

Spatial and temporal variation of bacterial population in ambient air particulate matters (PM_{2.5}, PM₁₀ and TSP) of Isfahan city, Iran

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ABSTRACT

Introduction: Airborne particles are considered as an important indicator of outdoor air quality. Many health problems have been linked to high concentrations of Particulate Matters (PMs) and their associated microorganisms. The aim of this study, therefore, was to investigate the population of bacteria in PMs in various times and locations.

Materials and methods: The PM samples including PM_{2.5}, PM₁₀ and Total Suspended Particles (TSP) were taken from 4 different stations of Isfahan city, Iran on February (cold season) and July (warm season), 2019 using an air sampling pump on culture media. The number of bacterial colonies was counted after 48 h growth in the incubator at 37 °C. The PMs concentration and some morphological characteristics of bacteria were also investigated.

Results: The highest number of bacterial colonies was in TSP followed by PM₁₀ and PM_{2.5}. The bacterial populations at two stations in north and east of the region in the warm season were higher than in the cold season, and the respective situation in the other two stations at south and center of the city was reversed, which seems somehow to have been the result of the PMs concentration of difference of pollution sources in various locations and seasons.

Conclusion: This study highlights the importance of PMs pollution especially PM_{2.5} (i.e. the main factor affecting the air quality of the study area) as the carrier of microbial pollution in the air which could adversely affect human health.

Introduction

Rapid growth of population has been associated with industrialization and urbanization have led to air and environmental pollution [1]. Indoor and outdoor air pollution may be associated with

various health effects, including respiratory and cardiovascular diseases, pneumonia, chronic obstructive pulmonary disease, lung cancer as well as premature human mortality worldwide [2]. World Health Organization (WHO) has reported that about 4 million people die every

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year prematurely from diseases caused by household biomass smoke [3]. The prevalence of many different respiratory, vision and skin diseases is the consequence of exposure to air pollutants such as Particulate Matters (PMs) and bioaerosols [4, 5]. It has been reported by WHO that the mean attributable death rate due to ambient and household air pollution particularly $PM_{2.5}$, (i.e. the fine particles with aerodynamic diameter $\leq 2.5 \mu m$) is 94.8 per 100,000 population [3]. Various organic and mineral substances such as heavy metals, sulfate ions, nitrate and ammonium, and organic compounds (e.g. Polycyclic Aromatic Hydrocarbons (PAHs)) associated with PMs, could adversely affect human health [6-9].

Air pollution caused by human activities is one of the most important destructive factors to the environment. Industries, power plants and domestic and commercial sources have caused regional problems by releasing ozone-depleting gases and the production of acid rain and have also led to global problems like climate change by releasing carbon dioxide and other greenhouse gases. These problems are the most important global challenges at present [1]. Air pollution has different sources and characteristics with respect to their combination and the situation, under which they are produced and consequently it would have different environmental and health effects [2].

Bioaerosols are airborne compounds or microfragments of plants, animals and microorganisms which are dead or alive [10]. Different types of bioaerosols including bacteria, viruses, fungal spores, mosses, ferns, pollens and small plant seeds as well as parts of arthropods and dander of pets in the air can be attached to PMs [11]. Bioaerosols

could be coated by liquid or solid secondary organic and inorganic materials that affect their physicochemical and biological properties. Bioaerosols may contain some pathogenic microorganisms (e.g. bacteria and fungi), which induce some side effects relative to health such as respiratory diseases [12, 13]. Air borne particulate matters can act as carriers of pathogenic microorganisms, and this interaction has serious consequences, such as deeper penetration into the lungs than expected. As a result, when chemical and biological components combine in PMs, harmful effects on health like chronic lung disease, asthma and lung cancer will increase [4].

The bioaerosols in the air may be found as separate particles or dense particles (agglomerates). Therefore, their dynamics in the air mainly depends on the physical properties of the particles, where the size and concentration are the most important ones [14]. Various parameters including temperature, humidity, and extent of air pollution may affect the composition and concentration of bioaerosols [15]. In this regard, previous researches showed that dust events as one of the main source of air pollution in Middle East countries could affect microbial concentrations in PMs so that the concentration in dust event days was more than normal days [16-18]. So far extensive researches have been conducted on the spatial and temporal changes of bioaerosols [19-21]. Given the current state of societies and the prevalence of the new Covid-19 virus, more attention has to be paid to micro-airborne particles and the role of bioaerosols in the transmission of pandemic diseases, especially through the respiratory tract and the infection of human population. So the investigation of microorganism populations associated with air

pollutants specially PMs having different sizes is very important issue.

Isfahan is one of the metropolises in Iran that has no clean and healthy air in most time of the year due to various industrial activities, such as iron and steel industries, power plants, brick production, cement factories, lime and gypsum mines, transportation and proximity to desert which is the source of natural dusts. Particulate matters, especially $PM_{2.5}$, are the most important factors influencing the air quality of the city. Considering the existence of various diseases, especially cardiovascular and pulmonary diseases in this city and its potential relationship with air pollution, it is important to study the PM characteristics, particularly identification of microbial population associated with fine particles. Therefore, this study was conducted with the aim of identification of spatial and temporal variation of bacterial population in PMs of ambient air with various sizes ($PM_{2.5}$, PM_{10} and TSP).

Materials and methods

Sampling area

Isfahancity, the capital of Isfahan province, is one of the metropolises of central Iran (Fig. 1). The city has a population of about 3 million. There are various urban, agricultural and industrial activities in the region including iron and steel industries, oil refinery, petrochemical industry, power plant, brick and cement production, lime and gypsum mines and transportation as well as natural centers of dusts in suburbs, especially in the eastern part which have been adversely affected air pollution. In this study, 4 sampling stations were selected based on the level of air pollution, proximity to urban and

industrial areas and population density. The station 1 (Isfahan University of Technology) is located in the north of city and close to Isfahan oil refinery, Isfahan petrochemical industry and power plant industrial zones). The station 2 (Jey station) is located in the east near the main bus station of the city and the closed to the desert area which is considered as natural dust sources. Station 3 (Soffeh station) is located in the south and near the cement, Iron and Steel Industries. Station 4 (Imam Hussein square station) is located in the city center and is mainly affected by transportation activities. The location of the stations is shown in Fig. 1.

PM sampling and measurement

Samples of Particulate Matters ($PM_{2.5}$, PM_{10} , TSP) on plates containing culture media were separately collected on February (cold season) and July (warm season) 2019 in one day and with three repetitions in each station. Sampling was done using an Anderson's one stage impactor (SKC, UK) and it was done continuously and actively for 20 min using the sampling pump with a volumetric flow of 28.3 L/min [22]. In the summer, due to the high density of the created bacterial colonies and their unaccountability, sampling took 10 min. An important principle in sampling of bioaerosols is to sterilize the equipments. Therefore, before the culture medium was placed inside the sampler, the sampler chamber and cyclones were sterilized by autoclave device. A plate was used as a control sample from the beginning of sampling to the end at each station. The sampling device had two particle-separating cyclones, where after passing of airborne particulates, only $PM_{2.5}$ and PM_{10} were sucked on the culture medium, and without them, TSP was collected on the culture medium. The samples were collected from the

height of 2 m above the ground (normal height for measuring pollutants and human respiratory height). A general culture medium, nutrient agar (Merck, Germany), was used to determine the bacterial population. After sampling, the plates (both control and main samples) was completely sealed with parafilm and aluminum foil (to eliminate any initial contamination). Between the samples, inside of impactors and cyclones was disinfected by ethanol (70 % v/v). At each station 4 plates were used the name of the sampling station, the date and time of sampling were recorded. After the sampling, the plates were transferred to laboratory. The number and concentration of $PM_{2.5}$ and PM_{10} , relative humidity and air temperature were measured using a portable device (CEM, DT-9881, Taiwan) during the sampling time.

Bacterial growth and colony counting

The collected samples were transferred to the incubator which was set at 37 °C. After 48 h the colonies formed on the culture medium were counted directly using the colony counter device and finally were reported in unit of colony forming unit CFU/m³ based on total air volume sucked. After that the colonies were characterized using the Gram staining and morphological shape [23].

Data analysis

SPSS 22 and Excel 2016 were used for statistical analyses and to draw the graphs. Duncan's average comparison test was used to compare the mean values.

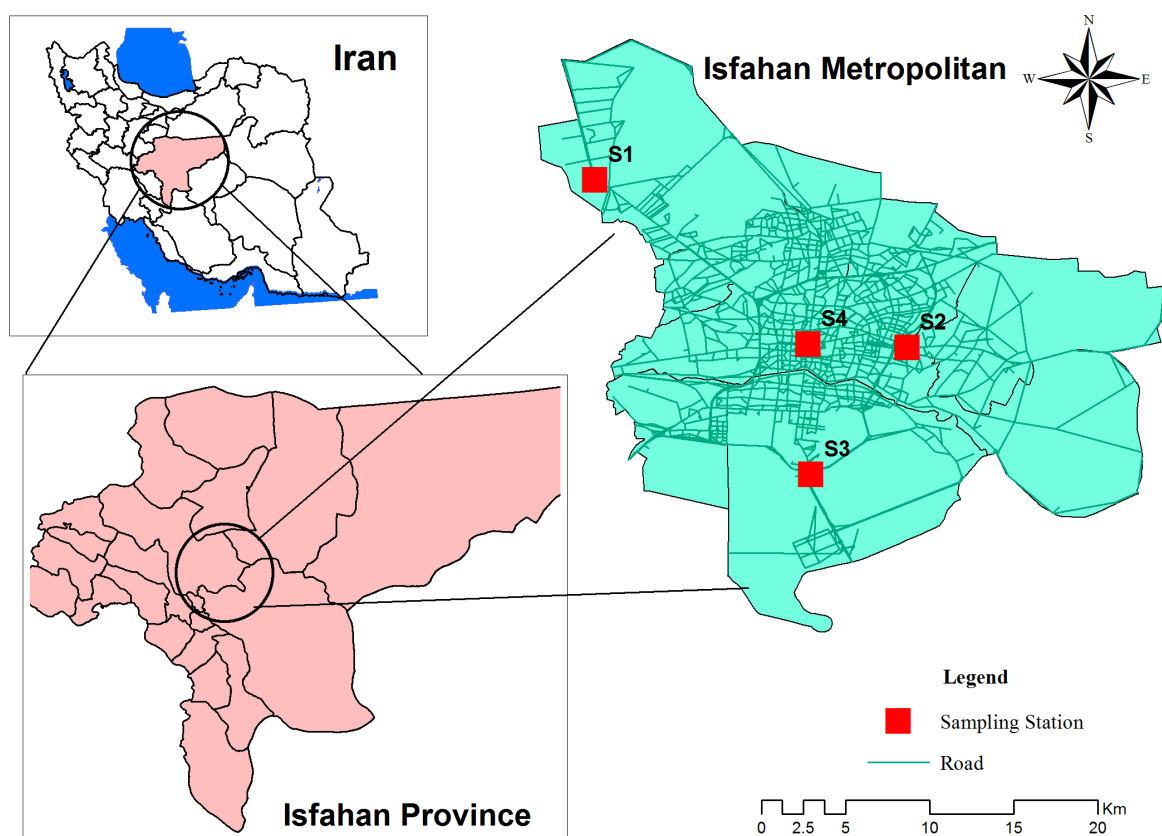


Fig. 1. Location of sampling points. S1: Isfahan University of Technology station; S2: Jey station, S3: Soffeh station; S4: Imam Hussein Square station

Table 1. The average concentration of particulate matters and count and some metrological parameters of sampling stations in winter and summer

	Station	PM _{2.5} concentration (µg/m ³)	PM ₁₀ concentration (µg/m ³)	PM _{2.5} (count/m ³)	PM ₁₀ (count/m ³)	Temperature (°C)	Relative humidity (%)
Winter	S1	74	53	595	40	7.5	30.1
	S2	28	71	708	33	9.8	27.2
	S3	7	19	164	10	13.4	13.5
	S4	4	42	222	15	13	11.6
Summer	S1	25	101	632	52	24.4	25.1
	S2	15	71	318	34	27.8	18.8
	S3	18	35	180	18	30.8	15.6
	S4	12	38	186	22	38.5	9.5

S1: Isfahan University of Technology station; S2: Jey station, S3: Soffeh station; S4: Imam Hussein square station

Results and discussion

PMs concentration and meteorological parameters

Temperature and relative humidity are two important environmental parameters that affect the survival of bioaerosols in the environment. In winter, these parameters were higher in station 1 (Isfahan University of Technology) and station 3 (Soffeh) and in summer, the same parameters were higher in station 1 and station 4 (Isfahan University of Technology and Imam Hussein Square, respectively) in comparison with the other stations (Table 1).

The average concentrations and number of PM_{2.5} and PM₁₀ in both cold and warm seasons at stations 1 and 2 were higher than stations 3 and 4. Station 1 had a higher concentration than other stations which might be because of the effect of industrial activities (e.g. Isfahan oil refinery, Isfahan petrochemical industry and Isfahan power plant) and the traffic of heavy vehicles in north highway of Isfahan city. Station 2 had also a high concentration and number of PMs due

to the transportation, as well as greater impact from the eastern centers of Isfahan which is the main source of natural dusts. The concentration of PM_{2.5} in stations 1 and 2 in cold season was higher than of that in warm season (Table 1). The location of these points and the activation of sources producing small particulate matters, such as industry and transportation, as well as their intensification due to the air inversion could increase PM concentration in stations 1 and 2. The concentration of PM₁₀ in all stations except station 4 located in the city center has an increasing trend in warm season compared to cold season (Table 1). One possible cause could be the impact of these areas from local dust, as well as increased vehicle traffic there during the warm season. The same trend is seen for the number of airborne particles. This makes sense given the relationship between the number of particles and their concentrations.

The trend of changes of bacterial population in different seasons and stations

In warm season, the population of bacteria

associated with particles of different sizes in stations 1 and 2 was higher than that in cold season. While in two next stations (station 3 and 4) the population of bacteria was higher in cold season or it didn't show any significant difference (Figs. 2-4). This trend was also consistent with the concentration of PMs at these regions (Table 1). It has been reported that there may be a direct link between microbial mortality and PMs concentration, and the microbial population may gradually increase as the air warms up [24, 25]. Of course, climatic conditions such as wind speed and direction, and the extent of their affection from the sources of PMs are influential in this regard. The mean bacterial concentration was less than of other parts of Iran, such as Ahvaz, Sanandaj and Ilam where more affected by dust storms and have higher PM concentrations [16-18]. The special location of station 3, its great distance from the surrounding industry and the lower traffic of cars there, has made the average concentration of particles and bacterial population less than those in other study areas. There is usually a positive relationship between human activity, vehicle density, and population density with increasing of particulate matter, dust, and smoke in the air which are places for bacteria to bond. More vegetation around areas 3 and 4 than other areas can also be effective in reducing bacterial populations in PMs. The presence of vegetation in an area not only reduces bacteria in the air, but also destroys bacteria by producing volatile compounds that have disinfectant properties. It should be noted that land-use change affects the formation and distribution of bioaerosols [11]. This doubles the need to maintain vegetation and to prevent land-use conversion, especially in densely populated urban areas. Furthermore, vegetation may reduce the soil erosion and

consequently decrease the PM concentrations which significantly affect microbial populations associated with particles [16-18].

In winter, the concentration of bacteria can be increased due to the lower air temperature and less exposure to sunlight, which has its own ultraviolet rays which have disinfectant and antimicrobial properties. However, in the present study, it seems that what has increased the bacterial population in winter in some places has been the increase in the concentration of PMs that has a direct relationship with the bacterial population. Factors such as the concentration of industrial activities, the air pollutants concentration, proximity to factories and industries, air temperature, air pollution on sampling days, and vehicle traffic are effective in increasing the population of bacteria in the air. On the other hand, climatic conditions in different places and times can be different, which affects the bacterial population. The relationship between environmental conditions and the distribution of bacteria in the air shows that microbial compounds can pose a higher health risk due to pathogenic factors or insensitive components of uncontrolled environmental bacteria [26]. In addition, due to weather conditions, including wind, humidity, and exposure to ultraviolet radiation, bioaerosols cannot survive for a long time. However, there are still concerns about exposure to bioaerosols in air polluted area.

The results also showed that the trend of increasing bacterial population in PMs from small particles to large particles, which was due to the increase in mass and concentration of particles with the same trend. Since all PMs can contain small and large particles, the presence of bacteria and other bio-aerosols having various sizes from 0.1 to 100 μm is very likely.

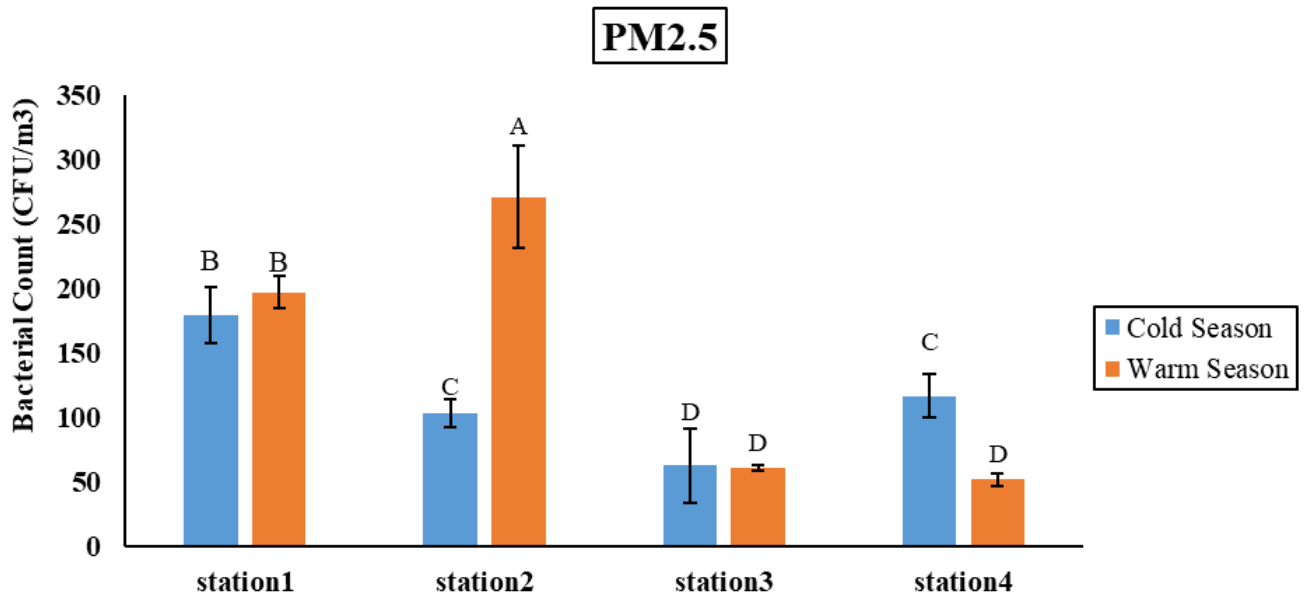


Fig. 2. Bacterial population in PM_{2.5} collected from ambient air from 4 stations in winter and summer

Similar letters show insignificant difference in Duncan test ($P < 0.05$). Columns represent mean and bars are standard deviation. S1: Isfahan University of Technology station; S2: Jey station, S3: Soffeh station; S4: Imam Hussein square station.

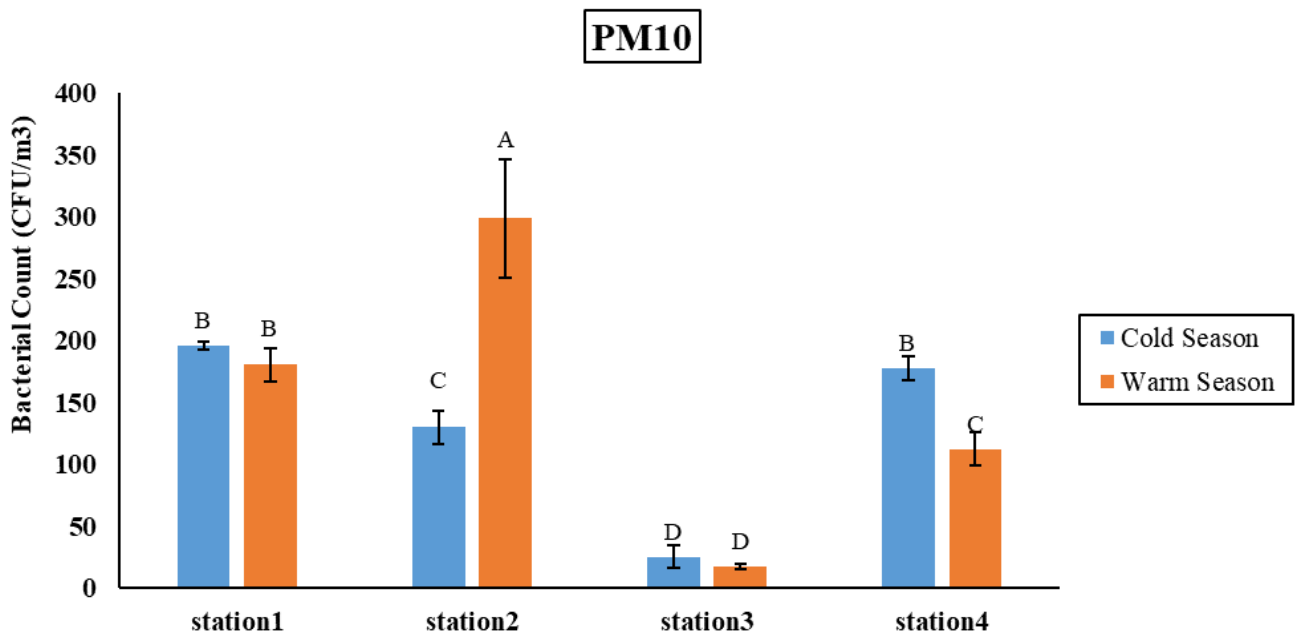


Fig. 3. Bacterial population in PM₁₀ collected from ambient air from 4 stations in winter and summer

Similar letters show insignificant difference in Duncan test ($P < 0.05$). Columns represent mean and bars are standard deviation. S1: Isfahan University of Technology station; S2: Jey station, S3: Soffeh station; S4: Imam Hussein Square station

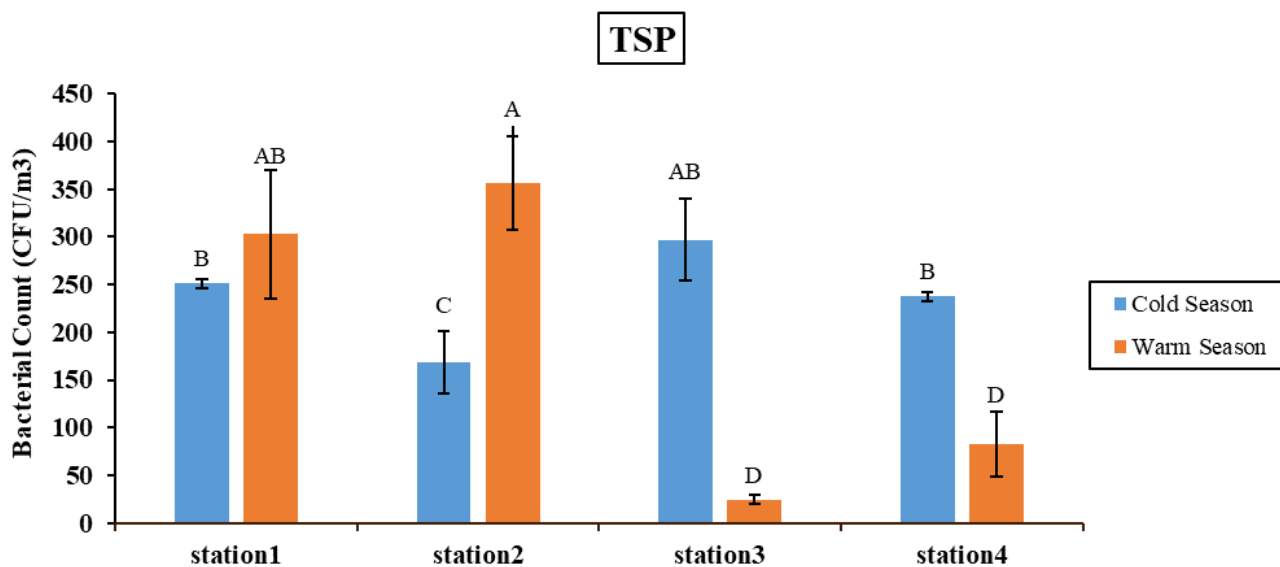


Fig. 4. Bacterial population in TSP collected from ambient air from 4 stations in winter and summer

Similar letters show insignificant difference in Duncan test ($P < 0.05$). Columns represent mean and bars are standard deviation. S1: Isfahan University of Technology station; S2: Jey station, S3: Soffeh station; S4: Imam Hussein Square station

The highest bacterial concentration was obtained in TSP samples of warm season which could have the enhancing factors including high temperature and PM mass concentration. The dust storms are one of the main sources of PMs in the region during the warm seasons which may significantly affect PM concentration and consequently increases bacterial population associated with the particles. Higher bacterial concentrations in stations 1 and 2 than those in the other stations could be originated from the effect of natural dusts as one of the main sources of PMs in those points.

Some morphological characteristics of bacteria

The percentage of bacteria associated with PM_{2.5}, PM₁₀ and TSP divided by gram-positive (G⁺) and gram-negative (G⁻) ones in different seasons is presented in figs. 5, 6 and 7, respectively. In general, in warm and cold

seasons, G⁺ bacteria showed higher populations than G⁻ bacteria in all stations (except station 3 in warm season). This is highlighted in PM₁₀ and TSP in summer (Figs. 5-7) which included particles larger than PM_{2.5} originated mainly from natural dusts in the region, while in winter the trend was inverse considering that the main potential sources of PMs could be anthropogenic sources such as fossil fuel combustion, transportation and industrial activities. Although this finding is in line with previous studies demonstrating dominance of G⁺ bacteria among the PMs associated bacteria [18, 27], however it should be noted that it not only could be due to the method of bacterial culture (i.e. culture-based) which primarily detect gram-positive bacteria [28] but also might be influenced by growth medium, temperature and inoculation time which change microbial community [29]. It is stated that the pathogenesis risk of G⁻ bacteria is higher than that of G⁺ ones, however there are pathogenic species in both groups [30].

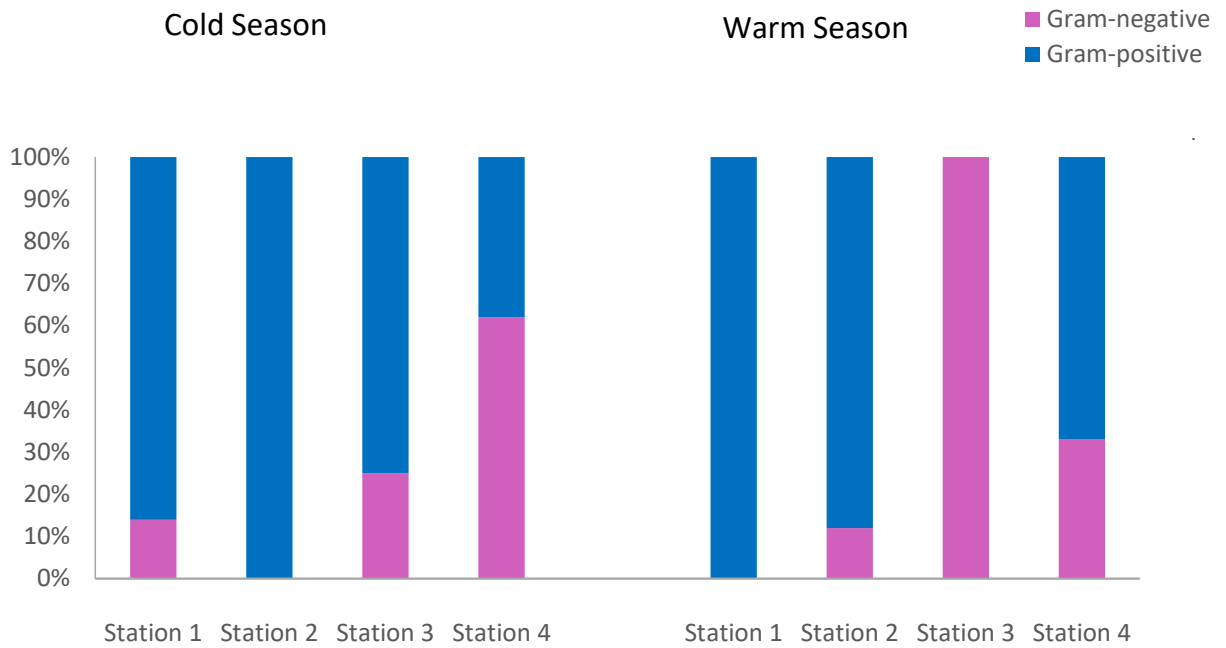


Fig. 5. The percentage of Gram-negative and Gram-positive bacteria in $PM_{2.5}$ collected from ambient air from 4 stations in winter and summer S1: Isfahan University of Technology station; S2: Jey station, S3: Soffeh station; S4: Imam Hussein Square station

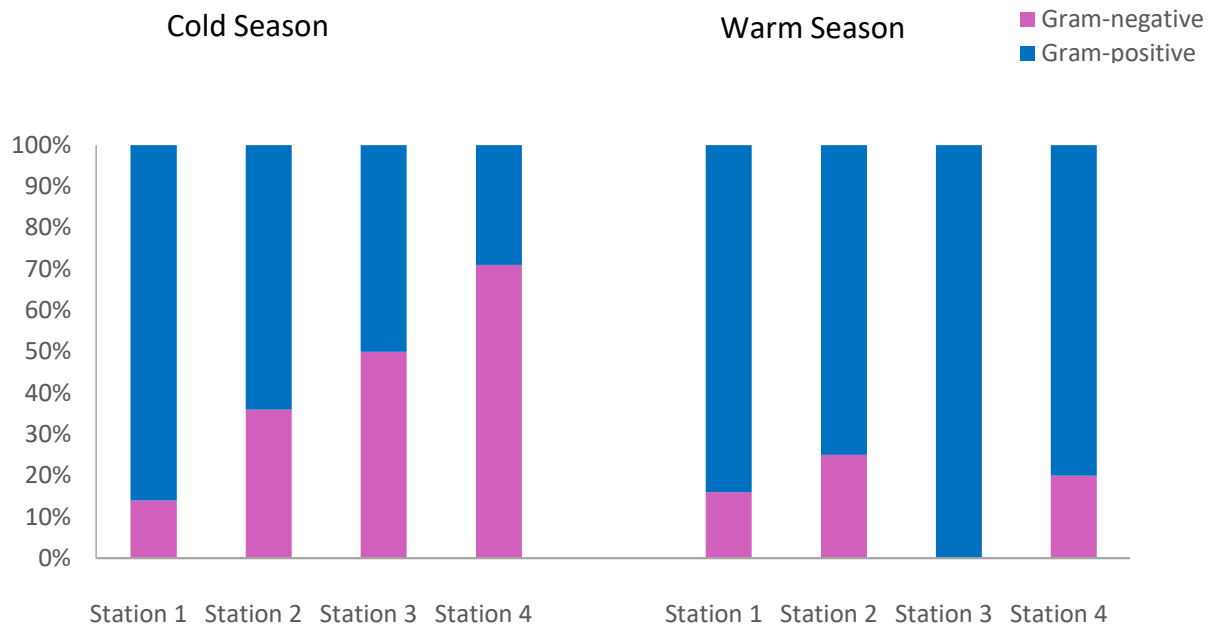


Fig. 6. The percentage of Gram-negative and Gram-positive bacteria in PM_{10} collected from ambient air from 4 stations in winter and summer S1: Isfahan University of Technology station; S2: Jey station, S3: Soffeh station; S4: Imam Hussein Square station

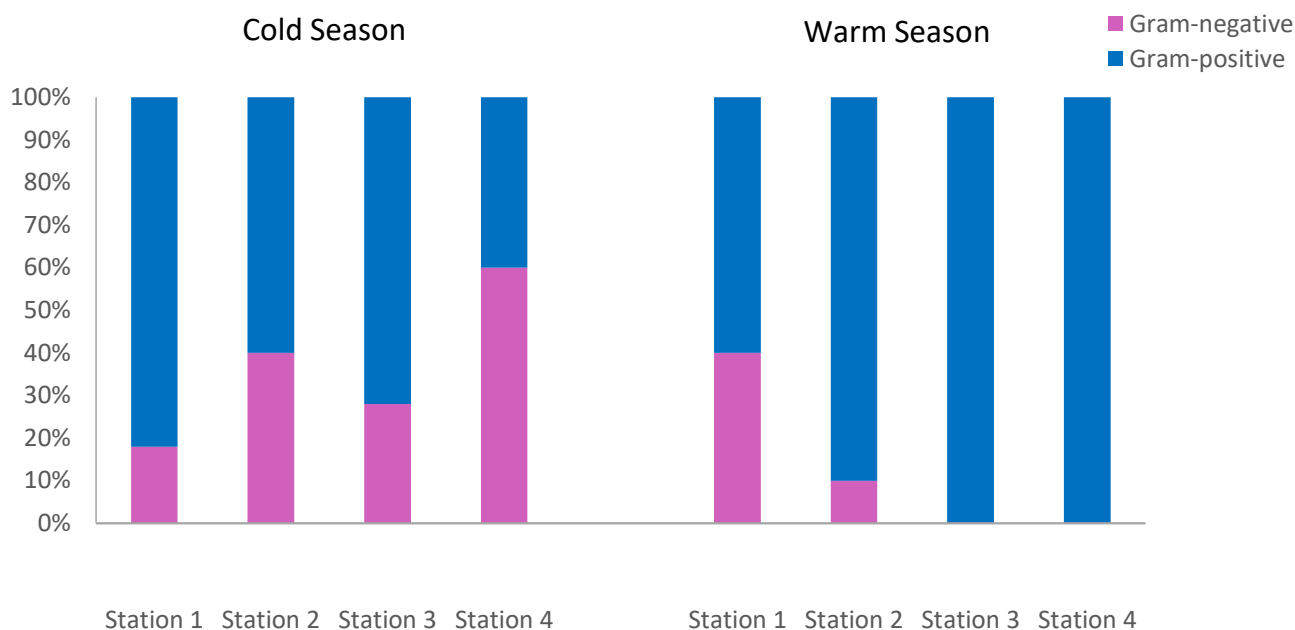


Fig. 7. The percentage of Gram-negative and Gram-positive bacteria in total suspended particles (TSP) collected from ambient air from 4 stations in winter and summer S1: Isfahan University of Technology station; S2: Jey station, S3: Soffeh station; S4: Imam Hussein Square station

According to the results obtained in the cold season, 32, 58, 66 and 44 percent of the bacteria had bacilli (rod) morphology in stations 1, 2, 3 and 4, respectively, while 68, 42, 34 and 56 percent of bacteria were cocci (spherical). The bacilli-shaped bacteria were 68, 32, 85 and 63 percent of total in station 1, 2, 3 and 4, respectively, and the correspondent amounts for cocci-shaped bacteria were 32, 68, 15 and 27 percent. However, it should be noticed that morphology of bacteria may change because of environmental conditions [31].

Since the bacilli-shaped bacteria have the potential of pathogenicity, the results may show the risk potential of the predominant bacteria associated with PMs particularly in summer throughout the study area. However, it needs identification of all bacterial species in PMs and it's not premature to conclude based on morphological characteristics in this regard. It should be noticed that during the warm season

we usually have several dust storm events throughout the country and the south, southwest and central regions are more affected. So more bacterial concentration is expected to see in PMs which mostly are G⁺ and spore-forming bacteria such as *Bacillus* and *Microbacterium* [18]. Previous researches showed that *Bacillus*, *Staphylococcus*, *Streptomyces*, *Kocuria* and *Corynebacterium* were the most dominant species in Ahvaz, Sanandaj and Ilam which are usually affected by dust storms and could be also occurred in Isfahan and other cities of Iran [16-18].

Conclusion

Given the importance of PMs, especially PM_{2.5}, in discussing air quality and human health, it is important to pay attention to the chemical and biological compounds associated with them to

assess their risk. This has double importance in developing countries such as Iran and industrial metropolises such as Isfahan where are severely facing PMs pollution in ambient and indoor air originated mainly from industrial activities, transportation and fossil fuel combustion as well as dust storms. The results of this study showed that depending on the time, place and the size of the PMs, the bacterial population of the air will also be different. The type and source of particles can affect the bacterial population associated with them. The area where had the higher PM concentrations was mostly in warm season because of natural dusts showed the higher bacterial concentrations than the area with lower PM concentrations. Furthermore, the bacterial concentrations in TSP was higher than PM_{10} and $PM_{2.5}$ which revealed the effect of PM size and mass on their associated microbial populations. Although gram-positive bacteria had a higher population than gram-negative bacteria in particles, both categories might be considered in terms of their negative health effects. This requires the identification of different bacterial species binding with particles, especially in an area that has been affected by a particular disease and the type of cause of that disease is clear. Furthermore, the interaction of PMs and other bioaerosols such as viruses particularly Covid-19 which has significantly affected the health of people throughout the study area as well as the world should be investigated. However, the presence of bacteria along with PMs suggests that controlling of these pollutants to reduce their negative effects on human health should be given serious attention by policymakers.

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Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Ethical considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

References

1. Kalisa E, Archer S, Nagato E, Bizuru E, Lee K, Tang N, et al. Chemical and biological components of urban Aerosols in Africa: current status and knowledge gaps. *International Journal of Environmental Research and Public Health*. 2019; 16(6):941. <https://www.mdpi.com/1660-4601/16/6/941>.
2. Schikowski T, Altug H. The role of air pollution in cognitive impairment and decline. *Neurochemistry International*. 2020; 104708. <https://www.sciencedirect.com/science/article/>

pii/S0197018620300991?via%3Dihub.

3. World Health Organization. Household air pollution and health. 2021. Accessed [22 September 2021] Available from: <https://www.who.int/news-room/fact-sheets/detail/household-air-pollution-and-health>.

4. KC R, Shukla SD, Gautam SS, Hansbro PM The role of environmental exposure to non-cigarette smoke in lung disease. *Clinical and Translational Medicine*. 2018; 7 (39): 1-12. <https://onlinelibrary.wiley.com/doi/full/10.1186/s40169-018-0217-2>.

5. Shamsollahi H R, Yunesian M, Kharrazi S, Jahanbin B, Nazmara S, Rafieian S, et al. Characterization of persistent materials of deposited PM_{2.5} in the human lung. *Chemosphere* 2022; 301: 134774. <https://www.sciencedirect.com/science/article/pii/S004565352201267X>

6. Jaafari J, Naddafi K, Yunesian M, Nabizadeh R, Hassanvand MS, Shamsipour M, et al. The acute effects of short term exposure to particulate matter from natural and anthropogenic sources on inflammation and coagulation markers in healthy young adults. *Science of The Total Environment* 735. 2020; 15(735):139417. <https://pubmed.ncbi.nlm.nih.gov/32498012/>

7. Soleimani M, Ebrahimi Z, Mirghaffari N, Moradi H, Amini N, Poulsen K G, et al. Seasonal trend and source identification of polycyclic aromatic hydrocarbons associated with fine particulate matters (PM_{2.5}) in Isfahan city using diagnostic ration and PMF model. *Environmental Science and Pollution Research*. 2022, 29(18): 26449–26464. <https://link.springer.com/article/10.1007/s11356-021-17635-8>.

8. Soleimani M, Amini N, Sadeghian B, Wang D, Fang L. Heavy metals and their source

identification in particulate matter (PM_{2.5}) in Isfahan City, Iran. *Journal of Environmental Sciences*. 2018; 72: 166-175. <https://www.sciencedirect.com/science/article/abs/pii/S1001074217325421>

9. Marjovvi, A.R. Soleimani, M., Mirghaffari, N. Karimzadeh, H.R., Yuan, Y., Fang, L. Monitoring, source identification and environmental risk of potentially toxic elements of dust in Isfahan Province, central Iran. *Bulletin of Environmental Contamination and Toxicology*. 2022, 108(5): 901–908. <https://link.springer.com/article/10.1007/s00128-021-03446-7>.

10. Douwes J, Eduard W, Thorne P S. *Bioaerosols*, Editor(s): Harald Kristian (Kris) Heggenhougen, International Encyclopedia of Public Health, Academic Press, 2008, Pages 287-297. <https://www.sciencedirect.com/science/article/pii/B9780123739605002811>.

11. Du P, Du R, Ren W, Lu Z, Zhang Y, Fu P. Variations of bacteria and fungi in PM_{2.5} in Beijing, China. *Atmospheric Environment*. 2018; 172: 55-64. <https://www.sciencedirect.com/science/article/abs/pii/S1352231017307136?via%3Dihub>

12. Baertsch C, Paez-Rubio T, Viau E, Peccia J. Source tracking aerosols released from land-applied class B biosolids during high-wind events. *Applied and Environmental Microbiology*. 2007; 73(14): 4522-4531. <https://pubmed.ncbi.nlm.nih.gov/17513591/>

13. Jahne M A, Rogers S W, Holsen T M, Grimberg S J, Ramler IP. Emission and dispersion of bioaerosols from dairy manure application sites: human health risk assessment. *Environmental Science & Technology*. 2015; 49(16): 9842-9849. https://www.researchgate.net/publication/279967317_Emission_and_

Dispersion_of_Bioaerosols_from_Dairy_Manure_Application_Sites_Human_Health_Risk_Assessment

14. Jones AM, Harrison RM. The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. *Science of the Total Environment*. 2004; 326(1-3): 151-180. <https://www.sciencedirect.com/science/article/abs/pii/S0048969703006752>
15. Gao M, Yan X, Qiu T, Han M, Wang X. Variation of correlations between factors and culturable airborne bacteria and fungi. *Atmospheric Environment*. 2016; 128: 10-19. <https://www.sciencedirect.com/science/article/abs/pii/S1352231015305768>
16. Goudarzi Gh, Shirmardi M, Khodarahmi F, Hashemi-Shahraki A, Alavi N, Ahmadi Ankali et al. Particulate matter and bacteria characteristics of the Middle East Dust (MED) storms over Ahvaz, Iran. *Aerobiologia*. 2014; 30: 345-356. <https://link.springer.com/article/10.1007/s10453-014-9333-7>
17. Nourmoradi H, Moradnejadi K, Mohammadi Moghadam F, Khosravi B, Hemati L, Khoshniyat R, Kazembeigi F. The effect of dust storm on the microbial quality of ambient air in Sanandaj: A city located in the west of Iran. *Global Journal of Health Science*. 2015; 7(5): 114-119. <https://ccsenet.org/journal/index.php/gjhs/article/view/46888>
18. Amarloei A, Fazlzadeh M, Jonidi Jafarie A, Zareif A, Mazloomi S. Particulate matters and bioaerosols during Middle East dust storms events in Ilam, Iran. *Microchemical Journal*. 2020; 152: 104280-104286. <https://www.sciencedirect.com/science/article/abs/pii/S0026265X19314602>.
19. Alghamdi M A, Shamy M, Redal M A, Khoder M, Awad A H, Elserougy S. Microorganisms associated particulate matter: a preliminary study. *Science of the Total Environment*. 2014; 479: 109-116. <https://www.sciencedirect.com/science/article/abs/pii/S0048969714001685>.
20. Sippula O, Rintala H, Happonen M, Jalava P, Kuusipalo K, Virén A, et al. Characterization of chemical and microbial species from size-segregated indoor and outdoor particulate samples. *Aerosol and Air Quality Research*. 2013; 13(4): 1212-1230. <https://www.researchgate.net/publication/266910205>
21. Cao C, Jiang W, Wang B, Fang J, Lang J, Tian G, et al. Inhalable microorganisms in Beijing's PM_{2.5} and PM₁₀ pollutants during a severe smog event. *Environmental Science & Technology*. 2014; 48(3): 1499-1507. <https://pubs.acs.org/doi/abs/10.1021/es4048472>
22. National Institute for Occupational Safety and Health (NIOSH). Bioaerosol sampling (Indoor Air), Method 0800. NIOSH Manual of Analytical Methods, Fourth Edition. 1998.
23. Colco R. Gram Staining. *Current Protocols in Microbiology*. 00 (1): Appendix 3C. 2005.
24. Pascal M, Falq G, Wagner V, Chatignoux E, Corso M, Blanchard M, Host S, Pascal L, Larrieu S. Short-term impacts of particulate matter (PM₁₀, PM_{10-2.5}, PM_{2.5}) on mortality in nine French cities. *Atmospheric Environment*. 2014; 95: 175-184. <https://www.sciencedirect.com/science/article/abs/pii/S1352231014004762>
25. Li Y, Lu R, Li W, Xie Z, Song Y. Concentrations and size distributions of viable bioaerosols under various weather conditions in a typical semi-arid city of Northwest China. *Journal of Aerosol Science*. 2017; 106: 83-92. <https://www.sciencedirect.com/science/article/abs/pii/S0021850216302907>
26. Brodie EL, Desantis TZ, Parker JPM,

Zubietta IX, Piceno YM, Andersen GL. Urban aerosols harbor diverse and dynamic bacterial populations. *Proceedings of the National Academy of Sciences*. 2007; 104(1): 299-304. https://www.researchgate.net/publication/6617176_Urban_Aerosols_Harbor_Diverse_and_Dynamic_Bacterial_Populations

27. Bowers RM, Sullivan AP, Costello EK, Collett JL, Knight R, Fierer N. Sources of bacteria in outdoor air across cities in the midwestern United States. *Applied and Environmental Microbiology*. 2011; 77(18): 6350-6356. <https://journals.asm.org/doi/10.1128/AEM.05498-11>

28. Fahlgren C, Bratbak G, Sandaa R-A, Thyrrhaug R, Zweifel UL. Diversity of airborne bacteria in samples collected using different devices for aerosol collection. *Aerobiologia*, 2011; 27(2): 107-120. <https://link.springer.com/article/10.1007/s10453-010-9181-z>

29. Fang Z, Ouyang Z, Zheng H, Wang X, Hu L. Culturable airborne bacteria in outdoor environments in Beijing, China. *Microbial Ecology*. 2007; 54(3): 487-496. <https://www.jstor.org/stable/25256220>

30. Ramachandran G. Gram-positive and gram-negative bacterial toxins in sepsis: A brief review. *Virulence*. 2014; 5(1): 213–218. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3916377/>

31. Van Teeseling MC, de Pedro MA, Cava F. Determinants of bacterial morphology: from fundamentals to possibilities for antimicrobial targeting. *Frontiers in microbiology*. 2017; 8:1-18.

<https://pubmed.ncbi.nlm.nih.gov/28740487>