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Original Article

Particulate Matter Concentration and Microbial Load in Heavy Traffic Areas of District Lahore, Pakistan

Farzana Rashid[1], Sadia Sarwar[1*], Samrin Habib[1] and Hunaiza Tahir[1], Iqra Noshair Ali and Tanwir Ahmad Abbas[2]

[1]Department of Zoology, Lahore College for Women University, Lahore, Pakistan
[2] Govt. Graduate College Asghar Mall, Rawalpindi

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***Corresponding Author:**
Sadia Sarwar
Department of Zoology, Lahore College For Women University, Lahore, Pakistan
sadisarwarss8@gmail.com

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ABSTRACT

A major threat to the environment and public health is air pollution. Previous research has looked at the harmful health impacts of both short- and long-term particulate matter exposure. In addition, PM (particulate matter) of fine or coarse size (PM 2.5 and PM 10) was linearly related with mortality, with PM 2.5 being associated with long-term mortality and PM10 being connected with short-term mortality. Traffic congestion is one of the foremost problems. **Objective**: To characterize gram positive and gram negative airborne bacteria from air samples of heavy traffic areas. **Methods:** These were assessed using direct as well as indirect sampling technique. A total of 40 samples 10 from each traffic areas (i.e., Bhatti Gate, Bohr wala chowk, Chauburji and Railway Station) were collected. These were collected with the help of Mini Vol portable air sampler. Filter papers containing particulate matter 2.5 and 10 were placed on prepared petri dishes. The petri dishes were then kept in an incubator for 24 hours at 37 °C, the appearances of colonies were characterized and proceeded for morphological and biochemical identification. **Results:** The results showed the presence of different bacteria including *Bacillus spp.*, *Micrococcus spp. Streptococcus* and *Staphylococcus aureus*, *Pseudomonas aeroginosa*. **Conclusion:** It was found that air was heavily polluted with PM 10 and *micrococcus aureus*.

INTRODUCTION

Air is a medium in which breath but pollution is increasing day by day in the environment, so healthy environment is a big challenge. A smooth air is hard to discover. Due to heavy traffic and combustion vehicles, the pollution and number of contaminants is alarmingly increasing. The more is the pollution in the environment, more is the bacterial contamination. Airborne microorganisms are abundant in the atmosphere of Earth. It is believed that these microorganisms display relationships with the weather and air pollution [1]. Because it lacks the requisite amounts of moisture and nutrients in a form that can be used by microorganisms, air really isn't a natural habitat for their development and reproduction. Despite the absence of a flora, organisms may nevertheless be detected in air. This could be a result of the fact that air, with its constant movement and mixing on a variety of scales, is an efficient medium for dispersing microorganisms[2, 3]. Different natural sources, including soil, animals, and people, are the source of airborne microorganisms [4-6]. Microorganisms are released into the atmosphere by human-made processes such sewage treatment facilities, animal rendering, fermentation, and agricultural operations [7-9]. Concern about air quality is spreading around the globe [10]. The amount of airborne pollutants affects air quality. Particulate matter (PM 10) is also produced in outdoor air by motor vehicle exhaust [11–13]. Although high numbers of

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bacteria in an air sample are a sign of overcrowding or inadequate ventilation, bacteria in the air do not often pose a health risk [14]. Certain kinds of asthma, allergic rhinitis, and hypersensitivity pneumonitis are brought on by biological pollutants [15]. These pollutants may also irritate human skin, which may worsen symptoms including headache, weariness, and irritation of the eyes, nose, and throat [16]. The airborne bacterial content, particle concentration, and size distribution remained constant within each setting throughout the investigation by Dybwad et al., from May to September 2010. The genera Bacillus, Micrococcus, and Staphylococcus comprised the bulk of the airborne bacteria that were present. PM, or particulate matter, is a mixture of tiny particles and liquids that are visibly suspended everywhere. Various materials make up particles. PM may be solids, such as a dry powder made up of minuscule fragments or residue. PM may also be solids suspended in liquid mixes or entirely liquid aerosols. Coarse PM (PM 10) with a diameter less than 10 m, fine PM (PM 2.5) with a diameter less than 2.5 m, and ultrafine PM (PM 0.1) with a diameter less than 0.1 m are the three categories used to categorize particulate matter [14]. Particulate matter (PM) is created by a variety of human activities such as building, agriculture, as well as industrial operations like cutting and grinding. In contrast, the main sources of PM 2.5 are stationary combustion sources and combustion sources such as the exhaust from cars, trucks, and other types of vehicles. The particles are either immediately released into the atmosphere or are created there as a result of gas combustion. This study's major goal was to identify and describe gram positive and gram negative airborne microorganisms that were prevalent on a public transportation system. The study will further describe the morphology of the colonies and bacteria from the culture media under the microscope. The present study has been conducted to assess the concentration of particulate matter (PM 2.5 and PM 10) at different traffic sites Bhatti Gate, Railway Station, Bohar wala chowk and Chauburji Pakistan.

METHODS

A detailed description of sampling locations, sampling methodology, instrumentation, parameters and procedure is divided into three parts:

1. Sampling of PM 2.5 and 10 from various traffic areas.
2. Measurement of concentration of PM 2.5 and 10 in various traffic areas.
3. Identification of bacteria in various traffic areas.

Total of 40 samples were collected in four different traffic sites (Railway Station, Bohar wala chowk, Chauburji and Bhatti Gate) in Lahore during the months of January and May 2018, between 2 p.m. and 3p.m. A cross-sectional study was carried out to determine the levels of PM 2.5 and 10 and to assess microbial counts with in outdoor air at selected roadside traffic areas. Samples collected by using MiniVol portable air sampler SN:3224. Nutrient agar plates were prepared for culturing of samples. Incubated for 24 hours at 37°C. Isolates obtained after incubation were characterized by colony morphology. The gram staining method performed to differentiate between Gram negative and Gram-positive bacteria. Different biochemical tests were performed for the biochemical characterization of the cultures. The tests include: Urease test, Coagulate plasma test, carbohydrate fermentation test, Motility test, Catalase test, Oxidase test.

RESULTS

A total of 40 samples were collected and analyzed for bacterial contamination from different traffic areas of Lahore i.e., Chauburji, Bhatti Gate, Bohr wala chowk and Railway station for three days a week. Bacterial colonies were characterized by the color appearance under the colony counter. The mostly observed colonies were the round white colonies, irregular white colonies and filamentous white colonies. In the present study the different bacterial faunas were observed in the Public Land Transport during peak hours. The concentration of PM (particulate matter) 2.5 and 10 in four traffic areas of Lahore was measured and analyzed as shown in figure 1 and 2. Among these four sites, the PM 2.5 concentration (µg/m3) was highest in Bhatti Gate and lowest in Railway Station while the PM 10 concentration (µg/m3) was highest in Bohr wala Chowk and lowest in Chauburji.

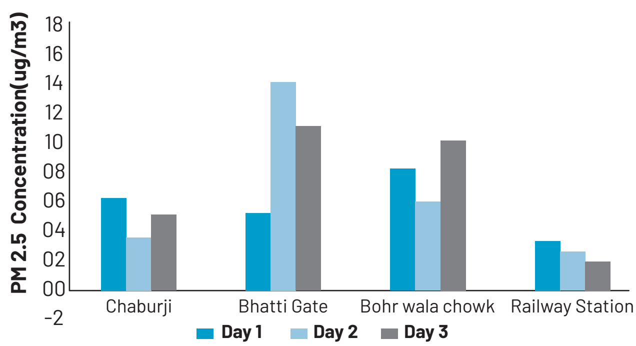

Figure 1: Graph showing the PM 2.5 Concentration (µg/m3) of four different traffic sites of Lahore, Pakistan.

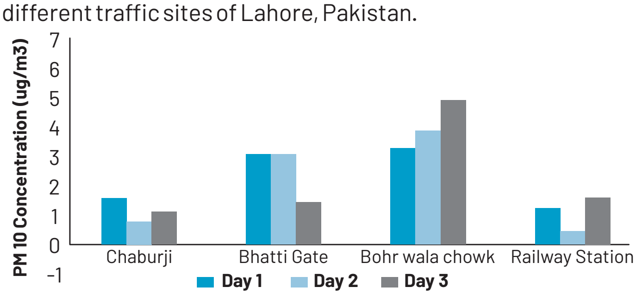

Figure 2: Graph showing the PM 10 Concentration (µg/m3) of from four different traffic sites of Lahore, Pakistan.

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The mean values of concentration of PM (particulate matter) 2.5 and 10 was measured in each of four traffic areas is shown in figure 3. Among the four sites, the concentration of PM 2.5 calculated was highest in Bhatti gate which was 10.18µg/m3 and was lowest in railway station which was 2.78 µg/m3 and the concentration of PM 10 calculated was highest on Bohr wala Chowk which was 4.22 µg/m3 and was lowest in Chauburji which was 1.12 µg/m3. After isolation and characterizing the bacterial samples, they were subjected to morphological and biochemical analysis to confirm and identify bacteria present in the traffic sites including Chuaburji, Bhatti Gate, Bohr wala chowk and Railway Station. In the traffic areas, different bacteria were identified shown in Table 1.

Source of samples	Total no. of samples	No. of samples devoid of bacteria	Type of bacteria identified
Chauburji	10	Nil	Micrococcus, Bacillus and Streptococcus spp.
Bhatti Gate	10	Nil	Micrococcu, Streptococcus and Staphlococccus spp.
Bohr wala chowk	10	Nil	Micrococcus, Staphlococcus and Bacillus spp.
Railway Station	10	Nil	Staphlococcu, Micrococcus and Bacillus spp.

Table 1: Bacterial analysis in the air of 40 air samples collected from heavy traffic areas of Lahore.

The large number of colonies isolated from the traffic areas of Lahore were of Micrococcus species while the smaller number of colonies isolated were of Streptococcus species. The number of colonies isolated is shown in Table 2.

Bacteria identified	Sources	No of colonies			Pathogenic/non Pathogenic
		D1	D2	D3	
Micrococcus	Railway Station	30	28	32	Harmless
	Bhatti Gate	40	91	15	
	Chauburji	35	30	22	
	Bohr wala chowk	73	57	30	
Staphyloco-ccus	Railway Station	60	18	21	Harmful
	Bhatti Gate	12	38	05	
	Chauburji	Nil	09	Nil	
	Bohr wala chowk	17	22	10	
Bacillus spp.	Railway Station	45	11	11	Harmless
	Bhatti Gate	Nil	Nil	02	
	Chauburji	07	08	12	
	Bohr wala chowk	11	18	10	
Streptococcus	Railway Station	Nil	Nil	Nil	
	Bhatti Gate	21	Nil	Nil	
	Chauburji	05	Nil	06	
	Bohr wala chowk	Nil	Nil	Nil	

Table 2: The number of colonies isolated and pathogenic status of bacteria from heavy traffic areas.

After bacterial isolation gram staining done. Staphylococcus aureus, Micrococcus and Bacillus subtillis show positive gram staining reaction. Bacterial colonies were counted and these colonies were calculated by the (Total colonies x 1,000)/100=Total counts (colony forming units/m3 or cfu/m3). The concentration (cfu/m3) of airborne bacteria from PM 2.5 and PM 10 at four different traffic sites of Lahore is shown in fig 3 and 4.

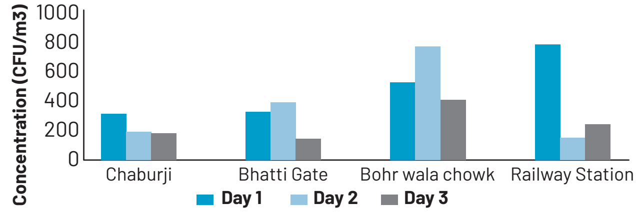

Figure 3: Graph showing the concentration (CFU/m3) of airborne bacteria from PM 2.5 at four different traffic sites of Lahore, Pakistan.

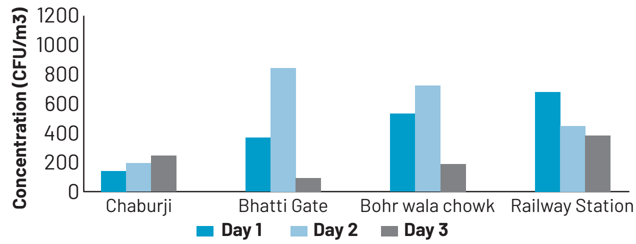

Figure 4: Graph showing the concentration (CFU/m3) of airborne bacteria from PM 10 at four different traffic sites of Lahore, Pakistan.

The biochemical characterization for strains is shown in Table 4.

Test	No. of strains (+)	No. of strains (-)
Coagulate plasma test	Staphlococcus	Micrococcus
carbohydrate fermentation test	-	Micrococcus
Motility test	Staphlococcus Micrococcus	Bacillus
Catalase test	Staphlococcus	Micrococcus

Table 3: Biochemical characterizations based on different tests for gram-positive and gram-negative bacteria strains

Air samples collected from the Railway Station and Bohr wala Chowk had the highest mean bacterial count 476.7 ± 151.4 and 493.3 ± 175.0 from PM 2.5 and 10 respectively while air samples collected from Chauburji had lowest mean bacterial count (203.3 ± 61.1 cfu/m3) and (196.7 ± 106.9) from PM 2.5 and 10 respectively

Sr No.	Locations	Mean ± Standard Deviation (cfu/m3) For Bacterial Count From PM 2.5	Mean ± Standard Deviation (cfu/m3) For Bacterial Count From PM 10
1	Chauburji	203.3 ± 61.1	196.7 ± 106.9
2	Bhatti Gate	470.0 ± 409.5	276.7 ± 138.0
3	Bohr Wala Chowk	456.7 ± 251.1	493.3 ± 175.0
4	Railway Station	476.7 ± 151.4	476.7 ± 285.4

Table 4: Mean and standard deviation of bacterial count from samples of different sites of traffic areas of Lahore, Pakistan.

DISCUSSION

Bacterial concentration in the air depends upon many factors such as humidity, temperature, and radiation that

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influence the bacterial viability. Different types of methods (air sampler or the prepared plates) were used to collect the bacteria. According to research that employed a SASS 3100 high-volume electret filter-based air sampler, airborne bacterial concentration levels were equivalent to those found in other studies conducted in subway settings, indicating that this instrument would be appropriate for collecting airborne bacteria[15-17]. The portable MiniVol air sampler with the SN:3224 was used to carry out the current investigation. In a different investigation, the mass concentration of particulate matter (PM 2.5 and PM 10) was measured using a MiniVol SN: 3224 air-sampler[18]. The automobile pollutants nitric oxide (NO), nitrogen dioxide (NO2), and total hydrocarbons were linked to suspended particulate matter [19]. In separate research, Andleeb et al., made the case that outdoor sources are a significant factor in indoor NO2 exposure in densely populated urban regions and that ventilation and infiltration have a significant impact on NO2 levels in constructed environments in cities [20]. According to the current research, Pakistan's Bhatti gate has significant levels of bacterial and particle pollution. According to research conducted in Bankok, Thailand, houses adjacent to highways or in metropolitan areas tend to have higher interior particulate matter concentrations, particularly PM 2.5. Research conducted by Chua et al., similarly discovered a significant concentration of PM 2.5 in an urban region [21]. Due to the fact that dust serves as a substrate for microbial development, the concentration of particulate matter or dust was linked to the quantity of microbes. Among the sources of particulate matter were cooking, dogs, and vehicle activity. Although airborne particles may travel great distances, peak concentrations usually happen near highways. The majority of individuals exposed are pedestrians and street vendors. PM 10 has been connected to diseases and fatalities brought on by heart or lung disease and has the potential to cause or exacerbate a range of health issues. Asthma and chronic bronchitis are only a few of the respiratory conditions that might make you more susceptible to contracting [22–24]. The regions in the current research were surrounded by traffic and dust particles that might lead to airborne infections. Short-term and long-term exposure to particulate matter may induce respiratory disorders and result in cardiovascular mortality and morbidity, this research was done on air pollution connected to traffic [25, 26]. It was stated that over the whole measured size range, higher particle concentrations were seen at the subway station during the day than at night. This is likely connected to anthropogenic daytime sources, such as people and train movement, which were mostly absent at night. In the current investigation, the daytime and higher concentration of particles were observed. Luksamijarulkul et al., reported that anthropogenic factors, such as people and train activity, were substantial contributors to airborne bacteria at the Norwegian subway station in their earlier investigation, which showed variations in bacterial diversity across the settings [27]. The gram-positive cocci (Micrococcus spp. and Staphlococcus spp.) and rods (Bacillus spp.) from high traffic locations were discovered in the current investigation. The majority of bacterial cultures in the research done at the Skyway stations in Bankok were gram-positive cocci (Staphylococcus spp.), while the majority of fungal cultures were Aspergillus spp., Botrytis spp., and Curvalarium spp. [28]. At RCEES and XZM in Beijing, larger microbial concentrations were found throughout the summer and fall, when the temperature and humidity offered the best circumstances for the airborne microorganisms. [29]. In our study, the higher bacterial density was detected in the Bhatti Gate of Lahore, Pakistan in the summer conditions as shown in figure 1. The mean (± SD) bacterial counts ranged from 319.3 ± 194.8 cfu/m3 to 547.7 ± 282.1 cfu/m3 and the mean fungal counts ranged from 89.6±47.2 cfu/m3 to 157.7±84.7 cfu/m3 [28]. The summary of mean ± standard deviation of the different traffic areas of Lahore, Pakistan where mean ± standard deviation (SD) of bacterial count was 203.3 ± 61.1 cfu/m3 to 476.7 ± 151.4 from PM 2.5 and 196.7 ± 106.9 cfu/m3 to 493.3 ± 175.0 from PM 10.

CONCLUSIONS

From present study it is concluded that the large number of airborne bacteria were identified Micrococcus, Bacillus, Staphylococcus and Streptococcus that can be associated with airborne diseases. Among the four sites, the PM 2.5 was highest in Bhatti Gate and lowest in Railway Station while PM 10 was highest in Bohr wala Chowk and lowest in Chauburji. It is also concluded that the large number of colonies isolated from the traffic areas of Lahore were of Micrococcus species while the smaller number of colonies isolated were of Streptococcus species. Different bacterial fauna isolated from heavy traffic areas were Micrococcus, Staphlococcus, Bacillus and Streptococcus species.

Conflicts of Interest

The authors declare no conflict of interest.

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