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RESEARCH ARTICLE

Role of metals in free radical generation and genotoxicity induced by airborne particulate matter (PM$_{2.5}$) from Pune (India)

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Abstract In the present study, metal-facilitated free radical generation in particulate matter (PM) and its association with deoxyribonucleic acid (DNA) damage were studied. The examined data showed that the concentration of fine PM in Pune exhibited seasonal variations. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) was used to examine the metal composition, which showed the presence of metals such as Cu, Zn, Mn, Fe, Co, Cr, Pb, Cd, and Ni. Fe metal was present in the highest concentrations in both the seasons, followed by Zn. The scanning electron microscopy-energy-dispersive spectrometer (SEM-EDS) results also demonstrated that the fine PM particles deposited in summer samples were less than those of winter samples, suggesting that the PM load in winter was higher as compared to that in summer. Elemental mapping of these particles substantiates deposition of metals as Fe, Zn, etc. on particles. The electron paramagnetic species (EPR) technique was utilized for free radical detection, and plasmid DNA assay was utilized to study the genotoxicity of ambient fine PM. Obtained g values show the presence of radicals in PM samples of Pune. PM contains the C-centered radical with a vicinal oxygen atom ($g = 2.003$). In addition to this, the g value for Fe was also observed. Therefore, we intend that the radicals related with fine PM comprise metal-mediated radicals and produce DNA damage. The plasmid DNA assay results indicated that the TM$_{50}$ values (toxic mass of PM causing 50 % of plasmid DNA damage) of PM exhibited seasonal variations with higher TM$_{50}$ values for summer and lower TM$_{50}$ values during winter.

Introduction

Particulate matter (PM) is one of the precarious air pollutants, which consists of a mixture of solid and liquid particles suspended in the air, varying in size, shape, surface area, morphology, solubility, origin, and chemical, physical, and biological characteristics (Mouli et al. 2003; Satsangi and Yadav 2014; Pipal et al. 2014). Epidemiological studies have shown the relationship between PM exposure and increased mortality and morbidity due to cardiovascular and respiratory diseases (Fierro et al. 1996, Abbey et al. 1995, Pope et al. 1995; Ghauri et al. 2012; Pope et al. 2002; Nawrot et al. 2007; Gotschi et al. 2008; Hsieh et al. 2011; Hung et al. 2012). Researchers have agreed that for a health-related aerosol study, probably fine (PM$_{2.5}$) particles would be the more appropriate size than coarse mode particles (PM$_{10}$) (Kupelwieser et al. 2010). Fine PM is considered to be more hazardous than larger-size PM due to its longer dwelling time in the atmosphere,

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and also, it can easily be deposited in the lungs in the course of respiration consequentially leading to many diseases. These $PM_{2.5}$ particles generally are of alveolar size range, thus able to penetrate into the deep area of the lungs, i.e., the alveolar region, leading to several well-documented health effects including cardiovascular diseases. Moreover, more toxic substances are absorbed by fine particles as compared to coarse particles (Ormstad 2010; Peters et al. 2001; Makkonen et al. 2010). Once deposited in the respiratory system, the action of PM depends on their chemical composition and toxicity.

According to some researchers, more than one constituent of PM may be responsible for its toxicity (Donaldson et al. 1998; Dreher 2000). These toxic substances include transition metal content, organic content, particle size and surface, free radicals, presence of endotoxins, or other biologicals (Dreher 2000; Donaldson et al. 1998; Monn and Becker 1999; Monn et al. 2002; Molinelli et al. 2006; Niu et al. 2010; Massey et al. 2013). According to the WHO nationwide analysis report in 2002, the main cause of death was chronic respiratory diseases (WHO 2002). Maharashtra State Health Resource Centre also published a report in March 2010 to examine the peak ten death causes in Maharashtra in which they found that chronic obstructive pulmonary disease (COPD) was the highest cause of death (Health Status Maharashtra 2010). Smoking is the major reason for COPD, but one of the reasons for COPD is also the fine particulate matter emitted by combustion sources and vehicular emissions. A significant association between outdoor air pollution and COPD admissions has been observed by several studies in the USA, in Europe, and worldwide (Ko and Hui 2012).

Pune is one of the most rapidly growing metropolitan cities of the country in Maharashtra, India, due to the establishments of various small- and large-scale industries like glasswork, electronic items, steel, rubber, agricultural products, etc. The industrial area covers nearly 10,000 industries that include TATA, Bajaj, Thermax, L&T, etc. (Sonaje et al. 2013). In addition to the faster rate of industrialization, it is emerging as an IT park city because it provides a source of employment and education resulting in the immigration of people from other states to it (Gidde and Sonawane 2012). Thus, Pune now has developed into a big city with more than three million inhabitants. The rapid rise in urbanization and population has tremendously increased the vehicular count by 54 % in the city from 16,22,675 (2008) (Gidde and Sonawane 2012) to 29,83,749 (2015) (Times City, 9 Dec 2015). Due to these facts, the air quality of Pune is getting worse especially in terms of particulate matter.

Although there are several studies on the particulate pollution of Pune, the toxicological studies from this city are almost lacking, clearly pointing towards the existence of a research gap from this city. Keeping this in view, the present study was framed with the aim to assess the genotoxicity of fine PM-bound metals in Pune City by characterizing their concentrations and quantifying the free radical generation and plasmid DNA damage. The study provides significant information on the baseline data and the importance of transition metals in the generation of free radicals from ambient PM and their potential health risks in the Pune urban area.

Material and methods

Sampling and analysis

Pune (18° 32′ N, 73° 51′ E) is located on the western margin of the Deccan plateau, 560 m (1840 ft) above sea level. It is situated on the leeward side of the Sahyadri mountain range, which forms a barrier between the Arabian Sea (Satsangi and Yadav 2014). The city is surrounded by hills and mountains. Just outside the city, Sinhagad fort is located at an altitude of 1300 m. Pune has a tropical wet and dry climate with average temperatures ranging between 20 and 33 °C (Satsangi and Yadav 2014). In summer, the temperature range was from 28.3 to 39.5 °C and in winter from 10.5 to 23.0 °C. Figure 1 shows the map of the sampling site. A detailed site description has been given elsewhere (Satsangi and Yadav 2014).

Sampling of fine PM was carried out at the terrace of the Department of Chemistry, Savitribai Phule Pune University (SPPU), Pune. A major traffic junction (i.e., university circle) and a railway line are nearly 1 and 3 km from the sampling site, respectively. The principal sources of contamination are traffic, biomass burning, and construction activities in and around the vicinity of the sampling site (Yadav and Satsangi 2013). The sampling was carried out at a height of ~12 m from the ground level, from December 2012 to May 2013. PM samples were collected for a period of 24 h on pre-conditioned (48 h at 25 °C and constant relative humidity around 55 %) and pre-weighed polytetrafluoroethylene (PTFE) filters (Whatman, 47 mm diameter, 2 μm pore size) using a Mini Vol - TAS air sampler (Airmetrics Co. Inc., 5 L min^{-1} flow rate, sample volume of 7.2 m^3) provided with $PM_{2.5}$ cutoff inlets. A total of 49 samples were collected in winter and 53 samples in the summer season (Yadav et al. 2015). After particle collection, the filters were preserved in the refrigerator at 4 °C till the analysis. In the present study, the filter was cut into two equal halves; one half of the filter was used for trace metal analysis and the other half for plasmid DNA assay, electron paramagnetic species (EPR) study, and scanning electron microscopy (SEM) analysis.

Gravimetric analysis

PM masses were calculated gravimetrically by subtracting the initial mass of the blank filter from the final mass of the sampled filter. The filters were weighed repeatedly (Shimadzu AUX 220;
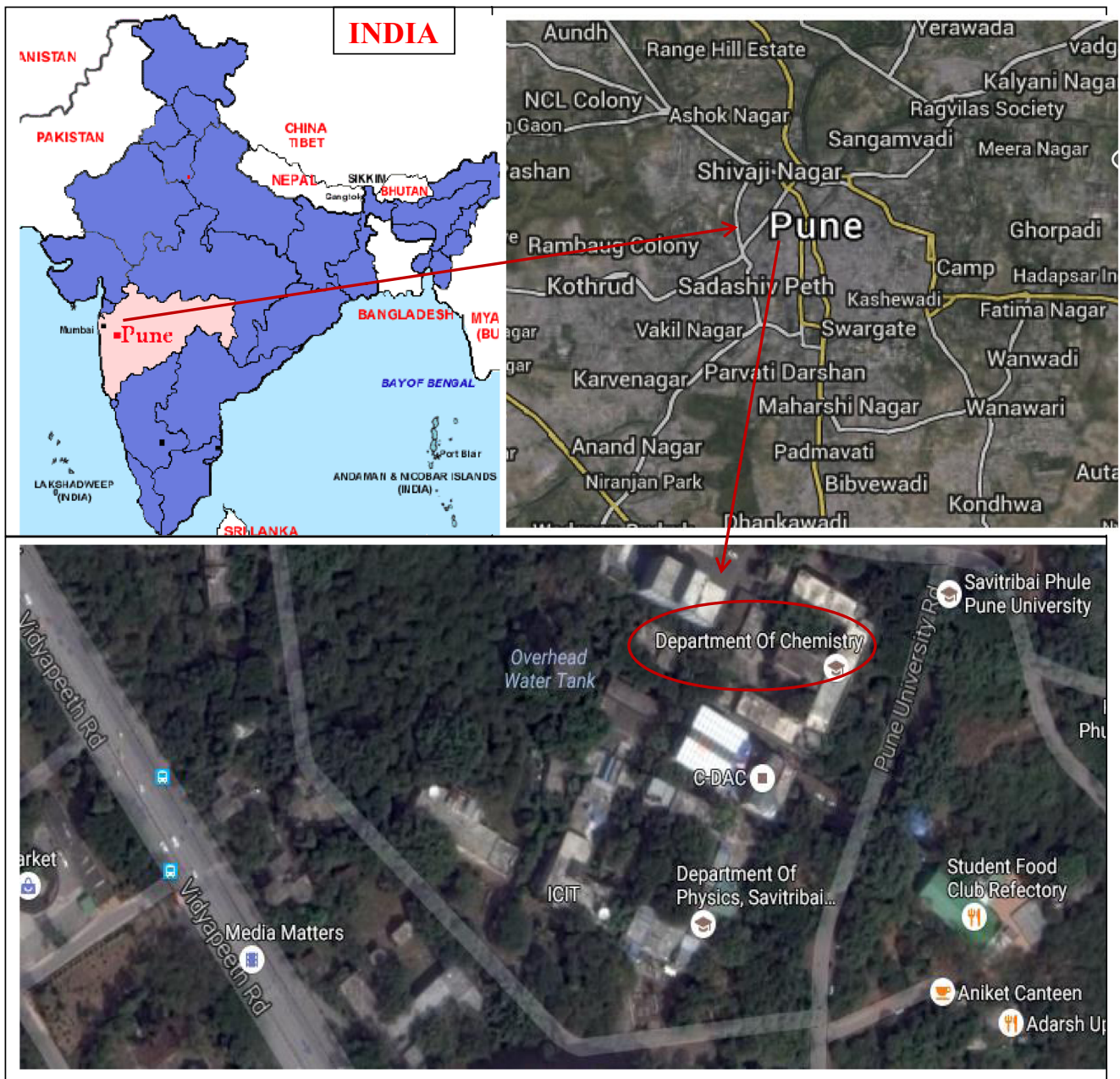
Fig. 1 Map showing sampling site

sensitivity ±1 μg) till three reproducible values were obtained (Yadav et al. 2015). The filters were equilibrated in silica gel desiccators at 25 °C temperature at a relative humidity of 35–45 % for 24 h before weighing to eradicate the effect of humidity and also to gain accurate particulate matter measurements. The filters were handled with tweezers coated with Teflon tape to reduce the risk of contamination (Yadav et al. 2015).

Trace metal concentration analysis

In this study, half of the filter samples were extracted using Milli-Q water at room temperature. The filters were

sonicated for 1 h with 15 mL of Milli-Q water (pH ~5.8). This extract was then acidified using ~10 % nitric acid to prevent the loss of metals and stored in clean polypropylene bottles to determine water-soluble metal concentration. In recent years, water is chosen as the simplest and most universal extraction solvent for evaluating the water-extractable fraction of total metals in PM (Heal et al. 2005; Shi et al. 2003; Yadav and Satsangi 2013). An inductively coupled plasma-atomic emission spectrometer (ICP-AES) (ARCOS, Spectro, Germany) was used for analysis of nine metals (Cu, Zn, Mn, Fe, Co, Cr, Pb, Cd, Ni) within a month of extraction.

SEM analysis

Scanning electron microscopy (JEOL JSM-6330F, JEOL Ltd., Akishima, Tokyo, Japan) was used for morphological characterization of fine particles. The samples were cut in sizes of 1 cm^2 from the PM collected sample filter. To make the samples electrically conductive, a thin film of platinum (Pt) was deposited on the surface of the samples (Satsangi and Yadav 2014). These samples were then placed and fixed on electron microprobe stubs. A computer-controlled field emission SEM equipped with an energy-dispersive spectrometer (EDS) was used for analyses of PM samples. In the present investigation, the SEM was used in its most common mode, i.e., emissive mode. Instrumental parameters were as detector, Si (Li); accuracy, 10 %; limit of detection, 500,000; and energy, 20 kV (accelerating voltages, 0.5–30 kV) (Satsangi and Yadav 2014). Particle morphology and particle count were assessed by a quantitative image analyzer (IA) using ImageJ software (Satsangi and Yadav 2014).

EPR analysis

Electron paramagnetic resonance (EPR) was utilized to evaluate the free radicals generated by PM. The filters containing the PM samples were cut into strips, rolled, and inserted in such a way that the PM collected side was the outer part into quartz EPR tubes. The EPR analysis of the same samples was done in triplicate for better detection of g values. A g value is a dimensionless quantity that describes the magnetic moment and gyromagnetic ratio of a particle or nucleus. The EPR spectra were recorded at low temperature using a JES-FA200 ESR spectrometer with the following instrumental conditions: microwave frequency, 9.3 GHz; X-band, 100 kHz; modulation amplitude, 2 G; attenuation, 7 dB; scan range, 400 G; receiver gain, 2500; time constant, 1 s; and scan time, 2 min. The g values were assessed using standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Roy et al. 2015).

DNA toxicity analysis

This assay examines the influence of extracts of fine PM to bring in strand breaks of phage DNA (Dellinger et al. 2001). Particles were separated from the filter substrate by immersing half of the filter in 500 μL phosphate-buffered saline (PBS) in microcentrifuge tubes. The sample was shaken evenly for 6 h and then vortexed for 30 min to discharge the PM into the solution from the filter. This was considered as the stock PM solution. This stock solution was further diluted with PBS to the required concentration. This concentration could be between 50 and 400 μg mL^{-1} depending on the original mass concentration of the PM (Shi et al. 2004; Xiao et al. 2013). Another two blank Eppendorf (EP) microcentrifuge tubes were taken and filled with the negative (unexposed blank filter

Table 1 Detection limits (μg L^{-1}), recovery (%), and reproducibility (mg L^{-1}) for different metals

	Detection limit (μg L^{-1})	Recovery (%)	Reproducibility (mg L^{-1})
Cu	0.6	101.3	1.2
Zn	0.2	96.2	0.43
Mn	0.1	98.2	0.82
Fe	0.5	108.2	0.83
Co	0.5	102.6	0.47
Cr	0.5	106.2	0.35
Pb	2.9	92.3	1.2
Cd	0.2	101.8	0.14
Ni	0.5	102.9	0.58

extracted in PBS) and positive (0.04 % H$_2$O$_2$) as controls, respectively. To all the experiment solutions as well as control solutions, 1 μL (200 ng mL^{-1}) of phiX174 supercoiled phage DNA (Bangalore Genie) was added. The microcentrifuge tubes were then incubated at room temperature by gently vibrating for 1 h onto a vortex (Vortex Genie2). After this, 3 μL of loading dye (bromophenol blue) was added to the above solution and homogenized. Then this solution was loaded in the gel wells and was run on 0.6 % agarose gel in 1 % tris-borate-EDTA (TBE) buffer for 6 h at 35 V. The gel was stained with ethidium bromide. Finally, the gels were visualized and photographed by computerized Geldoc (EC3 Chemi HR 410 Imagining System). The degradation and strand breaks of DNA were determined by the conversion of supercoiled øX174 DNA to the linear forms as previously reported by others (Kumagai et al. 1996, Senlin et al. 2008, Xiao et al. 2013). A quantitative procedure was set up to measure the relative proportion of the DNA damaged in each lane (in terms of a percentage of the total DNA in each lane). Visionworks life science software was used for calculating the percent of DNA in each lane. The PM-
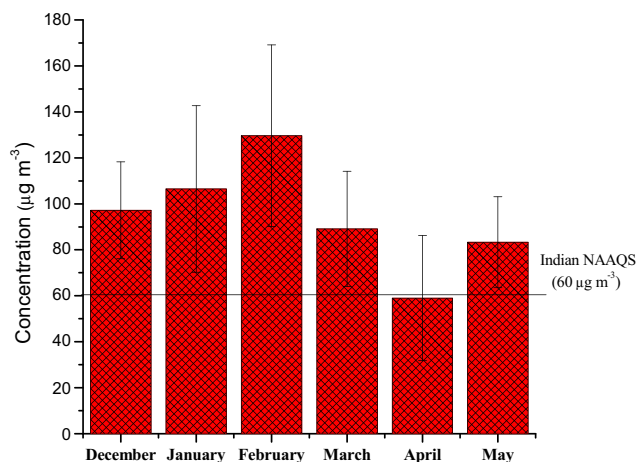
Fig. 2 Monthly variation of fine PM mass concentration at Pune (SPPU)

Table 2 Summary of meteorological parameters in Pune

Month	Temp (°C)	RH (%)	WS (km h^{-1})	PWD	WD (°)
Dec 2012	21.19	77.25	0.62	SSE	168.14
Jan 2013	19.85	65.71	0.52	SSW	197.98
Feb 2013	23.39	61.13	0.97	S	189.02
Average winter	23.81	67.36	0.71	S	185.05
Mar 2013	28.25	42.75	1.25	SSW	197.81
Apr 2013	30.06	49.25	1.96	SW	228.26
May 2013	36.50	52.06	3.23	SW	235.86
Average summer	31.60	47.35	2.15	SW	220.64

Temp temperature, *RH* relative humidity, *WS* wind speed, *PWD* prevalent wind direction, *WD* wind direction

induced DNA damage rate was calculated by subtracting the damage caused by the negative control. A regression equation was used to calculate the PM mass causing 50 % damage (i.e., TM_{50} toxic mass causing 50 % damage) to supercoiled DNA. The experiment was performed in triplicate to reduce the uncertainty on the PM dose.

Quality control parameters

A blank test for background contamination was performed by using unexposed filter papers, which were handled with field samples. The blank filters were stored and taken to the field to replicate the same atmosphere as the actual field samples used in the measurements. The blank filters were taken thrice during the season (Yadav and Satsangi 2013). After particle collection, the filters were well preserved in the refrigerator at 4 °C till the analysis. In this study, the background contamination was used to correct measurements. Field blank values were subtracted from the metal concentration to determine background contamination. Field blank values were very low, usually below or near the method detection limits. The recovery efficiencies were also calculated by spiking 10 % of the samples with a known amount of metal. The minimum detection limit and recovery test results are presented in Table 1 for all the metals. The recovery efficiency range was 92.3–108.2 %. Analysis for the same standard solution was repeated ten times for the reproducibility test, which verified the constancy of instruments.

For DNA assay, several batches of experiment were performed with different concentrations of samples collected throughout the study along with the negative (DNA + PBS) and positive (DNA + PBS + H_2O_2) controls. The experiment was performed in triplicate to reduce the uncertainty on the PM dose.

Results and discussion

Mass concentration of particulate matter

Mass concentrations of the exposed PM sample were gravimetrically determined during the period of Dec 2012 to May 2013 in the Department of Chemistry, Savitribai Phule Pune University (SPPU). The daily mass concentrations of particulate matter at this site varied from 59.8 ± 27.2 to 139.6 ± 38.6 µg m^{-3}. As depicted in Fig. 2, the concentrations of PM in the winter season (average = 111.1 ± 32.2 µg m^{-3}) (Dec–Feb) were about 1.4 times higher than the concentrations in summer months (average = 77.15 ± 24.03 µg m^{-3}) (March–May). The seasonal and monthly variabilities of the PM concentration were influenced by both the local anthropogenic activities and meteorological factors. The PM concentration may go up to numerous times higher than the normal level under poor meteorological conditions, i.e., inversion conditions (Yadav et al. 2015; Bathmanabhan and Madanayak 2010; Elminir 2005). The higher mass concentration was

Table 3 Comparison of the mass concentration of the present study with that of other studies

Place	Fine PM (µg m^{-3})	Site characteristics and season	Reference
Pune	111.1 ± 32.2 77.15 ± 24.03	Urban, winter Summer	Present study
Chennai	66.9 42.1	Urban, winter Summer	Srimuruganandam and Nagendra 2011
Agra (India)	185.7 90.1	Urban, winter Summer	Pipal et al. 2014
Raipur (India)	225.1 ± 53.6 97.4 ± 45.7	Urban, winter Summer	Deshmukh et al. 2013

observed in the winter season as compared to the summer season which can be explained on the basis of physical parameters like temperature (temp.), relative humidity (RH), and other meteorological variations (i.e., wind speed and direction) (Table 2). The wind speed and temperature were found to be low in the winter and gradually increased during summer. In winter, the average relative humidity was also comparatively high (67.36 %), contributing to higher collection of fine PM. Moreover, due to a lower mixing height, the dispersion of fine and submicron particulates is restricted and results in trapping of the particles nearer to the ground level (Salve et al. 2006; Yadav and Satsangi 2013) which leads to an elevated concentration of fine PM in the winter season. In addition, a high concentration of PM during winter can be due to biomass burning (excess of solid waste viz. waste paper, fallen leaves, and garden cuttings are burned by the side of roads) and increased heating activities in the proximity of the site (Yadav et al. 2015) The National Air Quality Standards (NAAQS) for ambient fine PM ($PM_{2.5}$) in India are set at 60 $\mu g\ m^{-3}$. The average concentration of the present study (94.1 ± 31.2 $\mu g\ m^{-3}$) was found to be 1.5 times higher than the NAAQS. The PM concentrations are much higher (3.7 times) than WHO standards (25 $\mu g\ m^{-3}$).

Fine PM mass concentrations in the present study were compared with earlier reported mass concentrations in India with similar locations (i.e., urban) (Table 3). The PM mass concentrations reported by Pipal et al. (2014) at Agra and Deshmukh et al. (2013) at Raipur were higher as compared to those of the present study, whereas the $PM_{2.5}$ mass concentration reported by Srimuruganandam and Nagendra (2011) in Chennai were less than those of the present study.

Trace metal concentrations and their source apportionment

A total of nine elements (Cu, Zn, Mn, Fe, Co, Cr, Pb, Cd, Ni) in the fine PM were analyzed by ICP-AES. Figure 3 shows the concentrations of metals distributed in PM during winter (Dec to Feb) and summer months (March–May) The concentration of trace metals in the winter season ranked in the following order: Fe > Zn > Cr > Cu > Pb > Mn > Cd > Co > Ni while in summer as Fe > Zn > Pb > Cr > Cu > Mn > Co > Ni > Cd. Fe was the most abundantly present metal in both the seasons with concentrations of 2.41 and 1.54 $\mu g\ m^{-3}$ in winter and summer, respectively. Principal component analysis was applied on the dataset of metals in PM for the source identification. Table 4 represents three rotated factor loadings explaining more than 82.4 % of total variance for PM. Factor 1 corresponds to 34.1 % loading with the components of Cu, Co, and Cr which can be accredited to road traffic emission. Cu is associated with the wearing of brakes and an automobile's oil pump or corrosion of metal parts coming in contact with oil. Cr is an important constituent of many metal
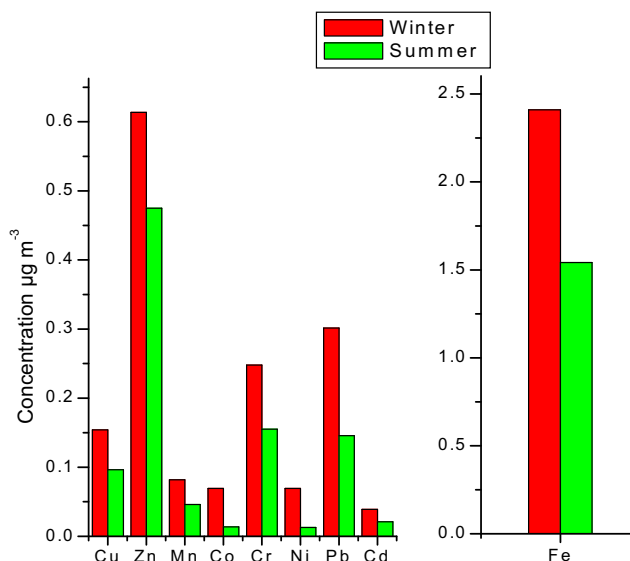

Fig. 3 Trace metal concentration ($\mu g\ m^{-3}$) in fine PM in Pune

alloys. It is also contained in cement and originating from construction activities (Chen et al. 2011), which were carried out in the vicinity of the sampling sites as new buildings are under construction in the university. The second factor 27.5 % shows high loading for Zn, Fe, Ni, and Cd which can be accredited to road dust. Its large milieu is soil and some other sources from vehicles such as tier stride, drum brake graze, etc. (Yadav and Satsangi 2013). Galvanized materials, wear of tires, and the usage of zinc compounds in rubber production could be probable sources of Zn. Fe is mostly emitted from soil (Yadav and Satsangi 2013). Ni is used as a fuel oil marker. Cd is linked with diesel engines and the taxing of brakes. The third factor 20.8 % shows high loading of Pb, Fe, and Zn which may be associated with resuspended road dust and fossil fuel combustion from vehicles. From the year 2000, Pb has

Table 4 Principal component analysis after varimax rotation for the trace elements analyzed in fine PM

	Factor 1	Factor 2	Factor 3
Cu	*0.990*	−0.102	−0.036
Zn	−0.147	*0.539*	*0.501*
Mn	−0.224	−0.254	*−0.526*
Fe	−0.142	*0.508*	*0.759*
Co	*0.990*	−0.100	−0.039
Cr	*0.995*	0.021	−0.008
Ni	0.023	*0.961*	0.028
Pb	−0.125	−0.279	*0.872*
Cd	−0.110	*0.919*	0.102
% variance	34.1	27.5	20.8
Ascribed sources	Constructional and vehicular activities	Road dust	Resuspended road dust and fossil fuel

Factor loading above 0.5 are denoted with italic font

Table 5 Oxidative damage induced by airborne fine PM collected in winter (Dec–Feb) and summer (March–May) seasons at Pune

Months	Sample	TM_{50} ($\mu g\ mL^{-1}$)	Months	Sample	TM_{50} ($\mu g\ mL^{-1}$)
December	1	100 ± 3.8	March	1	200 ± 3.8
	2	98 ± 4.3		2	109 ± 2.6
	3	200 ± 6.2		3	205 ± 3.5
	4	132 ± 5.1		4	171 ± 4.5
	Mean	132.5 ± 47.6		Mean	171.3 ± 44.1
January	1	160 ± 4.6	April	1	200 ± 4.0
	2	102 ± 2.0		2	249 ± 2.5
	3	99 ± 2.5		3	165 ± 4.4
	4	110 ± 2.9		4	122 ± 3.2
	Mean	117.7 ± 28.2		Mean	184.2 ± 53.7
February	1	105 ± 3.1	May	1	250 ± 3.6
	2	120 ± 4.0		2	210 ± 4.0
	3	104 ± 2.5		3	260 ± 3.5
	4	121 ± 3.1		4	200 ± 1.5
	Mean	112.5 ± 9.2		Mean	230 ± 29.4

been phased out of gasoline in India, but still, it perseveres in road dust from previous vehicular exhaust release due to its extensive dwelling time in the environment (Yadav and Satsangi 2013; Kulshrestha et al. 2009).

PM induced oxidative stress and DNA damage

To determine the PM-induced oxidative stress on DNA, phage phiX174 was selected and analyzed by plasmid assay. Percent

Fig. 4 Plasmid DNA assay— phiX174 DNA damage after exposure to different concentrations of PM$_{2.5}$ during **a** winter and **b** summer seasons along with **c** graphical representation of percentage DNA damage
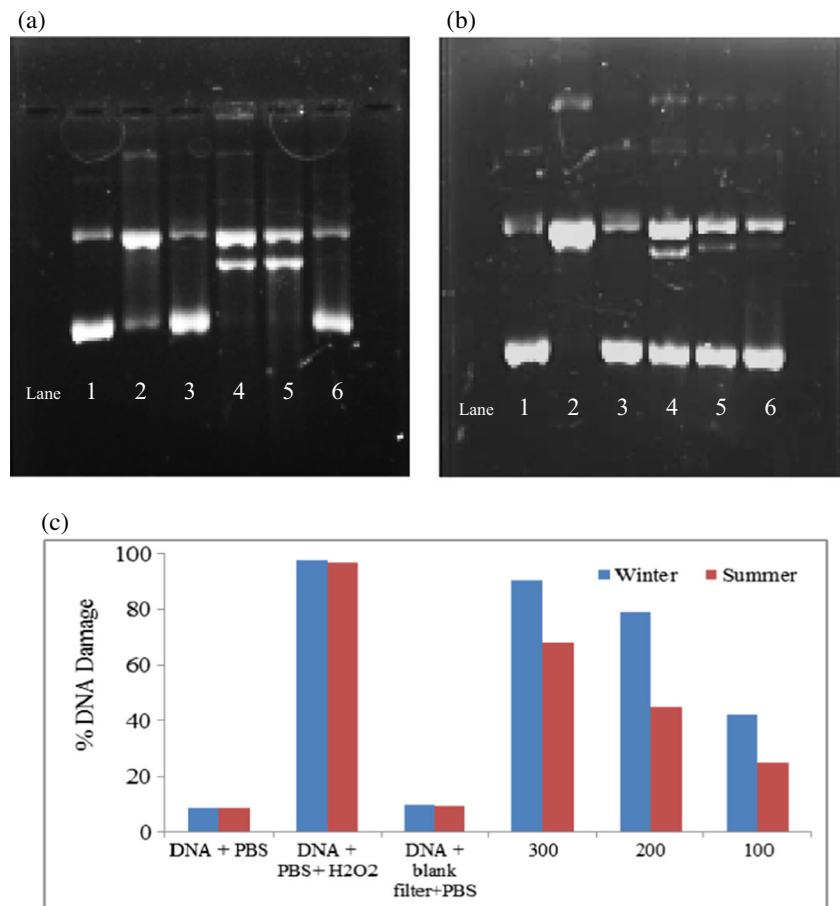
DNA damage and its TM_{50} (by linear regression) values were calculated for each plot. The term TM_{50} can be defined as particle mass causing 50 % damage to DNA. A lower TM_{50} value expresses a higher oxidative capacity or genotoxicity due to PM (Table 5). Several batches of experiment were performed with different concentrations (50 to 400 µg mL^{-1}) of samples collected throughout the study along with the negative (DNA + unexposed filter in PBS) and positive (DNA + PBS + H_2O_2) controls. The representative images of the DNA assay are shown in Fig. 4a, b which depicts the in vitro effects of PM extracts on phage phiX174 supercoiled DNA. Lane 1 in Fig. 4a, b shows the experiment with untreated DNA + PBS, lane 2 shows the positive control (i.e., with 0.04 % H_2O_2 which shows the entire DNA damage), lane 3 shows extracts from an unexposed filter incubated with DNA (negative control),

and lanes 4 to 6 depict DNA treated with different concentrations (100, 200, 300 µg mL^{-1}) of extracts of winter and summer fine PM.

In general, overall DNA damage for winter PM particles was between 40 and 90 % whereas for summer it was between 20 and 70 %. For comparison purposes, such PM extracts from both winter and summer seasons were selected and diluted to nearly same concentrations to detect the toxic content in PM samples. As depicted in Fig. 4a, b, at 300 µg mL^{-1} concentration, the DNA damage was found to be 90 and 68 % by PM samples collected in winter and summer seasons, respectively. In winter, 78 % of DNA damage was observed at 200 µg mL^{-1} PM concentration while summer samples exhibited 1.8 times (i.e., 45 %) lower damage than winter samples. At 100 µg mL^{-1} of PM concentration, DNA damage was found to be 42 and 25 % for winter and summer, respectively.


Fig. 5 SEM micrographs of PM loading in summer (**a**–**c**) and winter (**d**–**f**)
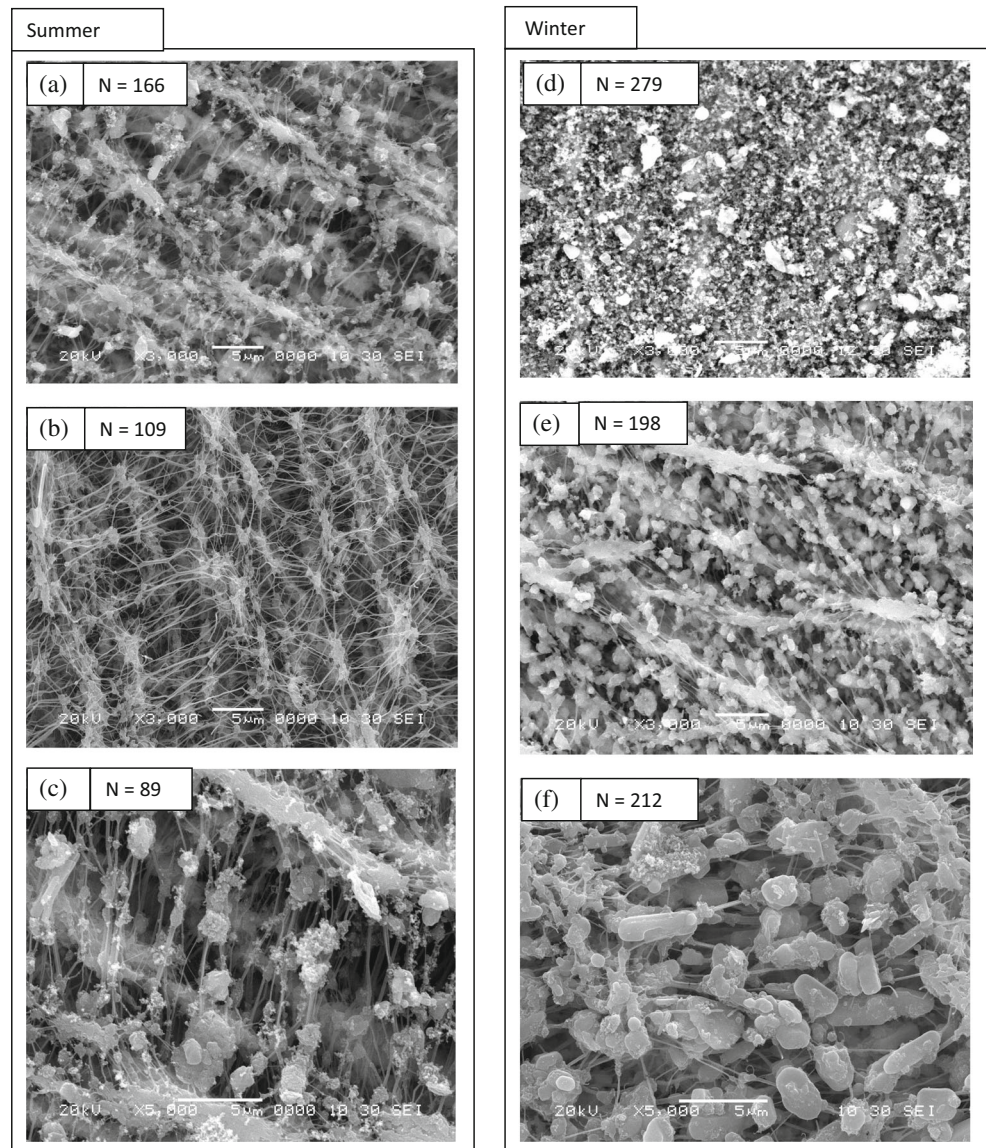
Meager DNA damage was observed even at 400 µg mL^{-1} for some summer samples (data not shown). All these data showed that at the same dosage of particles, the overall oxidative ability of the winter PM samples was higher than that of the summer samples. One of the possible reasons for this damage can be corroborated with the presence of a high concentration of metal content in the PM. The total concentration of metals was found to be 3.9 and 2.7 µg m^{-3} in winter and summer seasons, respectively. SEM-EDS analysis of several samples was done and representative graphs are shown in Fig. 5. The SEM-EDS results also demonstrated that the number and portions of fine PM particles in the summer samples (Fig. 5a–c) were less than those of the winter samples (Fig. 5d–f) suggesting that the PM load in winter was higher as compared to that of summer. The SEM-EDS mapping technique also confirmed the presence of different metals such as Fe, Zn, etc., in PM. As depicted in Fig. 5, masses of aggregated particles were observed which contained minerals with various shapes such as spherical, cuboids, soot aggregates, and fly ashes as main components in PM which can easily enter the respiratory tract. Elemental mapping of these particles substantiates deposition of metals as Fe, Zn, and Ti on the particles (Fig. 6). Metals such as Fe, Cu, Cr, and Ni can act as pro-oxidants and can produce Fenton-type reaction products along with the oxidative stress in biological systems, thus leading to oxygen-promoted DNA damage (Shi et al. 2003; Donaldson et al. 2005; Bräuner et al. 2007) leading to heritable mutations (Risom et al. 2005; Peters et al. 2006). These mutations may result from the chemical modification of histones (or binding proteins that back the structure of

Fig. 6 SEM images of the particles and their elemental mapping (a and b)
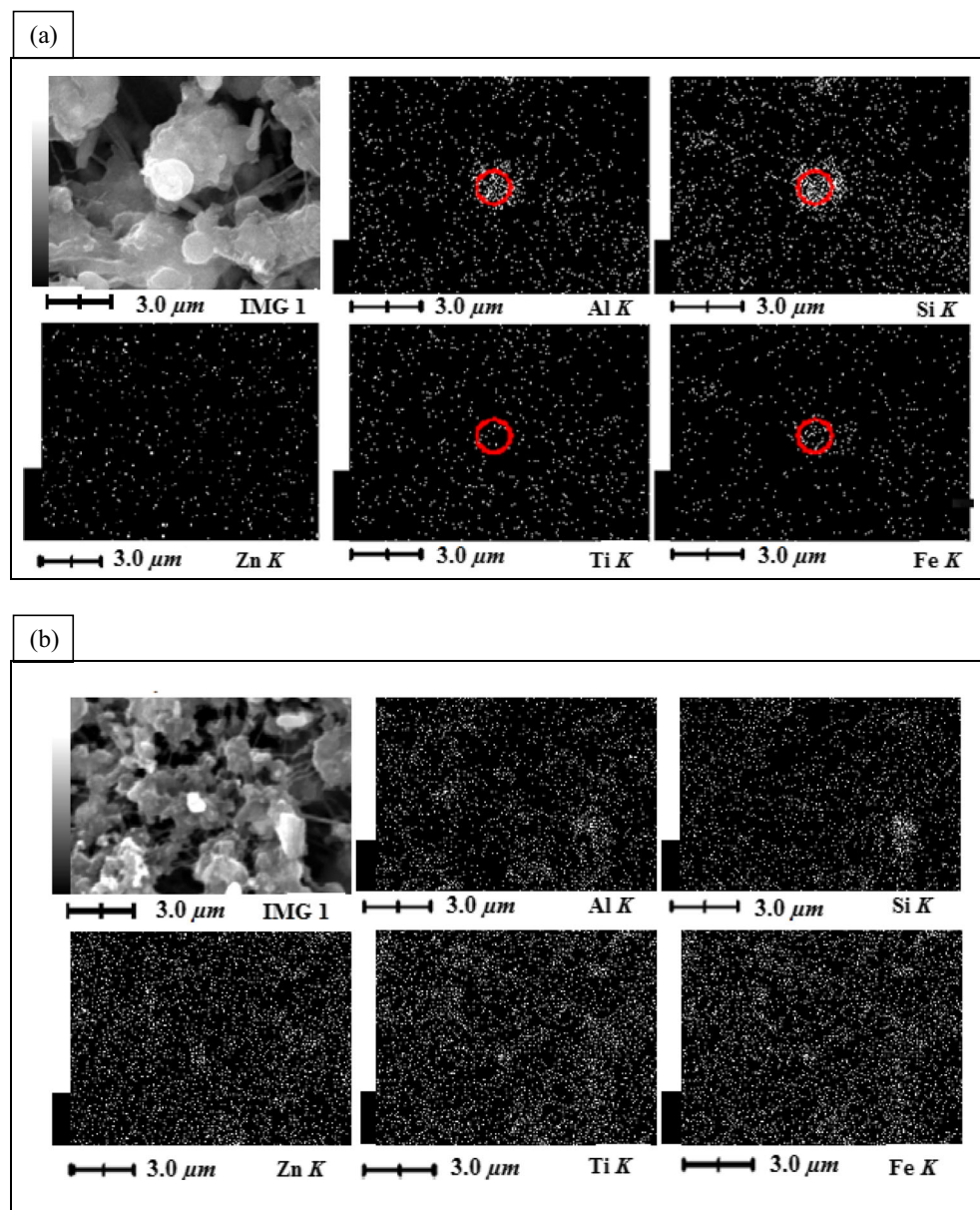
supercoiled DNA) which opens the coiled DNA to linear and allows its alteration (Donaldson and Ston 2003). The pyrimidine and purine base modifications are the major steps towards DNA damage by radicals, which leads to breaking of the DNA strand, cross-linkage of DNA-protein, and its basic site (Ji et al. 2005; Schyman et al. 2008). In addition to soluble metals, PAH or quinones also play an imperative role in oxidative damage (Li et al. 2008). Radical generation may be facilitated by metals in PM. As the present study is focused on metal concentrations and their genotoxicity, EPR was employed to assess which metal plays a vital role in the generation of free radicals. All EPR analyses were done at low temperature (77 K). Several samples were analyzed by EPR and showed similar peak patterns and g values, so representative graphs are shown in Fig. 7a–d. Figure 7a shows EPR spectra of field blank filters with no EPR signals (Roy et al.

2015). Figure 7b–d shows the EPR spectra of atmospheric fine PM along with their g values as 1.99 (i.e., ~2.0), 2.024, 2.0031, 2.0032, and 2.0036. EPR g values may change from one sample to another; this change depends on the number of paramagnetic species interactions present in that particular sample. The intensity of the signal is proportional to the amount of radicals released. A g value corresponding to 2.0031 (Fig. 7b) and 2.0032 (Fig. 7c) in fine fraction suggests the presence of free radicals in it. In the present study, a C-centered radical with a vicinal oxygen atom ($g = 2.0031$, 2.0036) was observed. It has been reported that C-centered radicals have a g value approximately near that of a free electron whereas C-centered radicals with a neighboring oxygen atom have slightly high g factors in the range of 2.003–2.004 (Tian et al. 2009; Dellinger et al. 2007). In general, carbon-centered free radicals are reactive when are in the company of
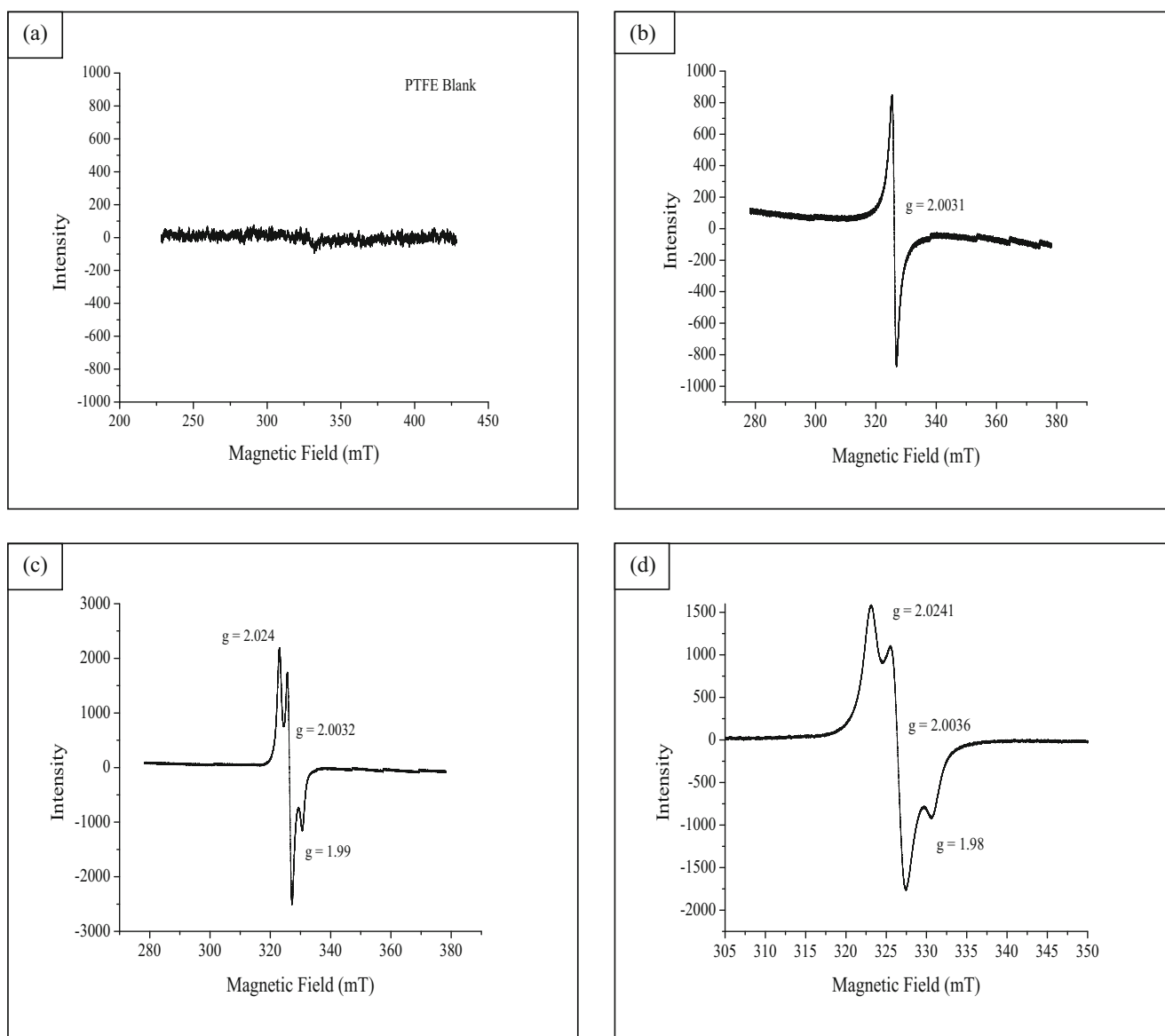

Fig. 7 EPR spectra of unexposed filter (a) and exposed $PM_{2.5}$ filter paper (b–d)

Table 6 Correlation coefficients between the TM_{50} values of fine PM and the concentration of metals in Pune

	TM_{50}	Cu	Zn	Mn	Fe	Co	Cr	Ni	Pb	Cd
$PM_{2.5}$	1.0	0.265	-0.797^{**}	0.374	-0.832^{**}	0.044	-0.519^{*}	-0.512	-0.424	-0.425

Statistical analysis (Spearman's test) performed using SPSS 10.0

*Correlation is significant at the 0.05 level (two-tailed)

**Correlation is significant at the 0.01 level (two-tailed)

living cells and are thus important for a biological system. Carbon-centered radicals are found to cause DNA alkylation, thus leading to DNA damage (Augusto 1993). In addition to this, a *g* value of 1.9 (~2.00) shows the presence of Fe (Fig. 7c). This suggests that in current study metals, particularly, Fe plays a vital role in the oxidant-generating activity. Available Fe can generate hydroxyl radicals via the Fenton reaction. The Fe concentration (2.41 $\mu g\ m^{-3}$ in winter and 1.54 $\mu g\ m^{-3}$ in summer) in Pune fine PM samples was higher than that of other metals which suggests that Fe may be responsible for DNA damage through radical generation. Many studies also reported that Fe is frequently related to oxidative damage (Valavanidis et al. 2000; Han et al. 2001; Di Pietro et al. 2009; Charrier and Anastasio 2011), and soluble Fe influences the oxidative capacity by the Fenton reaction (Shi et al. 2003; Risom et al. 2005; Nawrot et al. 2009). Numerous other "Fenton active" transition metals like Cr, Cu, etc. which typically arise in ambient PM are also known to induce $OH^{.}$ formation (Donaldson and Ston 2003; Shi et al. 1999). As the EPR study did not show any *g* value related to these metals, it has been ruled out from the discussion.

Moreover, plasmid assays of PM samples in the present study illustrate that TM_{50} (i.e., toxic mass causing 50 % DNA damage) of the majority of the winter samples are lower than those of the summer samples. Table 5 depicts the mean TM_{50} values of particulate matter in winter and summer seasons. It is inferred from the table that TM_{50} for the winter season: $132.5 \pm 47.6\ \mu g\ mL^{-1}$ (Dec 2013), $117.7 \pm 28.2\ \mu g\ mL^{-1}$ (Jan 2014), and $112.5 \pm 9.2\ \mu g\ mL^{-1}$ (Feb 2014) are lower than the TM_{50} values observed in the summer season: $171.3 \pm 44.1\ \mu g\ mL^{-1}$ (March 2014), $184.2 \pm 53.7\ \mu g\ mL^{-1}$ (April), and $230 \pm 29.4\ \mu g\ mL^{-1}$ (May). In general, low TM_{50} values show the high bioreactivity of PM (Shi et al. 2003; Xiao et al. 2013). Thus, it can be inferred that the bioreactivity of the winter PM samples is in general higher than that of the summer samples of Pune. These data further support the air quality of Pune was gravely polluted in winter (as shown in Fig. 2). When compared with other studies, we found that our study is in accordance with Shi et al. (2003) and Xiao et al. (2013) who also reported high oxidative damage and toxicities by winter samples. TM_{50} values were correlated with concentrations of total metals analyzed, and significant negative correlation was observed (Spearman correlation coefficient of -0.860) which

suggests that metals in PM are responsible for DNA damage to a great extent. Further, the TM_{50} was also correlated with the individual metals in fine PM to assess the contribution of particular metals in DNA damage (Table 6). Strong negative correlation was observed between TM_{50} and Fe which further indicates that Fe is responsible for the plasmid DNA damage. Beside Fe, Zn is another metal which showed statistical negative correlation (TM_{50}: Zn = -0.79). Zn has been considered as a bioactive element (Adamson et al. 2000; Richards et al. 1989) which can affect enzyme formation in an organism (Senlin et al. 2006). In the present study, Cu, Cr, Mn, and other such metal ions behave analogous in oxidant engendering activity but they are present in low concentration. Thus, the data shown by ICP-AES, EPR, SEM, and plasmid DNA assay together suggest that Pune PM shows significant genotoxicity.

The present study has some limitations. PM sources contribute to the total PM exposure as other pollutants viz. PAH, black carbon/soot particles, and mixture effects have not been assessed. Thus, further work on the effects of pollutant mixtures and secondary aerosols should allow better understanding of the pollution components and sources responsible for the genotoxic effects of air pollution exposure with more data points. Overall, this particular study is expected to present an important database for genotoxicity of PM which would be essential for the policy makers and environmentalists for proper planning.

Conclusion

The average daily mass concentrations of fine (PM$_{2.5}$) particulate matter in the present study varied from 59.8 ± 27.2 to $139.6 \pm 38.6\ \mu g\ m^{-3}$. The concentrations of fine PM in the winter season (Dec–Feb) were about 1.4 times higher than the concentrations in the summer months (Mar–May). The lowest physical concentration was observed in the month of April ($59.8\ \mu g\ m^{-3}$). High temperature and wind speed might be the cause of the lower levels of fine PM at these particular months. During the winter season, average relative humidity was also comparatively high (67 %), contributing to higher collection of fine PM. Beside the meteorological parameters, the high concentration of PM during winter can be due to biomass burning (excess of solid waste together with mostly garden cuttings, fallen leaves, and paper are burned by the side of roads) and increased heating activities. The ICP-AES

analysis indicated that, in the Pune PM samples, the Fe concentration was much higher than other metals in both winter (2.41 μg m^{-3}) and summer (1.54 μg m^{-3}) seasons. Strong negative correlation was observed between TM$_{50}$ with Fe and Zn. Thus, this suggests that mainly Fe may be responsible for DNA damage through radical generation including other metals such as Zn and Cu. Various sources which have been found responsible for a higher mass and metal concentration were vehicular pollution and combustion of fossil fuel, resuspension of road dust, biomass burning, soil-emitted particles, etc. EPR analysis shows the g value for free radicals and Fe in PM samples of Pune. The g values for a C-centered radical with a vicinal oxygen atom were also observed, which are reactive when they are in the company of DNA and cause DNA alkylation, thus leading to DNA damage. This suggests that in the current study, metals play a vital role in oxidant-generating activity. Therefore, we conclude that the radicals linked with fine PM comprise metal-mediated radicals and produce DNA damage.

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