

Study of metals in radical-mediated toxicity of particulate matter in indoor environments of Pune, India

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Abstract The concentration of coarse and fine indoor particulate matter (PM) was measured along with that of metals (Fe, Cu, Zn, Mn, Pb, Cd, Co, Ni, and Cr) collected from rural and urban residential environments of Pune City with the purpose of examining their free radical generation and its related toxicity. The average mass concentration of indoor PM₁₀ and PM_{2.5} was ~3.3 and ~4.5 times higher than the threshold limits of the Indian National Ambient Air Quality Standards (NAAQS) at urban and rural sites, respectively. The concentration of metals was found to be higher in the fine fraction of PM collected from the rural site. Metal analysis revealed the dominance of Fe concentrations in both PM₁₀ and PM_{2.5} followed by Zn, Ni, and Cu. Electron paramagnetic resonance (EPR) was used to evaluate the presence of free radicals in samples collected from both the sites. EPR spectra for these particles were attributed to both Fe³⁺ ($g = 1.99\text{--}2.0$) and free radicals ($g = 2.0023$ and $g = 2.003$) in PM samples. Dithiothreitol (DTT) assay was also performed which supports the presence of higher redox activity in the fine fraction of PM at the rural site. Significant DNA damage was observed by both fractions of PM when tested in the controlled experiment, with averages of 43 and 30.9 % for PM₁₀ and 55.3 and 40.5 % for PM_{2.5} at the rural and urban sites, respectively. This study articulates a significant amount of evidence to understand the importance of size and composition (especially metals) of indoor PM in the toxicological mechanism.

Keywords Indoor particulate matter · Free radical · Metals · EPR · DNA damage

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Introduction

Air pollution is a major environmental issue worldwide and recognized as a leading contributor to the global burden of diseases. There are emerging evidences from numerous scientific investigations that bridge the relation between air pollution and human health (Griffiths 2011; Nawrot et al. 2009). One of the most hazardous air pollutants is inhalable particulate matter (PM) which consists of solid and liquid particles dispersed into air, causing millions of deaths per year. There is a plethora of literature on outdoor PM and its consequences on health risk, while research on indoor PM and related health issues is very minimal and has largely been overlooked.

In recent times, the indoor air problem has become a pivotal concern due to the recognition that people spend a majority of their time indoors. Plenty of potential sources of indoor PM can exceed health thresholds and pose increased life risks (Jonathan et al. 2008). The concentration of indoor PM is an assimilation of various factors viz. sources of indoor particles, movement of pollutants from outdoors to indoors, and removal processes (Nagar et al. 2014; Kwangsam et al. 2004). Indoor PM creates a sense of discomfort and hazards which in turn results into various cardiovascular and respiratory lung diseases and ultimately increases the morbidity and mortality (Goyal and Kumar 2013). It is well documented that toxicological characteristics of indoor PM and the redox activity of these particles have been linked to their chemical composition especially transition metals and organic fractions (Deshmukh et al. 2013; Verma et al. 2009). The redox active transition metals have been identified as a priority concern due to their higher bioavailability and greater toxic potential as they influence both direct and indirect (through inflammation) capacities of particles to generate free radicals which are highly reactive (Wiseman 2015). These free radicals arise from both endogenous (metabolic processes, inflammation) and exogenous (environmental factors) sources

(Yang et al. 2015) primarily through three processes: (a) metal-induced free radical production via Fenton reaction, (b) inflammatory responses, and (c) metabolism of xenobiotics by redox cycling processes (Araujo 2011; Sing et al. 2007). Free radicals associated with soluble transition metals are followed by the generation of oxidative stress in airway epithelial cells (Valavandis et al. 2008). This oxidative stress generated by the formation of free radicals by various constituents of PM has been hypothesized as the primary pathway of PM-related health impacts (Yang et al. 2015). Among the various free radicals, the hydroxyl radical is of prime concern (Donaldson et al. 1998) as it is most potent for oxidative DNA damage (Nawrot et al. 2009).

Pune is one of the fastest developing metropolitan cities of the country. Air quality in Pune is deteriorating at a fast pace in terms of PM and becoming a subject of public health concern. Data collected from Central Pollution Control Board (CPCB) India revealed relatively higher levels of PM in Pune, exceeding the prescribed limit of Indian National Ambient Air Quality Standards (NAAQS) ($40 \mu\text{g m}^{-3}$ for $\text{PM}_{2.5}$ and $60 \mu\text{g m}^{-3}$ for PM_{10}) (CPCB 2010). Most of the studies exhibited a higher concentration of PM in the atmosphere of Pune (Sonaje et al. 2013; Satsangi et al. 2014), but few of those have been employed on indoor PM. Alongside, no such research has been found based on the toxicity assessment of indoor air quality in Pune. In order to fill this research gap, the present study is mainly focused on size, chemical composition, presence of free radicals, and toxicity assessment of indoor PM collected from rural and urban sites of Pune City.

Materials and methods

Site description

Pune ($18^{\circ} 32' \text{ N}$, $73^{\circ} 51' \text{ E}$), the cultural capital of Maharashtra, is situated on the western mountain ranges of Deccan Plateau. Pune has tropical wet and dry climate with average temperatures ranging between 20 to 41°C . Due to ample job opportunities in various sectors, the population level of the city is rising day by day. Pune has a mixture of various industries such as agricultural products, steel, glasswork, automobiles, electronic items, and many others. Growing industrializations along with urbanization have resulted in high loads of atmospheric pollution in Pune City.

Sampling and analysis

Sample collection

To study the physical, chemical, and toxicological characteristics of indoor PM, sampling of both PM_{10} and $\text{PM}_{2.5}$ was carried out at two sites of Pune, urban and rural (Fig. 1). Table 1 depicts the detailed sampling profile of the indoor

environments of both urban and rural sites. Urban houses are built with bricks and cement, with good flooring and painting, and rural houses are made up of mud, stones, and cow dung. The rural site is located in a region with relatively low traffic density and lots of greenery, whereas urban houses are situated in an area with high vehicular activities approximately about 2 km away from the sampling site. These urban houses use the conventional method for cooking (LPG), whereas at most of the rural houses, cooking is done mostly with traditional fuel such as wood, coal, oil fuels, and others. A low-volume air sampler (MiniVol TAS, Airmetrics, USA, flow rate 5 L min^{-1}) was used for the collection of both PM_{10} and $\text{PM}_{2.5}$ on pre-weighed quartz and PTFE filters (Whatman, 47 mm diameter), respectively, on a 24-h basis. Sampling was carried out during the period of December 2012–November 2013 from both urban and rural sites of Pune, covering the span of all three seasons, i.e., winter (December–February), summer (March–May), and monsoon (June–August). After collection, the particle-loaded filters were kept in filter cassettes, sealed in plastic bags, and refrigerated at about 4°C until analysis.

Gravimetric analysis

Before and after sampling, the filters were conditioned for 24 h for accurate measurement of PM. The filters were then weighed by using an analytical five-digit balance until three reproducible values were obtained. To reduce the possibility of contamination, the PM-loaded filters were handled with Teflon tape-coated tweezers.

Inductively coupled plasma-atomic emission spectroscopy analysis

In this study, extraction of half of the filter samples was done by using Milli-Q water at room temperature to determine water-soluble metals. Filters were ultrasonicated for 1 h with 25 mL of Milli-Q water (pH ~ 5.8) followed by centrifugation for analyzing the heavy metals (Pb, Zn, Ni, Fe, Cr, Mn, Cu, Cd, Co) by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (ARCOS, Spectro, Germany). The detailed method of the instrument is provided elsewhere (Satsangi et al. 2014).

Quality control

To check the consistency in the flow rate of the sampler, daily flow rate calculations (gas meter reading/timer reading) were done. No significant differences have been found in the flow rate. Cleaning of the sampler was done regularly so that no traces of dust can be counted in the mass concentration of PM samples. In addition to this, background contamination of the heavy metals was also measured in filter blanks. These values were found to be below method detection limits (Satsangi et al. 2014).



Fig. 1 Map of Pune city showing sampling sites (urban and rural)

Electron paramagnetic resonance study

PM-loaded filters were cut into small strips, rolled, and inserted into a quartz tube, and the electron paramagnetic resonance (EPR) spectra were recorded. The EPR spectra of all the samples were measured by means of a Varian E-109 spectrometer with the following parameters: modulation frequency 100 kHz, X-band microwave frequency 9.1 GHz, modulation amplitude 2 G, time constant 1 s, receiver gain 4000, scan time 4 min, and scan range 400 G. All EPR analyses were done at a low temperature (77 K). The radical concentration was calculated by double integration of the first derivative of the EPR spectra. The g values and radical concentration (spins/g) were determined by comparison with standard 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

Dithiothreitol assay

Dithiothreitol (DTT) assay was used in this study to measure the oxidative potential of indoor PM. This assay is based on the

measurement of DTT loss into its disulfide form by the redox active constituents of PM. DTT was incubated with phosphate-buffered saline (PBS)-extracted PM samples for 30 min at 37 °C followed by 20 min vigorous shaking. Trichloroacetic acid (0.50 mL) was added to stop the reaction, and the remaining DTT was allowed to react with 10 mM 5, 5'-dithiobis-(2-nitro benzoic acid) (DTNB) (made in 0.10 M phosphate buffer; pH 7.4) to form 2-nitro-5-thiobenzoate (TNB) which was detected by a UV-visible spectrophotometer (Shimadzu UV-2501PC) at 412 nm (Charrier and Anastasio 2012).

Plasmid DNA assay

Particles were separated from the filter substrate by immersing one fourth of the filter in 1 mL PBS (1×). The samples were first ultrasonicated for 15 min followed by vortexing. Agarose gel (0.8 %) and ethidium bromide (0.25 %) were put into the electrophoresis unit filled with Tris borate EDTA (TBE) buffer. PBS-extracted samples (10 μL) were incubated with 0.8 μL of

Table 1 Sampling profile of study areas

Activity	Urban site	Rural site
Building materials	House made up of bricks and cement and well painted	House made up of mud, bamboo, and cow dung and not well painted
Room size (<i>f</i>)	Dimension of room is 17 × 13 × 12	Dimension of room is 6 × 2 × 7
Window size	4.5 × 3	No windows
Ventilation mechanism	Large windows and exhaust fans	No ventilation
Fuels used in kitchen	LPG	Wood, thrash/grass, cow dung cakes, kerosene
Outdoor activities	Heavy vehicular emissions, road dust	Agricultural waste burning, biomass burning, re-suspension of dust due to dry open fields, construction activities, vehicular activities used for transportation as well as agricultural purposes
Distance from traffic source	~2 km	~8 km
Traffic count/day (counted manually)	~60,000	~20,000

Phi X174 supercoiled phage DNA (Bangalore GeNei) at 37 °C for 20 min. The mixture was carefully loaded into the wells of gel along with 2 µL of tracking dye (bromophenol blue). The same treatment was done with the OH radical scavenger (mannitol 4 mM). The gel was run at 80 V for 8 h. The gel image was visualized through Imagining System (EC3 Chemi HR 410), and the percent DNA damage induced by indoor particles was calculated with respect to control.

Results and discussion

Indoor air quality depends on numerous factors such as ventilation, pollutant sources, and infiltration of ambient pollution which makes indoor air pollution among the top category of environmental risks (Gibson et al. 2013; DeBrouwere et al. 2007). This study is based on the assessment of indoor air quality of residential areas: rural and urban sites of Pune.

Mass concentration of PM₁₀ and PM_{2.5}

Table 2 depicts the average mass concentration of PM₁₀ and PM_{2.5} at both urban and rural sites of Pune City. The mass concentration of PM₁₀ varied from 45.8 to 250 µg m⁻³ for the urban site with a mean value of 176 ± 57 µg m⁻³ and 83.3 to 630 µg m⁻³ for the rural site with a mean value of 283 ± 185 µg m⁻³ while the PM_{2.5} concentration varied from 27.8 to 263 µg m⁻³ for the urban site with a mean value of 105 ± 61 µg m⁻³ and 27.8 to 397 µg m⁻³ for the rural site with a mean value of 176 ± 126 µg m⁻³. The average mass concentration of PM₁₀ and PM_{2.5} was significantly higher at the rural site in comparison to the urban site during the study period. On comparing the average mass concentration of PM₁₀ and PM_{2.5} with Indian NAAQS (40 µg m⁻³ for PM_{2.5} and 60 µg m⁻³ for PM₁₀), our results exceeded 4.5 times for the rural site and 3.3 times for the urban site which indicates

poor air quality of Pune in terms of PM. PM₁₀ and PM_{2.5} concentrations were 3.5 and 7 times higher for the urban site and 5.6 and 3.5 times higher for the rural site, respectively, than the prescribed standard values of the US Environmental Protection Agency (USEPA) (50 µg m⁻³ for PM₁₀ and 15 µg m⁻³ for PM_{2.5}). The average PM_{2.5} to PM₁₀ ratio at the urban and rural sites was found to be 0.60 and 0.62, respectively. It suggests that PM₁₀ is composed of 60 % PM_{2.5} at the urban area and 62 % PM_{2.5} at the rural site, indicating fine particles comprised a large fraction in PM₁₀ at both sites. As regards health impact, slight domination of fine particles over coarse ones can be a threat as fine particles penetrate deep into the alveoli of human lungs through inhalation (Habil et al. 2013; Pipal et al. 2011). Table 3 represents the comparative study of mass concentration of indoor PM collected from different geographical locations. The mass concentration of PM₁₀ and PM_{2.5} at both urban and rural sites of Delhi City (Kulshreshtha and Khare 2011) is much higher than the mass concentration of the present study. The possible reasons for

Table 2 The average mass (µg m⁻³) and metal concentration (ng m⁻³) in PM₁₀ and PM_{2.5} measured at urban and rural sites of Pune

	Urban		Rural	
	PM _{2.5}	PM ₁₀	PM _{2.5}	PM ₁₀
Average mass	105.5	176.9	176.9	283.0
Cd	30.14	20.31	34.86	24.37
Co	30.45	29.67	35.56	29.35
Cr	30.03	27.00	41.35	30.66
Cu	39.00	25.62	37.28	36.64
Ni	33.58	33.00	45.30	34.30
Pb	26.78	24.56	42.36	32.54
Zn	32.77	34.38	62.62	41.20
Mn	17.59	30.05	31.16	28.80
Fe	495.62	570.84	498.68	1048.0

Table 3 Comparison of mass concentration ($\mu\text{g m}^{-3}$) of PM_{10} and $\text{PM}_{2.5}$ with other reported data

Location	PM_{10}		$\text{PM}_{2.5}$		Sampler used Make	Reference
	Urban	Rural	Urban	Rural		
Pune (India)	176.9	283.0	105.5	176.9	MiniVol TAS (Airmetrics)	Present study
Agra (India)	231.4	237.4	116.9	126.7	APM 550 (Envirotech 16.6)	Kulshrestha et al. (2014)
Delhi (India)	380.7	413.1	118.4	290.4	GRIMM (1.107)	Kulshrestha and Khare (2011)
United Arab Emirates	38.5 (median value)	41.3	6.0	6.1	Passive aerosol sampler (UNC)	Funk et al. (2014)
Klang Valle (Malaysia)	80.3	43.7	48.9	25.7	Dust track monitor	Nazariah et al. (2013)
Pakistan	273.0	298.5	156	175.5	GRIMM (1.107 and 1.108)	Nasir et al. (2013)
China	57.0	100.6	65.7	100.5	Digital dust indicator (P-5L ₂ C), site pack personal aerosol monitor (AM510)	Jiang and Bell (2008)

high concentration of PM found by them were cooking and improper ventilation for the rural site and infiltration of outdoor air at the urban site. The mass concentration of both fractions of PM at the rural site in the present study was higher than corresponding values at the rural site of Agra City (Kulshrestha et al. 2014). In addition to this, the overall mass concentration of PM is higher in the present study than the concentration reported at the United Arab Emirates (Funk et al. 2014), Malaysia (Nazariah et al. 2013), and China (Jiang and Bell 2008) and lower than the concentration reported in Pakistan (Nasir et al. 2013).

Seasonal analysis in mass concentration of PM_{10} and $\text{PM}_{2.5}$ showed significant variation at both sampling sites. Mass concentration of both sized PM was higher during winter ($157.4 \mu\text{g m}^{-3}$ for PM_{10} and $135.8 \mu\text{g m}^{-3}$ for $\text{PM}_{2.5}$) followed by monsoon ($124 \mu\text{g m}^{-3}$ for PM_{10} and $64.7 \mu\text{g m}^{-3}$ for $\text{PM}_{2.5}$) and summer ($82.45 \mu\text{g m}^{-3}$ for PM_{10} and $62.91 \mu\text{g m}^{-3}$ for $\text{PM}_{2.5}$) at the urban environment. This could be due to the lack of ventilation as windows are kept closed during the winter which results in the saturation of PM inside the houses, whereas for the rural site, mass concentration was

maximum in monsoon season ($753.4 \mu\text{g m}^{-3}$ for PM_{10} and $284.7 \mu\text{g m}^{-3}$ for $\text{PM}_{2.5}$) followed by winter ($250.94 \mu\text{g m}^{-3}$ for PM_{10} and $209.88 \mu\text{g m}^{-3}$ for $\text{PM}_{2.5}$) and exhibited the lowest during summer ($97.70 \mu\text{g m}^{-3}$ for PM_{10} and $92.59 \mu\text{g m}^{-3}$ for $\text{PM}_{2.5}$). During monsoon at the rural site, cooking activities are performed inside which may result in a higher concentration of PM indoors (Satsangi et al. 2014).

Table 4 presents a comparative study of seasonal variation in mass concentration of PM obtained in the present study with worldwide data. The average mass concentration (present study) exhibited higher values during winter for both PM_{10} and $\text{PM}_{2.5}$ in comparison with Pittsburg (USA) (Tunno et al. 2015), Athens (Greece) (Diapouli et al. 2011), Tallinn (Estonia) (Orru et al. 2014), and Agra (India) (Massey et al. 2012), whereas these values are lower in Pakistan (Ali et al. 2015) and Delhi (Kulshrestha and Khare 2011). In summer, the measured average mass concentration for both fractions of PM was higher than those of the USA, Greece, Estonia, and Pakistan but lower than those of Agra and Delhi (PM_{10}). The mass concentration of PM_{10} and $\text{PM}_{2.5}$ throughout monsoon was only measured in Agra. Therefore,

Table 4 Comparison of seasonal mass concentration ($\mu\text{g m}^{-3}$) of PM_{10} and $\text{PM}_{2.5}$ with other studies

Location	PM_{10}			$\text{PM}_{2.5}$			Reference
	Summer	Winter	Monsoon	Summer	Winter	Monsoon	
Pittsburg (USA)	–	–	–	25.8	18.9	–	Tunno et al. (2015)
Athens (Greece)	35.1	31.8	–	30.1	27.2	–	Diapouli et al. (2011)
Tallinn, Estonia	15	22.2	–	–	–	–	Orra et al. (2014)
Pakistan	71	300	–	31	240	–	Ali et al. (2015)
Indian studies							
Agra	254	137	111	146	80	80	Massey et al. (2012))
Delhi	216.5	546.1	–	62.5	346.2	–	Kulshrestha and Khare (2011)
Pune (rural)	97.7	250.94	753.4	92.6	209.9	284.7	Present study
Pune (urban)	82.4	157.4	124	62.9	135.8	64.7	

the comparison of both sized PM in the present study has been done only with respect to Agra City and was found to be higher.

Heavy metal concentration

Residential particulate matter was found strongly contaminated with Fe, Zn, Ni, and Cu followed by Mn, Co, Cd, Pb, and Cr. Table 2 shows average concentrations of metals in PM₁₀ and PM_{2.5} at both urban and rural sites during the entire study period. At both sites, Fe was the highest contributor for both PM₁₀ and PM_{2.5} while Cd and Mn provided the least contribution in both size fractions of PM. On average, the decreasing metal concentration trend in PM₁₀ was Fe > Zn > Cu > Ni > Pb > Cr > Co > Mn > Cd for the rural site and Fe > Zn > Ni > Mn > Co > Cr > Cu > Pb > Cd for the urban site, while in PM_{2.5}, it was Fe > Zn > Ni > Pb > Cr > Cu > Co > Cd > Mn for the rural site and Fe > Cu > Ni > Zn > Co > Cd > Cr > Pb > Mn for the

urban site. In both PM₁₀ and PM_{2.5}, Fe exhibited the highest concentration among all the analyzed elements at both the sampling sites. It could be a result of excessive usage of utensils and may also originate from re-suspension of dust from indoor human activities (Kwangsam et al. 2004).

Factor analysis (using SPSS 16) was applied to identify the principal sources which influence the concentration of metals at both the sampling sites. The urban site of Pune consisted of well-planned and modern types of houses with proper ventilation, many commercial activities going on in the surrounding areas, and high population density. Table 5 represents two rotated factors explaining more than 90 and 88 % of the total variance for PM₁₀ and PM_{2.5} at the urban site, respectively. Factor 1 with variance of 43.3 and 25.3 % in PM₁₀ and PM_{2.5} shows high loading of Co, Cr, Cu, Mn, Ni, and Pb, suggesting their emission from common sources like excessive usage of electric appliances and metallurgical products used in indoor environments (Nazir et al. 2011). Sources of Mn, Pb, Ni, and Cr are expected originate from household dust emitted by walls, cleaning, and other human

Table 5 Factor analysis of PM₁₀ and PM_{2.5} at urban and rural sites

Urban	PM ₁₀		PM _{2.5}		
	Factor 1	Factor 2	Factor 1	Factor 2	
Cd	-0.30	-0.92	-0.08	-0.99	
Co	<i>0.81</i>	<i>0.55</i>	<i>0.92</i>	0.22	
Cr	<i>0.77</i>	<i>0.61</i>	<i>0.91</i>	0.30	
Cu	<i>0.80</i>	0.58	<i>0.95</i>	0.25	
Fe	-0.77	-0.96	<i>0.94</i>	0.18	
Ni	<i>0.86</i>	-0.10	<i>0.96</i>	-0.01	
Pb	<i>0.85</i>	0.43	<i>0.75</i>	-0.09	
Zn	<i>0.55</i>	<i>0.69</i>	0.47	<i>0.73</i>	
Mn	<i>0.71</i>	<i>0.68</i>	<i>0.92</i>	0.35	
% variance	43.3	47.4	25.3	17.4	
Possible sources	Indoor daily activities	Paint, plastics, cooking fumes, and outdoor penetration	Cooking, smoking, and outdoor seeping	Indoor house dust	
Rural	PM ₁₀		PM _{2.5}		
	Factor 1	Factor 2	Factor 1	Factor 2	Factor 3
Cd	-0.31	<i>0.94</i>	0.21	<i>0.97</i>	-0.10
Co	<i>0.99</i>	0.05	<i>0.55</i>	0.43	-0.11
Cr	<i>0.98</i>	0.02	<i>0.93</i>	0.32	-0.07
Fe	0.39	<i>0.86</i>	-0.39	-0.12	<i>0.69</i>
Cu	<i>0.99</i>	0.04	<i>0.91</i>	0.04	0.12
Ni	<i>0.55</i>	0.29	0.20	<i>0.97</i>	-0.10
Pb	<i>0.93</i>	0.21	0.27	<i>0.88</i>	0.26
Zn	<i>0.70</i>	-0.06	<i>0.52</i>	0.20	<i>0.67</i>
Mn	<i>0.97</i>	-0.22	<i>0.94</i>	0.28	-0.15
% variance	65.7	20.2	43.2	34.1	11.9
Possible sources	Cooking activities	Combustion and biomass burning	Cooking fumes, food, cooking oil	Burning candles	Infiltration of dust

Factor loading above 0.5 are highlighted in italic

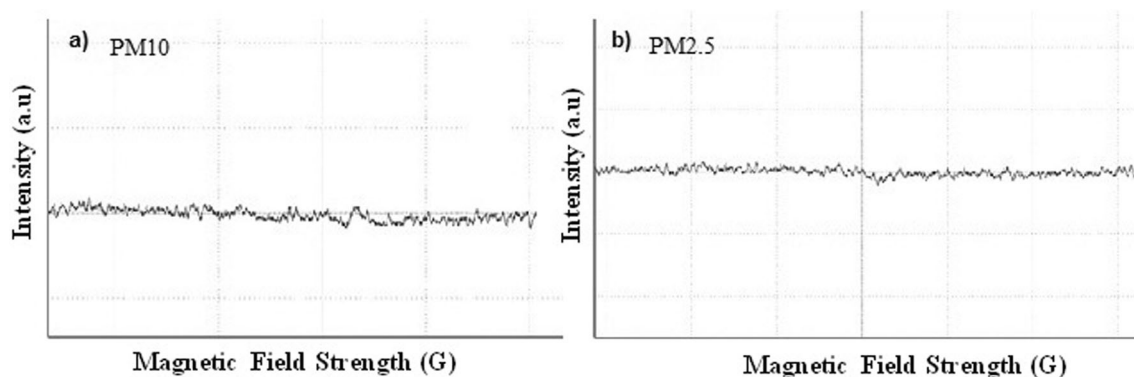


Fig. 2 a, b EPR spectra of unexposed filter paper

activities. Possible sources of Cu are primarily metallic cooking utensils because of the combination of corrosion, wear, and abrasion or emitted from the food during cooking (Balasubramanian et al. 2012). Additionally, $PM_{2.5}$ was also loaded with Zn and Fe in factor 1. Factor 2, with variance of 47.4 and 17.4 %, was dominated by Cr, Zn, and Mn in PM_{10} and Zn in $PM_{2.5}$. Zn is mostly released from cooking fumes and various other indoor building materials like paint and plastics (Taner et al. 2013) whereas Cr is derived from smoking of cigarettes, cosmetics, and paint chips (Madany et al. 1994). Mn is used in unleaded gasoline as antiknocking agent and can be also used as an additive in petroleum products (Kulshrestha et al. 2014). This metal may be profoundly influenced by ambient air that infiltrates into houses as this site is located near the roadside.

Rural sites are characterized by low traffic density and agricultural activities in close vicinity with improper ventilation. At rural site, the factor analysis suggested contribution from two factors for PM_{10} with a total variance of 85.9 %. Candles and fuel lamps are used as alternative methods of lightening as this site usually faces power outage and three factors for $PM_{2.5}$ with the total variance of 89.2 % (Table 3). Elements like Cr, Cu, Co, Pb, Zn, and Mn represent factor 1 with 65.7 % variance in PM_{10} whereas Cu, Cr, and Mn represent 43.2 % variance in $PM_{2.5}$. Pb, Mn, and Zn may be attributed to vehicular activities that are generally used for agricultural purposes at these sites (Park and Dam 2010). Stainless steel used indoors primarily consists of Cu and Cr. Therefore, the probable common sources of these two metals may be associated with

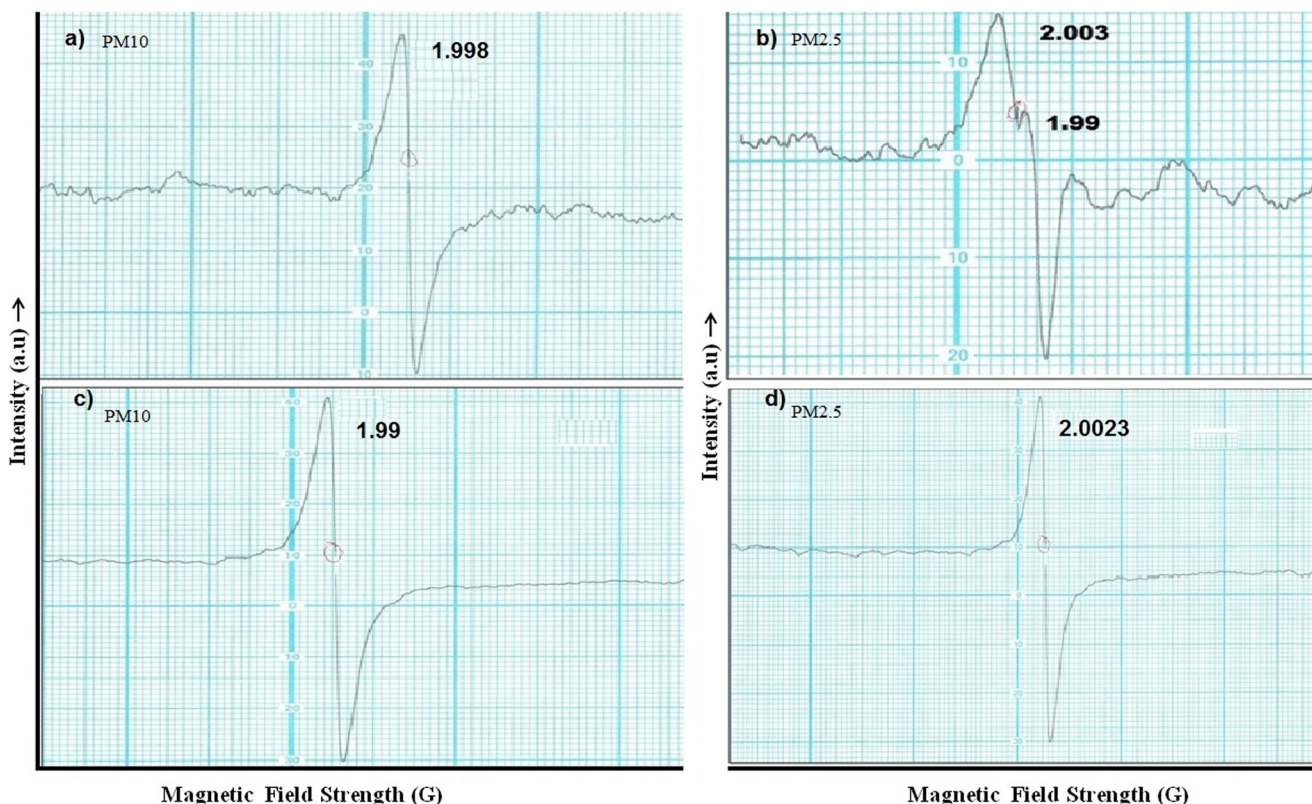


Fig. 3 a–d EPR spectra of various samples

cooking utensils. Cd and Fe represent factor 2 with 20.2 % variance in PM₁₀. Both of these species predominantly originate from fossil fuel combustion and biomass burning which are frequently carried out at this site (Watson et al. 2001), whereas in PM_{2.5}, factor 2 was enriched with Cd, Ni, and Pb with a variance of 34.1 %. The candles with lead wicks have the potential to generate indoor airborne Pb particles. Combustion of coal and other fuels used for cooking and lighting purposes can be the possible common origin of Ni and Cd at this site. Factor 3 (PM_{2.5}) represents 11.9 % of total variance for the loading of Zn and Fe attributed to the infiltration of dust.

Free radical generation and EPR

Generation of free radicals in PM has been suggested as one of the most important mechanisms underlying the toxicity of particulate matter. For assessment of this activity, EPR method was used to characterize PM according to their paramagnetic properties. Figure 2a, b shows the EPR spectra of unexposed filters, where no signals were found. Figure 3a–d shows EPR examination of both PM₁₀ and PM_{2.5} which display a singly unstructured similar kind of spectra with only differences in peak intensities. The average ΔHp–p (peak to peak width) was found to be 8–10 Gausses. The broadness of these EPR signals indicates the presence of several paramagnetic radical species and organic-metal interaction (Gehling et al. 2013). *g* values for all the spectra were calculated. For PM₁₀, the observed *g* value in all samples was 1.99 (i.e., ~2.0) which indicates the presence of high-spin ferric ion (Fe³⁺), whereas in PM_{2.5}, a set of different *g* values, i.e., 1.99 (i.e., ~2.0), 2.0023, and 2.003, were observed. EPR data revealed that both particulate sizes contain Fe³⁺ which is corroborated by the ICP data (Table 2). In presence of Fe, formation of hydroxyl radicals (·OH) via Fenton-type reactions has been suggested as a key feature of the PM toxicity (Schins et al. 2004). In addition to this, PM_{2.5} contains both purely C-centered radical (*g* = 2.0023) and C-centered radical with vicinal oxygen atom (*g* = 2.003). In the present study, radical concentration of the paramagnetic center was also calculated with reference to the stable free radical, i.e., DPPH. In indoor samples, radical concentration was found in the range of 10¹⁷–10¹⁸ radicals/g of PM for both PM₁₀ and PM_{2.5}. Therefore, these calculated values indicate that indoor PM of Pune is composed of free radicals in high concentration at both urban and rural sites.

EPR gives direct quantification of radical species in the samples, but the complexity and high price of this instrument makes its use very limited (Hedayat et al. 2015). In addition to this, EPR gives only the qualitative information about the free radical generation but, in order to quantify redox-active moieties in PM responsible for the oxidative stress through ROS generation, DTT assay was done. The DTT assay is considered biologically relevant because DTT loss has been correlated

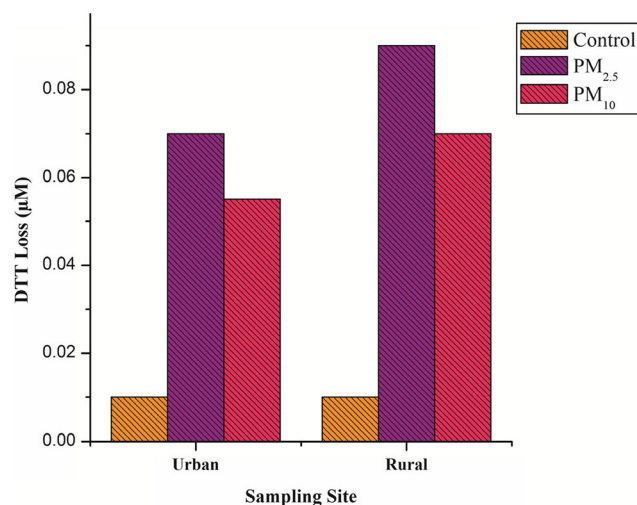


Fig. 4 DTT loss of PM₁₀ and PM_{2.5} samples

with cellular oxidative stress in vitro. In this study, DTT activity has been measured in terms of DTT loss for both PM₁₀ and PM_{2.5} at the urban and rural sites of Pune. For both fractions of PM, significant rise in DTT loss (in µM) was observed as compared to the control, suggesting that collected indoor PM samples are highly oxidative in nature. Figure 4 shows DTT loss of both the fraction of PM at two sampling sites. DTT loss exhibited the highest value in PM_{2.5} mode at both sites. Various studies have reported similar observations regarding the effect of particle size on per mass DTT loss (Ntziachristos et al. 2007). Among these two sites, DTT loss was found to be maximum at the rural site for both sized PM which is directly influenced by the high concentration of metals at this site (Table 2). It is speculated that DTT loss increases with the presence of more redox active species in samples; that is why it is needed to assess the significance of metals towards this loss of indoor particles. Pearson's coefficients also have been calculated for both PM₁₀ and PM_{2.5} (Table 6). A significant correlation of water-soluble Cu and Ni concentration with DTT loss was observed with *r* values of 0.64 and 0.67, respectively. Our results indicate the significant contribution of metals in

Table 6 Correlation matrix of metals with DNA damage and DTT loss

	DNA damage	DTT loss
Cd	0.44	−0.1
Co	0.28	0.12
Cr	0.26	0.12
Cu	<i>0.58</i>	<i>0.64</i>
Fe	<i>0.68</i>	0.34
Ni	<i>0.76</i>	<i>0.63</i>
Pb	0.12	−0.03
Zn	0.11	−0.04
Mn	0.33	0.04

Significance level, 0.05. Factor loading above 0.5 are highlighted in italic

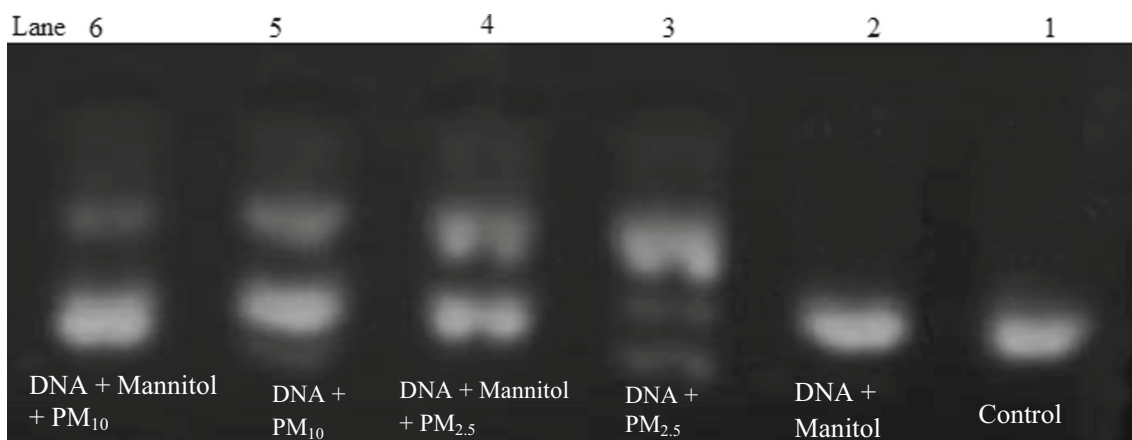


Fig. 5 DNA damage profile of indoor PM

DTT loss of indoor PM (Kam et al. 2011) as these metals have potential to form ROS such as OH[•] and HOOH by participating in Fenton reaction (Charrier and Anastasio 2012). In contrast to Cu and Ni, Fe concentration was not correlated with DTT loss, even though Fe is known to form reactive oxygen species (ROS). The possible reason for this may be due to the binding of Fe with organic ligand, altering the redox activity of Fe on formation of Fe-DTT complexes. Similar results were also reported by Lin and Yus (2011), suggesting the loss of DTT due to Cu metal and not by Fe. In the context of this present study, the significant correlation of DTT loss with Cu and Ni concentrations emphasizes the significant contribution of these metals to the generation of free radicals.

Plasmid DNA assay

Metals are implicated as one of the possible causes of the adverse effects of PM on human health. The presence of a high concentration of metals in PM is one of the main contributors to free radical generation and these radicals cause oxidative stress and ultimately damage the DNA. To evaluate the genotoxic effect of Pune PM, indoor samples have been extracted in PBS and DNA strand breakage was quantified by gel electrophoresis technique. Figure 5 depicts the typical output from DNA plasmid assay where there is a strand breakage of DNA by indoor PM samples and protection effect by mannitol. Among various ROS, the OH radical is the most potent to cause DNA damage formed via Fenton reaction in the presence of H₂O₂ and metals (Halliwell and Gutteridge 1999). In the present study, mannitol has been

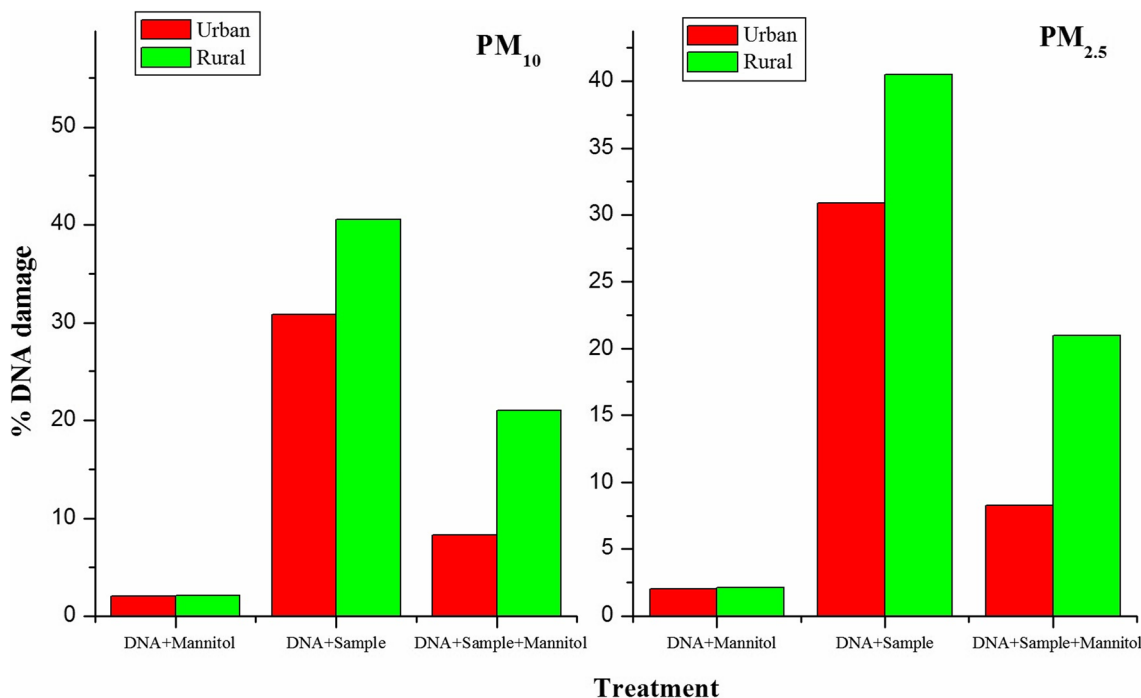


Fig. 6 a, b Percentage DNA damage by PM samples

used as $\cdot\text{OH}$ radical scavenger and tested to determine whether its presence could protect DNA scission and measure the role of $\cdot\text{OH}$ in the degradation of DNA. On treatment of Phi X174 supercoiled phage DNA with PM, samples collected from urban and rural sites resulted in significant strand breakage with an average of 43 and 30.9 % for PM_{10} and 55.3 and 40.5 % for $\text{PM}_{2.5}$ at the rural and urban sites, respectively (Fig. 6). On the basis of these findings, $\text{PM}_{2.5}$ caused more oxidative DNA damage than PM_{10} . Fine particles are generally considered as more toxic because of the abundant presence of reactive oxygen species (Hung and Santschi 2001). In comparison to the rural PM samples, urban PM samples were found to be less potent in inducing oxidative DNA damage due to the low concentration of metals (total metal concentration was 736–795 ng m^{-3} in urban and 829–1306 ng m^{-3} in rural). It can be visualized in Fig. 6 that co-incubation with mannitol caused a significant reduction of DNA strand breakage. The average DNA strand breakage was reduced to 28.5 and 20.9 % in PM_{10} and 10.5 and 8.3 % in $\text{PM}_{2.5}$ for the rural and urban sites, respectively, suggesting the involvement of $\cdot\text{OH}$ in mediating a proportion of DNA damage via Fenton-type reaction. In the present study, the observed DNA breakage showed significant correlation with the concentration of soluble metals such as Fe ($r = 0.68$), Cu ($r = 0.58$), and Ni ($r = 0.76$), indicating the ability of PM to cause DNA damage. It may be due to the water-soluble metals which promote radical generation via Fenton reaction (Table 6). Fe is a well-known Fenton active metal and considered to exert its effect via formation of $\cdot\text{OH}$ radical (Charrier and Anastasio 2011). The ICP-AES analysis indicated that Fe concentration in PM_{10} and $\text{PM}_{2.5}$ samples was much higher than that of other metals which suggests that Fe may be responsible for DNA damage through radical generation. However, other metals such as Cu and Ni in PM have potential to perform Fenton chemistry and have been reported to elicit DNA damage (Oikawa et al. 2002). Our data demonstrated the key role for Fenton-driven $\cdot\text{OH}$ in induction of DNA damage. DNA scission was not completely protected by the mannitol, as it is an effective scavenger for $\cdot\text{OH}$ and not capable of scavenging other radicals (like peroxide, superoxide) during the reaction..

Conclusions

The quality of indoor air and the resultant risk associated with regular exposure to particle-bound metals are major concerns for building occupants. This paper is aimed at expanding the present knowledge about the physico-chemical and toxicological characteristics of indoor particles in both coarse and fine size ranges at urban and rural sites of Pune City. The average mass concentration of indoor PM_{10} and $\text{PM}_{2.5}$ was higher than the Indian NAAQS and USEPA standards. The monitoring data revealed that the mass concentration of PM_{10} and $\text{PM}_{2.5}$ was maximum in winter season at the urban site whereas for

the rural site it showed a higher value in monsoon. Among the metals, Fe showed the highest concentration for both particulate sizes at both sampling sites followed by Zn, Ni, and Cu. Heavy metal concentration was observed to be higher in fine fraction of PM at the rural site. Factor analysis identified the possible common sources such as combustion of fossil fuels, coal, re-suspension of household dust, waste dumping, paint, candle burning, and outdoor impact influencing the metal concentration at the sampling site. EPR study divulged the presence of free radicals in PM samples collected from both the sites of Pune. In both PM_{10} and $\text{PM}_{2.5}$, the g value of 1.9 corresponding to Fe^{3+} shows that Fe plays a pivotal role for oxidant-generating activity. The oxidative capacity measured by DTT assay exhibited fine PM as highly oxidative in nature as compared to PM_{10} . Toxicity of indoor PM was evaluated by gel electrophoresis, and significant DNA strand breakage was caused by both fractions of particulate matter, slightly higher in the fine fraction, which was inhibited to some extent in the presence of mannitol suggesting the $\cdot\text{OH}$ radical was the main cause of this damage. Presented results suggest a mechanism of genotoxicity of indoor PM in Pune is based on $\cdot\text{OH}$ radical generation through Fenton reaction. Plasmid DNA breakage showed significant correlation with metals such as Fe, Cu, and Ni, indicating that oxidative DNA damage induced by PM_{10} and $\text{PM}_{2.5}$ was mainly due to the water-soluble metals.

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