Indoor Particulate Matter Pollution on Oxidative Stress Pathways: Role of Chemical and Biological Constituents

Milindkumar Yashvant Bhatkar, Shreya Roy, Ritwika Roy, and P. Gursumeeran Satsangi

Abstract The present study aimed to evaluate the relationship between physico-chemical characteristics and toxicological assessment of indoor PM bound chemical and biological constituents. The average concentration of both indoor PM_{10} and $PM_{2.5}$ exceeded the NAAQS standards of India. Metals of geogenic origin (Fe and Mn) are predominant in PM_{10} samples, while the metals of anthropogenic origin (Ni, Co, Cd, Pb, Cu, Cr, and Zn) were dominant in $PM_{2.5}$. Among all analyszd metals, Fe exhibited the maximum concentrations in both sized PM followed by Mn, pb, Cr, Cu and Ni suggesting considerable health risk to the exposed inhabitants due to their ability to generate reactive oxygen species through Fenton's reaction, mainly responsible for causing PM toxicity. Commonly isolated bacteria and fungi identified in the indoor environment were *Bacillus sp., Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus sp. and Penicillium sp.,* respectively. Amongst them, *Pseudomonas aeruginosa* and *Aspergillus sp.* were found to be predominant in both sized PM revealing a significant health risk due to their association with various respiratory tract infections. The cytotoxic profile of indoor PM and their constituents determined by MTT assay on A549 cells exhibited a significant decrease in cell viability indicating the cytotoxic behavior of particles. Oxidative reactivity of indoor PM in terms of DTT assay showed considerable DTT depletion suggesting the oxidative nature of PM. Results of the present study will be effectual to raise awareness and address the better indoor air quality for the residents.

Keywords Indoor PM · Metals · Microbes · Reactive oxygen species · Cytotoxicity

M. Y. Bhatkar · S. Roy · R. Roy · P. G. Satsangi (✉)
Department of Chemistry, Savitribai Phule Pune University, Pune 411007, India
e-mail: pgsatsangi@unipune.ac.in

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1 Introduction

Indoor air quality depends on many contributing factors *viz.* individual home characteristics, routine habits and personal activities. As compared to ambient particulate pollution, indoor air pollution is always less explored in terms of sources and health effects [1]. It should be noted that humans spend the majority of their time in (approximately, 90%) different indoor conditions such as homes, offices, schools [2]. These indoor environments are rich in particulate concentration due to routine activities performed by individuals. However, spending a plenty amount of time in various indoor settings, ultimately increases the inhalation rate of particles. Particulate matter (PM) is a heterogeneous mixture of many constituents like dust, metals, bioaerosols, carbonaceous matter including VOCs and PAHs. PM bounded with bioaerosols and metals have considerable adverse health outcomes. Human respiratory tract inflammation, asthma, influenza, chronic obstructive pulmonary disease (COPD) and oxidative stress can be considered major indicators for bioaerosol-bound microbe exposure [3, 4]. Similarly, metals bound with PM are capable of inducing malfunctioning and disturb cellular activities. Considering Fe, excess concentration is responsible for initiating Fenton's reactions in human body by catalyzing the conversion of hydrogen peroxide to hydroxyl free radicals [5]. Production of these radicals is accounted by the oxidation of ferrous ions (Fe^{2+}) with hydrogen peroxide. The unstable nature of these free radicals makes them highly toxic, having the capability of reducing disulfide bonds present in proteins. Thus, it is necessary to take into account the toxicological profiling of these PM, which is considered a crucial process for evaluating their health effects. In view of this, the present study was aimed to investigate the role of bioaerosols and metal concentration for inducing toxicity to PM, collected from the indoor atmosphere of urban houses of Pune. Results obtained in this study will be useful for spreading awareness and addressing the need for better air quality for residents of Pune city.

2 Materials and Methodology

2.1 Sampling and Extraction Procedures

In the present study, three houses based on different characteristics i.e., construction, living standards, etc. were selected. Samples of PM_{10} (N=36) and $PM_{2.5}$ (N=36) were collected from June, 2021 to May, 2022 by Mini Vol TAS (Airmetrics, USA) with a constant flow rate of 5 LPM in Pune. A detailed survey of these selected houses is reported in Table 1. Properly desiccated (for 48 hrs), sterilized, pre- weighed nucleopore filters were used for sample collection. Post-sampling, these filters were stored in a refrigerator unit at 4 °C. For extraction, PM filters were cut into two halves; one portion was digested in 10 ml of HNO_3 for 2 h on a hot plate followed by dilution was used for the analysis of total metal content by ICP-AES (ARCOS, Spectro,

Table 1 Household characteristics of selected sampling locations

Particulars	Pune		
	H1	H2	H3
Building characteristics	Bricks, plastered walls and well painted	Built with bricks, cemented but not painted	Built with bricks with plastered walls
Cooking gas usage	L.P.G	L.P.G	L.P.G
Other activities	Incenses sticks, mosquito vaporizers	Tobacco smoking, Mosquito vaporizers	Tobacco smoking, Incenses sticks
Ventilation	Window and exhaust fan	Windows	Windows, AC, exhaust fan
Pets/Plants	Dog/Yes	No/Yes	No/yes
Outdoor influence	Road dust and dump yard	Vehicular emissions, construction activities and road dust	Vehicular emissions
No. of occupants	2	4	3
Floor type	Tiles	Old tiles	Tiles
Temperature (°C)	25.8	26.9	24.5
RH (%)	51.3	57.5	52.7

Germany) The second portion was transferred to sterile culture tubes containing a mixture of 0.1% sterile peptone water and 0.01% Tween 80, vortexed for about 5 h and stored at 4 °C in the refrigerator. Temperature (°C) and relative humidity (%) were simultaneously measured by using an Aeroqual air quality monitor (IQM 60) which is having a flow rate of 1.0 ± 0.05 LPM).

2.2 Cultivation of Microbes Associated with PM and Their Toxicological Profiling

Fungal and bacterial isolates were cultivated on potato dextrose agar (PDA) and nutrient agar, respectively. 0.4 $\mu g ml^{-1}$ Chloramphenicol and 0.5 $\mu g ml^{-1}$ Cyclohexamide were added to inhibit bacterial and fungal growth, respectively. On sterile petri plates poured with required culture media, 50 µl of each microbial preparation was spread-plated in triplicates. An incubation period of 3 and 7 days for bacterial and fungal growth, respectively was followed for each petri plate. Manual counting of culturable microbial colonies in the form of average colony-forming units (CFU) was done for each incubated petri plate.

When the antioxidant power of the human body is unable to nullify the harmful effects of excessive ROS generated, this untoward situation is termed as oxidative stress, which is the ultimate mechanism of PM toxicity [6]. Dithiothreitol assay can be considered a promising approach towards quantification of oxidative stress. Collected PM samples were incubated with DTT for about 30 min at 37 °C. After vortexing for 15 min, 0.5 ml of trichloroacetic acid as a quenching agent was added, further, this reaction mixture was treated with 5, 5-dithiobis-(2- nitro benzoic acid) (DTNB, 10 mM) to form 2-nitro-5-thiobenzoate (TNB). Formation of TNB was measured at 412 nm by using a UV–Visible spectrophotometer (Shimadzu UV-2501PC).

Combined visualization of cell viability, cytotoxic effect and predominant microbial species can be achieved by MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. PM samples in triplicate were treated with A549 cells for 24 h. Then, MTT solution is added and incubated for 3 h. After centrifugation media was removed and DMSO was added. Finally, absorbance was measured at 570 nm by a well plate reader (PerkinElmer EnSpire 2300).

2.3 Quality Control

From the initial phases of the reported research work, precautions were taken to rule out any possibility of error. All the weighing activities and laboratory analyses were performed in triplicates. The flow rate of the sampling instrument was checked every time before sampling. Glass-wares and reagents used were of proper grades and cleaning measures were followed throughout the study period. Toxicological assays and microbial analysis were performed under aseptic conditions. Field blanks were analyzed periodically to reduce any chances of uncertainty in the upcoming analysis.

3 Results and Discussion

Annual concentration of both, $PM_{2.5}$ and PM_{10} were 82.6 ± 2.7 μgm^{-3} and 105.1 ± 7.08 μgm^{-3}, respectively during the study period. Average annual concentration for all the three houses (H1, H2 and H3) has exceeded the annual average standard of NAAQS specified by CPCB, which is 60 μgm^{-3} (PM_{10}) and 40 μgm^{-3} ($PM_{2.5}$) (Fig. 1). Similarly, standards fixed by United States Environmental Protection Agency (USEPA) are 15 μgm^{-3} ($PM_{2.5}$) and 50 μgm^{-3} (PM_{10}). On comparing our measured PM values with the USEPA standard, these are 5.5 ($PM_{2.5}$) and 2.1 (PM_{10}) times higher than the set values. Indoor $PM_{2.5}$mass concentration for H2 was found to be highest among all the three houses, recorded as 85.4 μgm^{-3}. In case of PM_{10}, H3 witnessed the highest particulate count as 112.4 μgm^{-3}, followed by H2 and H1. Sources responsible for higher mass concentrations in H2 and H3 may include nearby construction activities, vehicular emissions and lack of ventilation.

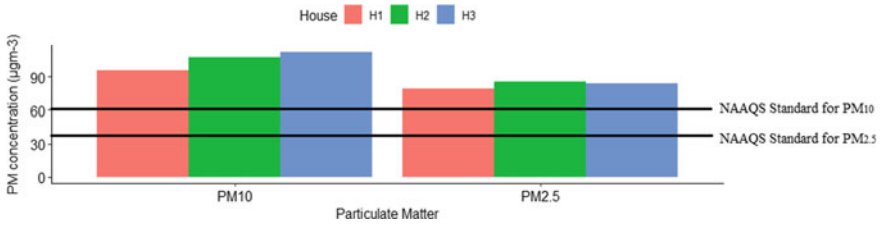

Fig. 1 Average annual concentration of PM

From these house, commonly isolated bacteria and fungi were Bacillus sp., Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus sp., and Penicillium sp., respectively. Amongst them, Pseudomonas aeruginosa, and Aspergillus sp. were found to be predominant in both sized PM revealing a significant health risk due to their association with various respiratory tract infections. Quantitative analysis for total culturable bacteria (TCB) revealed that the average TCB associated with $PM_{2.5}$ was 1325 ± 150 CFU m^{-3} and the concentration of TCB bound with PM_{10} was 1878 ± 187 CFU m^{-3}. In H2, level of TCB associated with $PM_{2.5}$ and PM_{10} were exhibited as 1165 ± 137 CFU m^{-3} and 1290 ± 266 CFU m^{-3} respectively. High CFU count for the bacterial species can be attributed to the number of individuals staying in H2. Individual routine activities i.e., talking, coughing, cooking and sneezing can be correlated with higher concentration of bacterial species in H2. Average fungal load observed in H2 was higher than H1 and H3, the key reason behind this can be natural ventilation. Only source for air ventilation in H2 was through windows which may increase the levels of fungal loads because of high wind flow from outdoor environment which leads to higher air exchange between outdoor and indoor environments. Walls of H2 were not painted and presence of cracks and leaks may increase the air flow, which may encourage the fungal growth in the indoor environment of H2.

Individual response to particularly inhaled microbe is diverse in nature, thus there is no provision for minimum standard values for inhalable microbes and their health effects. Therefore, outcomes from the current study were compared with familiar research works done in India and guidelines provided by WHO. Microbial concentrations recorded in this study were above the decided values provided by WHO. This study clearly specifies the increased level of microbial pollution in indoor environments of Pune. Identification of microbes in the indoor environment was done on the basis of their morphology and further confirmed by the gene sequencing.

The annual average concentrations of metals *viz.* Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in both PM samples were analyzed and showcased in Fig. 2a, b. Based on the concentrations, metals are categorized as crustal (Fe), trace (Co, Mn, Zn and Cu) and carcinogenic (Pb, Ni, Cr and Cd) [7]. Furthermore, based on their origin we can segregate metals as geogenic (Fe and Mn) and anthropogenic (Ni, Co, Cd, Pb, Cu, Cr and Zn). As evident from the Fig. 2, that the metals of geogenic origin were predominant in PM10, whereas PM2.5 samples were rich in metals of anthropogenic origin. Fe is having the highest annual average concentration of 1.152 µgm^{-3} ($PM_{2.5}$)

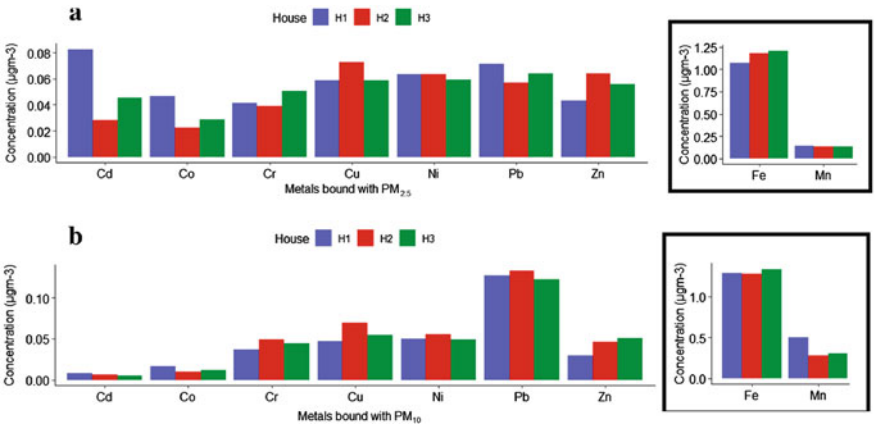

Fig. 2 **a** Average annual concentration of metals in $PM_{2.5}$. **b** Average annual concentration of metals in PM_{10}

and 1.299 μgm^{-3} (PM_{10}) due to its crustal origin. Another abundant metal in both PM was Mn, with annual average concentration of 0.139 μgm^{-3} ($PM_{2.5}$) and 0.363 μgm^{-3} (PM_{10}).

Reactive oxygen species (ROS) play a vital role in causing potential health. Numerous studies determining the oxidative potential of various components of PM have escalated and the most common method for quantification is through the DTT assay. Figure 3 shows the DTT loss of the PM_{10} and $PM_{2.5}$ samples of all the houses. Average DTT loss for $PM_{2.5}$ and PM_{10} was found to be 0.0349 µM and 0.0411 µM, respectively. Higher DTT loss in PM_{10} samples are corroborated with high concentrations of bioaerosol and metals found in this size fraction range. The concentration of redox active metals mainly Fe, Mn are more than non-redox metals *viz.* Cd and Co indicating significant risk for the exposed population. Further, MTT assay was also performed on indoor PM samples and cell viability was found to be significantly decreased as compared to the control. Finding from the present study indicates the proposing role of chemical and biological constituents in inducing the oxidative stress ultimately causing the significant health risk to the exposed population.

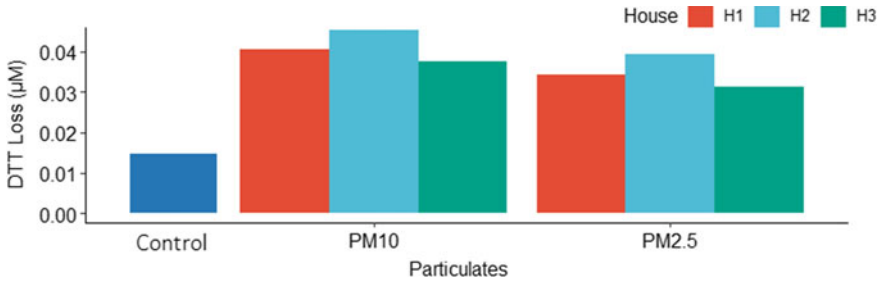

Fig. 3 Average DTT loss for PM samples

4 Conclusion

In summary, the present study revealed the combined effect of metals bound with PM and bioaerosols collected from three urban households in Pune. On a conclusive note, these microbial constituents are dependent on household characteristics, personal activities, routine habits of residents and as well as outdoor activities going on in close vicinity of these households. Associated species of microbes are proven for their ability to cause severe health conditions i.e., acute and chronic diseases. Detailed toxicological profiling of these PM may assist the policymakers to frame future guidelines regarding the health hazards of bioaerosols. These particulates are having a complex composition and thus it is of much importance to investigate both culturable and non-culturable microbial species. Therefore, it should be noted that a complete analysis of these microbes must be considered for estimating the overall harmful effects of these PM. Similar research studies should be encouraged to understand more about the air that we breathe, which will ultimately help to build more safe future for mankind.

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