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Original Research

Assessment of Bioaerosols and Metal Contaminants in Car Cabin Filters Dust of Lahore, Pakistan

Hazrat Zaman[1,2], Zobia Safdar[1], Madiha Habib[1,3], Farhad Ali Shah[1], Muhammad Imran[4], Zaigham Abbas[1]*

[1]Institute of Microbiology and Molecular Genetics, University of the Punjab, Pakistan
[2]School of Biomedical Sciences, The University of Hong Kong, Hong Kong
[3]School of Life Sciences, The Chinese University of Hong Kong, Hong Kong
[4]School of Chemistry, University of the Punjab, Pakistan

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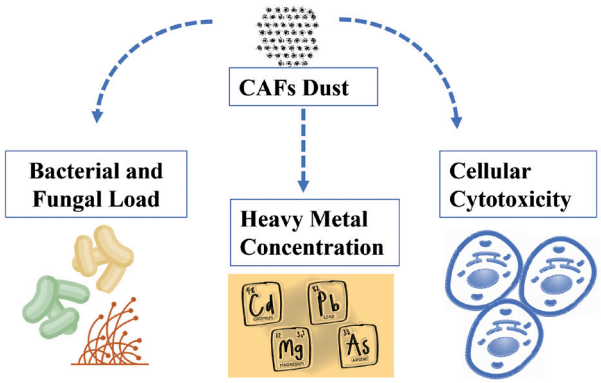
Abstract



A higher concentration of airborne micro-organisms and toxic trace metals in the air is a potential risk for human and environmental health. Suspended dust and microbes in the air are the main inducers of respiratory symptoms. This study aimed to estimate and characterize the microflora and heavy metal contaminants in the car cabin filters (CAFs) dust of Lahore, Pakistan. Moreover, toxicological characterization of the dust was done using relevant cell culture of Human Lung Alveolar Epithelial Cells A-549 through MTT assay. Culturable bacteria and fungi populations were quantified using the standard spread plate method and heavy metals lead, chromium, and cadmium concentrations were checked through atomic absorption spectroscopy. Kirby-Bauer disk diffusion method was used to determine the antibiotic resistance profile of the isolated bacterial species. The mean concentration of culturable bacteria and fungi was $4X10^6$ and $5X10^5$ CFU/g of dust respectively.

*e-mail: zaigham.mmg@pu.edu.pk

Both Gram-Positive and Gram-Negative bacterial species with substantial antibiotic resistance were present in the dust. The bacteria present in the CAFs dust were resistant to ampicillin, cefoxitin and gentamycin while susceptible to ciprofloxacin and gentamycin. The heavy metal concentrations were as high as 273 mg/kg for Pb, 192 mg/kg for Cr and 11.4 mg/kg for Cd. The cell viability decreased significantly with the increase in dust concentration from 100 to 400 µg/mL. The findings of this study will be useful to policymakers and health care professionals to develop and implement effective interventions to prevent adverse health impacts of bioaerosols and trace metals on the local population.

Keywords: air quality, car cabin filters, bioaerosols, heavy metals, cytotoxicity

Introduction

Air pollution is an emerging problem caused primarily by industrial and vehicular emissions [1] rapid urbanization, cooking, and heating [2, 3]. The situation of air pollution in the Asian continent is adverse as most of the polluted cities in the world are in South Asia. In countries like Pakistan, India, Bangladesh, and China, almost 100% of the population lives in areas having pollutant concentrations well above the World Health Organization (WHO) guidelines [4]. Pakistan, air quality has been poor for many decades because of the inadequate initiatives taken by the policymakers and the lack of proper monitoring systems that have left this region under serious health risks [5, 6]. Additionally, Pakistan, which is ranked third globally on the list, suffers a loss of 128,000 lives per year as a result of air pollution (7). Due to a lack of monitoring stations and information pertaining to healthcare settings, it is difficult to assess the state of air pollution in Pakistan [8]. On top of that, Lahore is the most polluted city in Pakistan and the second in the world. During winter, the city was mostly engulfed by a heavy blanket of smog and fog which create a great concern among the occupants [9] and the authorities had to close the schools for an extended period [8].

Air pollution can adversely impact people's health and has been linked with cancer, infertility, negative pregnancy outcomes, cognitive decline, stroke, respiratory diseases, and cardiovascular diseases [10-12]. Furthermore, it also deteriorates air quality, our environment, and the ecosystem [13-15]. In the atmosphere, many forms of dust particles exist such as visible grains of sand, dust and sub-micron aerosols [3]. Different sources emit different types of dust and chemical pollutants into the air where its chemical composition is further affected by existing pollutants and their transformation products. Moreover, road dust is an important source of environmental contamination since it contains heavy metals, organic and inorganic materials, animal dander, pollen grains, mould spores and hydrocarbons [16]. A study reported that heavy metal traces like lead (Pb), chromium (Cr) and cadmium (Cd) present in the dust even at low concentrations can cause significant risk to human health [17]. Additionally, bacterial, and fungal contamination in the air may release harmful substances including allergens, endotoxins, microbial cell fragments, and mycotoxins that trigger adverse immunological reactions and cause cardiovascular and respiratory diseases [18-20]. Biological testing assays can be utilized to assess and characterize the toxicity of complex mixtures such as atmospheric dust. In such a context, in vitro tests using the relevant cell cultures can provide useful information related to health effects [21].

Deposition of the atmospheric dust on car cabin filters (CAFs) makes it a useful tool to monitor and assess urban air quality [22-24]. It is a necessary component of the automobile and reflects the ambient air pollution of a location. Similarly, several studies have utilized heating, ventilation, and air conditioning (HVAC) filter dust to investigate air pollution in different locations and cities [25-28].

To the best of our knowledge, no study has reported microflora contaminants and heavy metal traces from Lahore using CAF's dust collected from the cars that operate only in the city. This study was designed to examine both bacterial and fungal load along with its identification, quantify Pb, Cr and Cd trace metals, and assess the toxicity of the CAFs dust on A549 cells.

Materials and Methods

Sampling Site and Collection

To determine the concentration of bioaerosols and heavy metals in the metropolitan city of Lahore, CAFs filters were collected from the Punjab Air conditioning workshop located in Lahore city as can be seen in Fig. 1. CAFs were collected from the vehicles that only operate in the city of Lahore. Ten used CAFs were directly collected from the vehicles by a qualified mechanic from November 2021 to March 2022 and stored in a sterile plastic box at 20ºC in the laboratory prior to analysis.

Dust Extraction and Collection From The CAFs

Dust was obtained from each filter through shaking and scrapping with the help of sterile forceps and then by sweeping with a smooth brush. A total of 4-5 g of dust was collected in a 15 ml Falcon tube and was stored at -4ºC for further analysis [27].

Isolation and Characterization of Bacteria

To determine the bacterial load in the CAFs dust, 1 mg dust of each filter was properly dissolved in 1 ml sterile phosphate-buffered saline (PBS). For smooth dissolution of dust, the suspension was vortex for 15 minutes. Serial dilutions were performed in normal saline as per standard protocol to obtain the optimal level of colonies on Nutrient agar plates for colony-forming unit (CFU) calculation. A 100 µL of each dust suspension was plated using the standard spread plate method. A sterile spreader was used for spreading the sample to obtain uniform growth and for 4 days the inoculated plates were incubated at 30ºC. To calculate the CFU/g, each plate colony were counted manually. Moreover, to obtain pure growth, bacterial colonies were further subcultured on nutrient agar plates followed by bacterial identification through macroscopic characteristics (bacterial morphology, reaction to Gram and Spore staining) and by standard biochemical tests [29].

Furthermore, the antibiotic susceptibility test was performed for purified bacterial strains using the standard "Kirby-Bauer disk diffusion" method on Mueller Hinton Agar (MHA) [30]. The commercially available discs "(OXOIDS)" of five commonly used antibiotics include Ampicillin (10 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Gentamycin (10 µg), and Oxcillin (1 µg) were used to screen the antibiotics-resistant profile of the isolated bacteria. The MHA plates were inoculated and placed in incubation at 37ºC for 24 hours, colony inhibition zone was measured using a metric ruler and was compared against standards described by "Clinical and Laboratory Standard Institute (CLSI) guidelines. Based on the zone of inhibition, each bacterial isolate was categorized into resistant (R) and sensitive (S) with the given antibiotics [17].

Fig 1. Physical Map of Lahore Division (source of the map: Government of the Punjab, Pakistan webpage).

Isolation and Characterization of Fungi

To measure the fungal load and its species distribution in the CAFs dust, potato dextrose agar (PDA) plates were prepared to have antibacterial streptomycin to avoid bacterial contamination. A total of 100 µl suspension from each sample was inoculated on PDA plate and was incubated for 5-7 days at 30ºC. After the growth, each separated colony was further purified on SDA plates for its classification purposes. The identification of fungi was carried out through morphological and microscopic characteristics using the lactophenol cotton blue method [28].

Heavy Metal Determination

To analyse the concentration of heavy metals like lead, cadmium, and chromium, 1g of each filter dust was dried in the oven at 105ºC for 24 hours followed by its digestion in 1:3 mixtures of HCL and HNO_3 for 2 hours at 150ºC. The digested solution was cooled down at room temperature and was filtered through a filter membrane of a 0.45-µm diameter. The total volume of the digested solution was made up to 50 mL by adding double distilled water. The atomic absorption spectrophotometer was used to examine the concentration of Pb, Cd and Cr [31].

Cell Viability Assay

The A549 cell line was purchased from American Type Culture Collection and was maintained in Dulbecco's Modified Eagle's Medium (DMEM) media, supplemented with 10% of fetal bovine serum (FBS). The "MTT (3-(4, 5-dimethylthiazoyl-2-yl) 2,5 diphenyltetrazolium bromide)" (Sigma, St. Louis, MO, USA) assay was used to determine the cell viability. In each well of the 96-well plates, 1 x 10^5 cells/mL of A549 cells were seeded and after 24 hours, the culture medium was changed. Suspension of CAFs dust was prepared in a sterile PBS separately followed by vortex to ensure the suspension is properly dissolved. Cells were treated with the dust suspension at concentrations of 100, 200, 300 as well as 400 µg/ml and were incubated for 24 hours at 37ºC. On the next day, MTT solution was added to the wells and incubated further for 4 hours. The "formazan crystals" of the MTT assay were dissolved by adding 100 µL of Dimethyl sulfoxide (DMSO), and the mixture was shaken at room temperature for 30 minutes. Then, the micro-plate reader was utilized to take the absorbance of the samples (O.D) at 570 nm and the cell viability percentage was calculated using the following equation [32].

Toxicity% = 1- [mean OD of sample/ mean OD of control] x 100
Viability% = 100−Toxicity%

Statistical Analysis

Prism software version 6 and Excel 2019 were used to plot the graphs and data charts. For groups involved in cytotoxicity assay, One-way ANOVA was performed for analysing the differences between groups. The values of experimental groups were considered significant when p-value was <0.05 as compared to the control group (**; $p<0.05$; ***: $p<0.001$) .

The values were compiled as means±standard deviation.

Results and Discussion

Lahore, (31º15′-31º45′N and 74º01′-74º39′E) is the capital of the Punjab province, the second largest city of Pakistan, the fifth largest in South Asia and twenty third largest in the world. Currently, in the city, several small and large industries are operating in addition to the main train and bus stations [33]. It is densely occupied and has a population of 10 million with a total area of 1172 km^2. Overall, the average temperature of Lahore is 24.3ºC and the weather is a hot, semi-arid climate with an annual rainfall of 600 to 800mm [34, 14].

Microbial Contaminants Level and Composition

Dust collected from CAFs was first evaluated for the presence of total bacterial and fungal load along with the types of species and then the antibiotic resistance pattern of bacterial species was also determined. The mean concentrations of bacterial and fungal was $4X10^6$ CFU/g and $5X10^5$ CFU/g respectively and this concentration varied from filter to filter (Fig. 2). Overall, across all CAFs, the Gram-positive bacteria were more prevalent as compared to Gram-negative bacteria and the predominant species included *Staphylococcus*, *Bacillus*, *Streptococcus*, *Pseudomonas*, and *E. coli*.

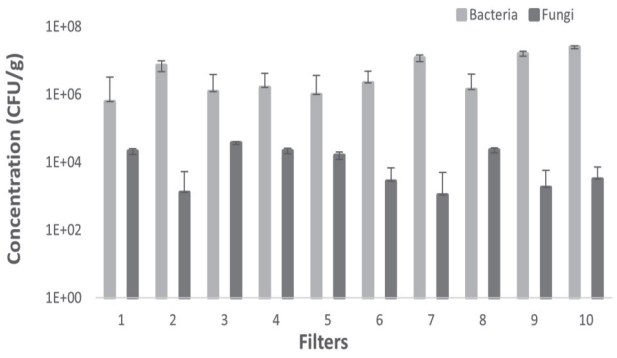


Fig 2. Total Bacterial and Fungal Count (CFU/g) in the car cabin filters (CAFs) Dust

Furthermore, the fungal species identified were *Penicillium*, *Alternaria*, *Cladosporium*, *Trichoderma* and *Aspergillus*. As far as the sources of airborne bacteria are concerned, major portion is contributed by plants, animals, soil, decaying animal bodies, sewage, waste disposable and treatment plants [35].

In addition, to assess the health risks associated with the presence of these bacterial contaminations in the CAFs dust, an antibiotic sensitivity pattern was checked for all of the isolated species against five commonly used antibiotics. Ciprofloxacin and gentamycin were the most effective while ampicillin, cefoxitin and oxacillin were the least effective antibiotics against the isolated bacterial species (Table 1). Most of the bacterial isolates are multi-drug resistant which shows the potential health hazards of residents' exposure to pathogenic bioaerosols [17, 36]. Several studies have reported a similar higher prevalence of antibiotic resistance in bacterial species isolated from ambient air [37, 38]. For proliferation and transport of air-borne antibiotics-resistant bacteria, the hospitals provide a suitable environment because the indoor environment accumulates the infectious bioaerosols that spread to the outside environment by air and cause nosocomial infections in the community [35].

Likewise, many studies have been conducted from different parts of the world including Lahore about the microbial communities present in the air and the atmospheric dust. A study from Lahore assessed microbial air quality of waste disposable stations and plants and found total bacterial and fungal counts in the range of 10^4 to 10^5 and 10^2 to 10^3 CFU/m^3 respectively. The major bacterial species identified were *Staphylococcus, Bacillus, Pseudomonas*, and *Mycobacterium* while the fungal species comprised *Penicillium*, *Aspergillus*, and *Fusarium* [39]. Another study reported bioaerosols from houses of asthmatic patients in Lahore and found *Bacillus* and *Staphylococcus*, the most common bacterial species while fungal species included *Aspergillus*, *Trichoderma*, *Alternaria*, *Cladosporium* and *Penicillium* [40]. Colbeck et al. [30] examined the indoor levels of bacteria and fungi in houses of Lahore city and found bacterial concentrations that ranged from 275 to 14469 CFU/m^3 in the living rooms while 472 to 9829 CFU/m^3 in the kitchens. Similarly, the fungal concentration varied from 315 to 1887 CFU/m^3in the living room while in the kitchen it ranged from 236 and 1887 CFU/m^3. Furthermore, they identified a total of 7 bacterial species that comprised *Staphylococcus* and *Bacillus* whereas the major fungal species were *Aspergillus*, *Alternaria*, and *Trichoderma*. The above-mentioned studies from Lahore reported similar bacterial and fungal compositions as we found in the current study. Similarly, Noris et al. [28] observed bacterial and fungal concentrations in heating, ventilation, and air conditioning (HVAC) filter dust varied from 10^4 to 10^7 and 10^3 to 10^7 CFU/g respectively. The growth of the microbes can vary from filter to filter depending on

Table 1. Antibiotics resistance profile of the isolated bacteria from CAFs dust (R= Resistance; S = Susceptible).

Antibiotics Discs	Potency (µg)	Bacterial Isolates in CAFs Dust				
		Staphylococcus	*Bacillus*	*Streptococcus*	*P. aeruginosa*	*E. coli*
Ampicillin	10	R	R	R	R	R
Ciprofloxacin	5	S	S	S	S	S
Cefoxitin	30	R	R	R	R	R
Gentamicin	10	S	S	S	S	S
Oxacillin	1	R	R	R	R	R

the moisture content, presence of organic matter and nutrients in the filters dust particles [17].

Inhalation serves as an important route of disease transmission therefore the presence of bioaerosols in the air may elicit hazardous human health effects such as allergic reactions, infections, inflammation, and respiratory diseases [40-45]. It should be noted that the majority of the bioaerosols isolated during this study were opportunistic and allergenic species [46, 47]. Information on the microbial count in ambient air is not only required to estimate the health risks associated with its inhalation but also to strengthen and formulate strategies to decrease microbial air pollution. In addition, this study further points out the importance of appropriate safety measures to avoid the resident's exposure to pathogenic bioaerosols.

Heavy Metal Concentration

Heavy metals including Pb, Cr and Cd become more concentrated in urban dust because of increased urbanization, industries, vehicular emissions, and human activities that pose risk to public health and the environment. Heavy metals are poisonous, non-biodegradable, persistent and may build up in the bodies of the animals. Residents, particularly the elderly and children can be highly affected by the long-term exposure to the polluted dust. It can disrupt the functioning of internal organs such as the kidney and can harm the reproductive, nervous, and the circulatory systems as well as retard IQ development [8].

Table 2 summarizes the concentration of heavy metal (Pb, Cr, Cd) in the CAFs dust of Lahore along with the acceptable limits set by World Health Organization (WHO). The Pb concentration was high across all samples with the mean values of 201.2±55 mg/kg. CAFs dust concentrations for Cr and Cd were lower than Pb, with the mean concentration of 137.8 ±29 and 7.6±2.7 mg/kg respectively. In our study, the mean values for Pb, Cr and Cd (201.1, 137.8, 7.6 mg/kg) were significantly higher than the tolerable limits set by WHO (100, 100, 3 mg/kg) respectively [8]. From Pakistan, other similar studies have also reported higher concentrations of heavy metals from school and road dust [8, 33, 48, 49]. The trace metal concentrations in the air of Lahore city are higher because of the new coal power plants, rapid industrialization, anthropogenic activities, and a high number of vehicular emissions [50].

The local population have a great potential exposure to these toxic heavy metals that can accumulate through inhalation, dermal contact and ingestion upon long term exposure and may elicit chronic damage, especially in children [17]. This is a frightening situation, and it is highly advisable that related authorities should develop and implement an air pollution abatement policy to protect the people and the environment.

Cytotoxicity Analysis

For toxicological characterization, in vitro tests using the relevant cell cultures can provide useful information related to health effects. The A549 cell

Table 2. Selected heavy metal concentrations (mg/kg) Pb [a], Cr [b] and Cd [c] levels in CAFs [d] dust.

Filter	Pb (mg/kg)	Cr (mg/kg)	Cd (mg/kg)
1	273	121	9.2
2	249	136	6.7
3	155	192	3.4
4	127	87	11.4
5	203	129	8.1
6	213	116	7.8
7	179	141	6.3
8	253	158	9.7
9	119	171	3.3
10	241	127	10.1
Mean	201.2	137.8	7.6
Maximum	273	192	11.4
Minimum	119	87	3.3
Standard Deviation (SD)	55	29	2.7

[a] Lead (Pb), [b] Chromium (Cr), [c] Cadmium (Cd), [d] CAF (Car Cabin Filter)

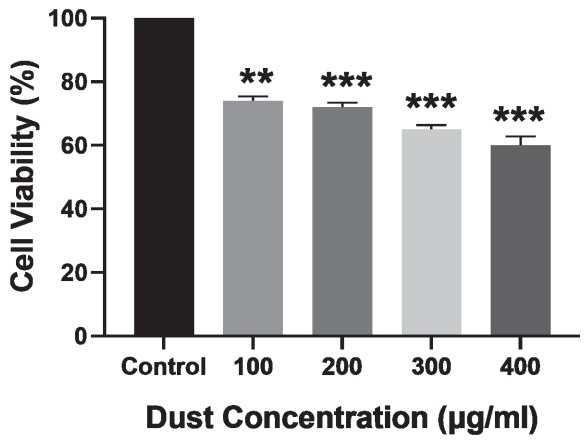


Fig. 3. Cell viability of A549 cells in MTT assay exposed to car cabin filters (CAFs) dusts collected from Lahore as calculated by % of the negative control (Dulbecco's Modified Eagle Medium(DMEM). A549 cells were treated at concentrations of 100-400 µg mL^{-1} for 24 hours. The values of experimental groups (CAFs dust) were considered significant when p-value was <0.05 as compared to the control group (**; $p<0.05$; ***: $p<0.001$).

culture was chosen because air pollution mostly triggers inflammatory conditions in the respiratory system, particularly the lungs. The purpose of this experiment was to assess the real exposure scenarios as previously a study reported adverse impacts of the pollution on the lungs of the traffic wardens from Lahore city [51]. In our study, the viability assay showed that exposure of A549 cells to CAFs dust decreased its viability significantly (Fig. 3). The cytotoxicity results were measured as the percentage decrease in the viable cells with respect to negative controls. There was a linear relationship between dust concentration and cell viability as the dust concentration increased from 100-400 µg/mL, the cell viability decreased accordingly. Similarly, another study conducted by Mohseni et al. [34] found the same toxicity level of dust on A549 cells where the viability percentage of cells decreased with the increase in dust concentration. Another study conducted by Faraji et al. [12] found similar results in which the peripheral blood mononuclear cells (PBMCs) treated with dust storm samples decreased the cell viability significantly with the increase in dust concentrations. The induced cytotoxicity on A549 cells by the dust is because of its composition particularly the heavy metals (e.g., Pb and Cr) and airborne microbes and the mechanisms involved are enhanced production of cellular oxidative stress levels, DNA damage, production of inflammatory cytokines and mutagenicity [52-54].

Overall, the CAFs dust samples showed a significant cytotoxic effect on A-549 cells that points to the potential risk to the respiratory system of the exposed inhabitants. Moreover, it indicates the importance of preventive and control measures regarding public health safety and the reduction of urban air pollution.

Conclusions

The air quality monitoring of Lahore city was found to be poor. The level of bioaerosols and trace metals demonstrated higher than the prescribed limits. The microflora composition of the city comprised both opportunistic bacteria and fungi that may affect the exposed residents. On a priority basis, air quality monitoring stations and maintenance policies should be implemented and most importantly, practical measures need to be taken to combat the increase in air pollution in Pakistan. Additionally, awareness campaigns should be carried out to inform policymakers and the general public about the adverse health impacts of air pollutants.

Although CAFs filters provide better air quality but these air conditioner filters may act as a contamination source too once they got contaminated, therefore, they need to be replaced on regular basis. Moreover, CAFs can be utilized to study air quality, environmental contaminants, urban health, and residents' exposures to the pollutants present in the air. Furthermore, this study can be extended to animal models to see the in-vivo effects of air pollution.

Highlights

1. The air quality monitored in Lahore city was found to be poor.
2. The concentration of bioaerosols and heavy metals demonstrated higher than the prescribed limits.
3. Contaminated CAFs may act as a contamination source once contaminated.
4. CAFs as sample sources provide a better understanding of urban air quality.
5. Policies need to be implemented to ensure environmental and public health safety.

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Conflict of Interest

The authors declare no conflict of interest.

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