0.01	0.00	0.00
Cr	0.26 ± 0.21	0.11	0.05	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Co	0.07 ± 0.10	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mn	34.4 ± 24.5	14.62	6.21	2.64	1.12	0.48	0.20	0.09	0.04	0.02	0.01
Ni	1.54 ± 1.58	0.65	0.28	0.12	0.05	0.02	0.01	0.00	0.00	0.00	0.00
Sr	11.15 ± 7.54	4.74	2.01	0.86	0.36	0.15	0.07	0.03	0.01	0.01	0.00
Pb	131.94 ± 204.32	56.07	23.83	10.13	4.30	1.83	0.78	0.33	0.14	0.06	0.03
Se	1.69 ± 1.19	0.72	0.31	0.13	0.06	0.02	0.01	0.00	0.00	0.00	0.00
V	0.77 ± 1.07	0.33	0.14	0.06	0.03	0.01	0.00	0.00	0.00	0.00	0.00
Sb	5.10 ± 5.97	2.17	0.92	0.39	0.17	0.07	0.03	0.01	0.01	0.00	0.00

Viability percentage was calculated through absorbance recorded at 492 nm using a Multiplate Reader (Multiskan Ascent, Thermo Labsystems, Finland). The absorbance values were automatically converted into percentage of cell viability through software Graph Pad Prism 5. Complete dose-response curves were also plotted for all aerosol samples using Graph Pad Prism 5.

1.7 MTS cytotoxicity assay

Tetrazolium compounds such as MTT, XTT and MTS are extensively used as convenient, non-radioactive methods for determining the number of viable cells in proliferation and cytotoxicity assays. The colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) -2H tetrazolium salt) assay is based on the ability of viable cells to convert a soluble tetrazolium salt to a formazan product that is soluble in culture medium. The MTS assay offers a convenient, less expensive, non-radioactive alternative to using thymidine radioisotope incorporation assays (Fig. 2).

1.8 Method for cell exposure to soluble toxic metals contaminants

Serial dilutions of soluble extract were employed in 96-well flat bottomed microtitre tissue culture plates. For preparation of serial dilutions of sample directly in 96 well plates, the following dilution protocol was used:

The culture media (DMEM/F12; 60 μL) supplemented with FCS (5%; *V*/*V*) and antibiotic mixture (1%; *V*/*V*) was added to all 96 well microtitre plate. As per our scheme, column #1 had cell (20 μL) and media (60 μL) only. This acts as quality control for 100% viability of cells.

In column #2, there were media (60 μL) with MTS (30 μL) only. The soluble extract of a sample (60 μL) prepared as per procedure described in Section 1.3 above, were then added to the column #3 and mixed 3–5 times with the micro-pipette. Aliquots of the mixed solution (60 μL) were then transferred to the column #4 wells and mixed. Again aliquots of the mixed solution (60 μL) were transferred to the column #5 wells and mixed. This procedure continued until serial dilutions were prepared in all columns. At the end, 60 μL extra solution in the column #12 were removed. Then 20 μL of cell mix containing a predetermined cell density of 70,000 cells/100 μL were used in all columns except column #2 (IC_{100}, 100% inhibitory concentration; media only).

Cells with concatenation of 70,000 cells/100 μL were added to serial dilutions of water extract of $PM_{2.5}$ prepared in 96-well microtitre culture plates in four replicates. The concentration of various toxic elements in 60 μL extract-were diluted to 140 μL upon addition to each well of column #3 of 96 well microtitre plate shown in Fig. 3. Columns #11 and #12 had minimum concentration of toxic elements to demonstrate minimum toxicity towards the living cells as shown in Table 1. The absorbance values (492 nm) of column #1 which had no toxic elements were comparable with those of columns #11 and #12. This shows minimum or negligible toxicity towards subject cells in these two columns (#11 and #12). This means the osmosis effect on cells would be negligible since columns #1, #11 and #12 show 100% viability of cells. It was assumed that as the serial dilution was done in media mainly using a small volume of the water extract and mainly medium, the osmosis effects would be minimal.

For each test concentration, blank wells were also prepared separately in eight replicates in which no cell suspension were added for considering any possible reactions induced by the water extract samples and the assay reagents. For each experiment, two internal controls were set up including IC_0 (0% inhibitory concentration; cells only) and an IC_{100} (100% inhibitory concentration; media only). The MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H tetrazolium) *in vitro* assay was then performed.

1.9 *In vitro* dose-response relationship

Basically, toxicity assessment aims at the estimation of the relationship between dose or concentration and magnitude of a response or an effect. Establishment of the dose-response is one of the important components of chemical risk assessment. Several important reference concentrations can be determined from the dose response relationship using *in vitro* assay systems (Fig. 4).

1.10 MTS-tetrazolium salt assay

The MTS assay is based on the ability of viable cells to convert a soluble tetrazolium salt to a formazan product. This assay has been used in our laboratory for toxicity

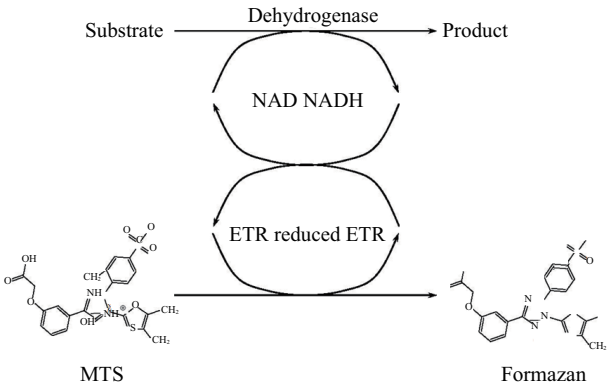

Fig. 2 Cellular metabolism resulting in the conversion of MTS to formazan.

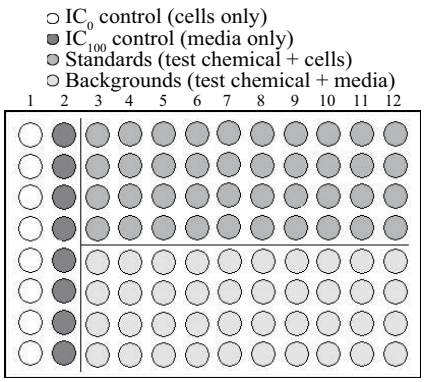

Fig. 3 Typical layout of 96 well microtitre plate for *in vitro* assays.

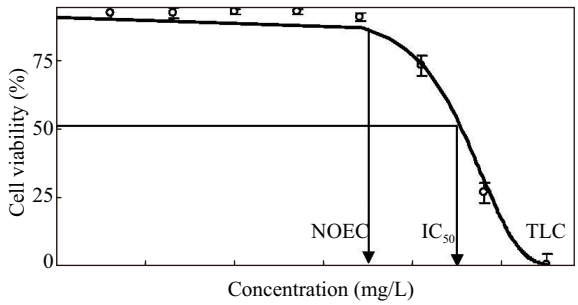

Fig. 4 Dose-response relationship using *in vitro* methods. IC_{50}: concentration of test chemical that requires inhibition of 50% of cell viability; NOEC: no observed adverse effect concentration; TLC: total lethal concentration.

testing of toxic trace metals in microtitre well plates (Frampton et al., 1999; Griem et al., 1995). The MTS/PMS reagent was added and cell cultures were incubated for one hr at 37°C. After the incubation period, absorbance was recorded at 492 nm using a multiplate reader (Multiskan Ascent, Thermo Labsystems, Finland). Complete dose-response curves were plotted for all aerosol samples using Graph Pad Prism 5 software. The cytotoxicity endpoints including the NOAEC (no observed adverse effect concentration), inhibitory concentration) and TLC (total lethal concentration; IC_{100}) values were determined.

The average IC_{50} (50% inhibitory concentration values) were also determined in selected A549 human cells with the MTS assay. These are summarised in Table 2 based on obtained IC_{50} values, relative toxicity ranking of all these was performed, this is given in Table 2.

2 Results and discussion

Rapid urbanization and lack of efficient monitoring and control of pollution make the South Asian atmospheric chemistry a very complex one.

2.1 $PM_{2.5}$ concentrations

$PM_{2.5}$ samples are regularly being collected as part of air quality monitoring at Lahore, Pakistan. During the total sampling period May 2008 and April 2009, the mean $PM_{2.5}$ concentration was (113 ± 76) μg/m^3. The highest mean concentration was observed in winter (385 μg/m^3 in Jan, 2009) while the lowest was observed in summer/ fall (Fig. 5). The mean $PM_{2.5}$ concentration in Lahore was several folds higher than those measured in the developed countries. Around 67% of $PM_{2.5}$ mass at Lahore was reported to be of carbonaceous in natures which were originated from fossil fuel combustion and biomass burning. Although vehicular traffic and activities including industrial emission as localized sources continue all along the year, the monthly trend of atmospheric concentrations of $PM_{2.5}$ show many fold rise in winter compared to summer. This is apparently because of decrease in mixing heights during winter when atmospheric conditions are more or less stable (higher atmospheric pressure). This results in building of particulates concentration in the atmosphere. While in summer, the atmospheric conditions are reversed, enhanced vertical mixing and thereby less $PM_{2.5}$ concentrations. The RH during this month remained close to super saturation (around 95%).

2.2 Metal concentrations

Concentration of water soluble (water extract) and non soluble (acid extract) trace metals content of $PM_{2.5}$ are summarised in Table 1. Results are expressed in ng/m^3 which are indicative of amount of metal that is taken in by the exposed human being. Since atmospheric particles at a given sampling site receive the apportioned from various potential sources with varying composition. Trace metal solubility difference is important from a bio-available point

Table 2 Experimental results of IC_{50} and particulate concentrations

Sample No.	IC_{50} (mg/L)	R^2	Conc. of $PM_{2.5}$ (ppm)*
1	39.3	0.89	167.8
2	38	0.70	152.3
3	32.8	0.64	113.8
4	25.1	0.82	99.7
5	12.24	0.77	225
6	10.76	0.87	78.7
7	7.77	0.71	55.6
8	7.3	0.84	82
9	6.67	0.80	99
10	6.4	0.86	71.9
11	5.6	0.68	92.5
12	3.45	0.78	171.4
13	3.11	0.71	277.7
14	2.87	0.70	58
16	2.73	0.73	42
17	2.7	0.65	72.4
18	2.66	0.73	53
19	2.23	0.85	51.3
20	1.8	0.82	32.5
21	1.54	0.84	55.6
22	1.21	0.76	62.5
23	1.18	0.74	113.8
24	1.17	0.75	30.4
25	1.01	0.74	91.1
26	0.99	0.77	52.2
27	0.97	0.87	52.6
28	0.8	0.82	30
29	0.75	0.70	66
30	0.28	0.66	9.8
31	0.25	0.76	18
32	0.14	0.75	66
33	0.1	0.70	12.4
34	0.05	0.80	9
35	0.016	0.80	25
36	0.01	0.70	7.3
37	0.002	0.90	8.6
38	0.0		

* Concentration of $PM_{2.5}$ at which the cells were exposed.

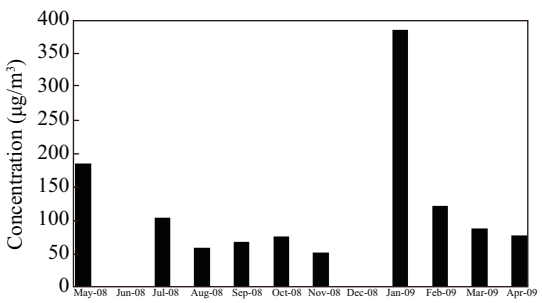

Fig. 5 Variations in $PM_{2.5}$ concentrations.

of view perspective because airborne PM is an important exposure a person through air and lung fluid.

The chromium forms ionic compounds and their vapour pressure can be considered negligible. Similarly, Manganese has both, crustal and anthropogenic sources and while in urban centres, it is attributed to road dust, soil, road transport and fossil fuel combustion. Most of the elements such as Cd, Pb, Co, Ni, V and Sb are linked to fossil fuel combustion in motor vehicles, brake linings for Sb and tyre abrasion for Mn (Morawska and Zhang, 2002). The rest of the elements (As, Sr) are more related to re-suspension of crustal material or dust. The industrial component includes a wide variety of toxic metals such as combustion processes of fossil fuel emit variable amounts of V, Pb, Ni, Cr and Co. Metallurgical and pigment activities in Lahore are responsible for the presence of As, Pb, Cr, Ni, Mn, Pb, Zn and Cd in the atmosphere. These elements mostly emanate from the steel works. Elevated concentration of some of these (Co, As, Cr, V and Ni) are partly linked to intrusion of polluted air masses from distant sources in neighbouring India, although local industrial, vehicular and re-suspension processes also have role to play. For example brick kilns around the city (Lahore) use a mixture of locally available biomass and powdered coal, fuel related elements were expected to be emanating from these sources as well as from burning of and wood. A majority of power generated in neighbouring India (approximately 55%) is fuelled by coal. There is a large coal-fired power plant near Rupar, India (northwest of Chandigarh). There are also a few power plants in southwest Punjab, northern Rajasthan, Uttar Pradesh in India, and near Faisalabad in Pakistan.

While comparing the metal concentrations in soluble and insoluble fractions, the following elements have been found to have greater proportion of metal in water soluble fraction than in acid digested fraction, i.e., As, Cd, Co, and Sb (Table 1) reported almost all of (80% to 100%) of their trace metals were leachable. Se and Pb were 50: 50 in both extraction method, revealing that it had equally important natural and manmade sources Janssen et al. (1997).

2.3 Cytotoxicity of toxic trace elements

To evaluate the cytotoxicity of soluble components of particulate matter, namely As, Cd, Cr, Co, Mn, Ni, Sr, Pb, Se, V, Hg and Sb water extract of $PM_{2.5}$ were prepared. Dose dependency of toxic effects of water soluble transition heavy metals was studied in human A549 lung derived cell lines The confluent monolayer's of type II alveolar epithelial cells A-549 were used as a model for the alveoli, and toxicity was quantified via the MTS assay. Cells cultured in cell sterile cell culture flasks and were then exposed to the different quantities of water extracts of the field samples having varied concentration of these toxic trace elements. The MTS/PMS reagent was added and the cell culture was incubated for one hour to attain the end point. Even within 30 min of exposure, these metal extract resulted in a significant decrease in cell viability. After one hour exposure, close to zero viability was observed in most of the cells which were exposed to various samples having concentrations from 9 to 278 ppm of $PM_{2.5}$ concentrations (water extract concentration prepared in 5 mL). The dose-response curves of individual experiment using these samples on A-549 cells with MTS are presented in Fig. 6a, b. The IC_{50} (50% lethal concentration) values for these experiments are also presented in Table 2 along with R^2. The water extract of field blank representing one of filters from the same lot was also experimented to see for any toxic effect. The experiment resulted in absence of IC_{50} that is absence of any cell toxicity of the filtering media.

The results of toxicity of individual sample as indicated in Table 2 varied from absolutely non toxic blank to highly toxic one. $PM_{2.5}$ sample collected on 14 May 2008 has shown least toxicity (IC_{50} value of 39.3 ppm) towards A-549 lung cells compared to $PM_{2.5}$ sample collected on 22nd March, 2009 having IC_{50} of 0.002 ppm.

In our study, sample with higher $PM_{2.5}$ concentration have not always found to be highly toxic. For example sample #28 (Table 2) having $PM_{2.5}$ concentration of 30 ppm (59 μg/m^3) had higher IC_{50} of 0.8 ppm whereas sample #32 having $PM_{2.5}$ concentration of 66 ppm (128 μg/m^3) has been found more toxic with its IC_{50} at 0.14 ppm. Similarly samples #4 and #5 have behaved in this manner having IC_{50} of 25.1 and 12.2 mg/L, respectively (Table 2). Which means that the particulate mass of individual sample does not always represent the mass of toxic metals present in that particular sample. $PM_{2.5}$ mass contributions from secondary particles, diesel, and two-stroke emission have shown higher contributions to $PM_{2.5}$ concentrations when the particle mass concentrations were greater than 200 μg/m^3 (Zelikoff et al., 2002). The $PM_{2.5}$ measurements (May 2008-April 2009) have been less than 200 μg/m^3 which indicate contribution of distant sources rather than the immediate local ones. These sources could include coal combustion and industrial emissions having

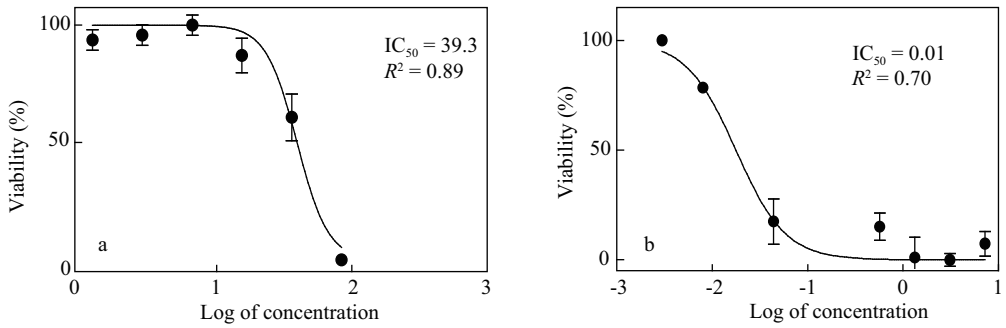

Fig. 6 Dose response of sample #5 (a) and sample #3 (b).

higher contributions to $PM_{2.5}$ mass particularly when the particle mass concentrations were lower than 100 μg/m^3. Therefore, it can be concluded that at Lahore, $PM_{2.5}$ mass is contributed by fine fraction of particles originating from distant industrial sources which are rich in heavy metals and can travel long distance before deposition.

Considering the chemical analysis of water soluble fraction, samples with higher concentration of heavy metals such as sample 05, had higher IC_{50} value compared to those samples with lower metal concentrations (Tables 1 and 2).

3 Conclusions

Toxic materials present in airborne particles are known for adverse health effects. The magnitude of health varies with their type and concentration of the toxic material one is exposed to. In this study, it is investigated that an aqueous solutions of ambient particles, collected at an urban site in Lahore, Pakistan from May 2008 to April 2009 varied with respect to heavy and trace metal contents, cytotoxicity for respiratory epithelial cells, and induction of pro-inflammatory cytokine expression. The data supported the argument that respiratory health effects are controlled by the variation in particle source and it chemical composition as well as in exposed concentration. Our study provided sufficient evidence as to the importance of size and composition of $PM_{2.5}$ in *in vitro* cytotoxic and toxicological mechanism. The water soluble constituents such as transition metals are known to be absorbed onto the surface cavities of $PM_{2.5}$ and can be released into lung alveoli and deposit in the lung parenchyma. This has clear implications for future regulations; identifying of composition of the $PM_{2.5}$ samples that mediate toxicity which allow regulatory efforts to potential sources that shares the most toxic particles.

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