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Microorganisms associated particulate matter: A preliminary study

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HIGHLIGHTS

- We determined the microbiological quality of particulate matter in an urban area.
- We found fungi and actinobacteria in low counts.
- 1/$PM_{2.5}$ concentration was the main determinant of microbial concentrations.
- Negative correlation was found between O_3 and $PM_{2.5}$.
- Temperature had negative effect on microorganisms associated $PM_{2.5}$.

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ABSTRACT

This study aims to determine the microbiological quality of particulate matter (PM) in an urban area in Jeddah, Saudi Arabia, during December 2012 to April 2013. This was achieved by the determination of airborne bacteria, fungi, and actinobacteria associated PM_{10} and $PM_{2.5}$, as well as their relationships with gaseous pollutants, O_3, SO_2 and NO_2, and meteorological factors (T°C, RH% and Ws). High volume samplers with PM_{10} and $PM_{2.5}$ selective sizes, and glass fiber filters were used to collect PM_{10} and $PM_{2.5}$, respectively. The filters were suspended in buffer phosphate and aliquots were spread plated onto the surfaces of trypticase soy agar, malt extract agar, and starch casein agar media for counting of bacteria, fungi and actinobacteria-associated PM, respectively. PM_{10} and $PM_{2.5}$ concentrations averaged 159.9 μg/m^3 and 60 μg/m^3, respectively, with the ratio of $PM_{2.5}/PM_{10}$ averaged ~0.4. The concentrations of O_3, SO_2 and NO_2 averaged 35.73 μg/m^3, 38.1 μg/m^3 and 52.5 μg/m^3, respectively. Fungi and actinobacteria associated PM were found in lower concentrations than bacteria. The sum of microbial loads was higher in PM_{10} than $PM_{2.5}$, however a significant correlation ($r = 0.57$, $P \leq 0.05$) was found between the sum of microbial loads associated PM_{10} and $PM_{2.5}$. *Aspergillus fumigatus* and *Aspergillus niger* were the common fungal types associated PM. Temperature significantly correlated with both PM_{10} ($r = 0.44$), and $PM_{2.5}$ ($r = 0.5$). Significant negative correlations were found between O_3 and $PM_{2.5}$ ($r = -0.47$), and between SO_2 with PM_{10} ($r = -0.48$). Wind speed positively correlated with airborne microorganisms associated PM. The regression model showed that the inverse $PM_{2.5}$ concentration (1/$PM_{2.5}$) was a significant determinant of fungal count associated PM. Chemical processes and environmental factors could affect properties of PM and in turn its biological quality.

1. Introduction

Particles with both biological and non-biological origins are transported together with air currents in the atmosphere. Particles originate from various natural and anthropogenic sources, and affect visibility, climate, air quality, and human health (Fuzzi et al., 2006). Particle concentrations are influenced by meteorological conditions, long-range transport of pollutants, and new particle formation in the air (Sippula et al., 2013). Particles are removed from the air either by sedimentation or precipitation (Despres et al., 2012).

Biological particles/bioaerosols are particles of biological origin suspended in the air such as: bacteria, fungi, viruses, microbial toxins, proteins and enzymes (ACGIH, 1999). Such particles may be suspended in the air either as individual organisms or attached to dust particles or tiny droplets of water (Lighthart, 1997). Bioaerosols tend to attach in

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coarser PM fraction, however fungal spores, fragmented pollen, and non-agglomerated bacteria are found in the fine fraction as well (Meklin et al., 2002), due to the mechanism of reaction between biological agents and PM (Oikonen et al., 2003).

Biological particles have received less attention in the atmosphere than other aerosol particles such as: sulfates, mineral dust and ash (Friedlander, 2000), because its average concentrations have been assumed to be insignificant compared to non-biological particles (Penner et al., 2001; Kuhn and Ghannoum, 2003). Fungi accounted for up to ~10% of organic carbon, and ~5% of PM_{10} at urban and suburban locations (Bauer et al., 2008). In pristine tropical rainforest airborne fungal spores accounted for up to ~45% of a coarse PM (Despres et al., 2012). Biological materials above land constituted ~25% of the total particulate matter (Jones and Harrison, 2004).

Bioaerosols undergo daily and seasonal changes depending on environmental factors, and human activities (Rossi et al., 2005). The survival of airborne microorganisms may be affected by hydrocarbons, NO_2 and SO_2 (Ho et al., 2005), and trace elements (Jackson et al., 1978). PM bound with airborne pollen and fungal spores (Glikson et al., 1995) could alter their biological and morphological characteristics. Physical, chemical and biological compositions of suspended dust may be changed depending on dust source, whether it originated from desert or dried wetland (Soleimani et al., 2013). Smoke contains deleterious compounds that may either kill microorganisms or modify their antigenic properties (Abdel Hameed, 2003). PM may change microbial dispersal pattern, and alter their aerodynamic diameters (Monn, 2001).

T°C, RH% and wind speed affect concentrations and viability of airborne microorganisms (Jones and Harrison, 2004). Climate change could alter the timing and abundance of aeroallergens and the growth and distribution of organisms that produce them (Burge and Rogers, 2000).

Less information is available on microbial community associated PM in arid regions. However few studies have been directed to investigate the factors affecting microorganisms associated non-biological particles and their health effects. A number of studies provide interesting information pertinent to evaluate bioaerosols in contributing to health effects associated with exposures to ambient PM (Stevanovic and Nikic, 2006). Health responses may be enhanced when chemical and biological constituents of particulate matter are combined together (USEPA, 2004).

The purposes of the present study were to 1) gain information on the microbial community associated PM_{10} and $PM_{2.5}$, with particular focus on fungi, and 2) determine relationships between microbial community associated PM with air pollutants (PM, O_3, NO_2, and SO_2), and meteorological parameters in an urban–arid region.

2. Materials and methods

2.1. The sampling site

Jeddah, 21.4869°N; 39.39.2517°E, is a costal city located in the western region of the Kingdom of Saudi Arabia on the Red Sea (Fig. 1). Jeddah's climate is warm and moderate in winter, and high temperature and humidity in summer (Khodier et al., 2012), with spare or no rainfall. Traffic, power stations, oil refinery and desalination plants are the main sources of air pollution.

The sampling site was located at the King Abdulaziz University campus (a sensitive place). It is an urban area characterized by high traffic density and barren with no vegetation or farmland. The air samplers were positioned at a height of ~8 m above the ground on a rooftop of the Faculty of Meteorology, Environment and Arid Land Agriculture Building, during the period between December 2012 and April 2013.

2.2. Particulate matter sample collection

$PM_{2.5}$ and PM_{10} samplers (Staplex Air Sampler Division, USA) operated at flow rate of 1.13 m^3/min were used to collect $PM_{2.5}$ and PM_{10}. The daily (10 AM–10 AM) $PM_{2.5}$ and PM_{10} samples were collected on

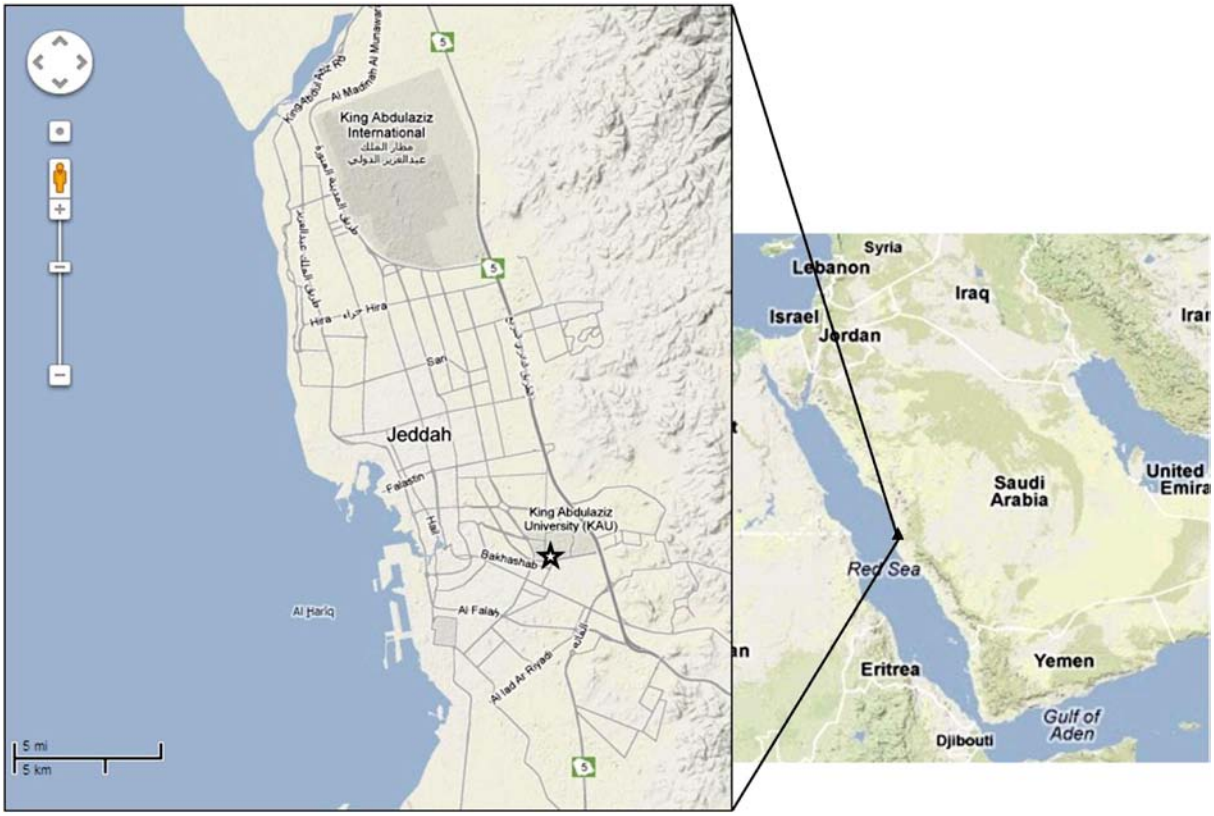

Fig. 1. Map of Jeddah with the sampling site marked with a star. Map data ©Google, 2013 Terra Metrics.

pre-weighed sterilized glass fiber filters, because glass fiber is robust and inert. The samplers were sterilized with isopropyl alcohol before each sampling set. PM_{10} and $PM_{2.5}$ samplers were operated 2–4 times per month (once per week). The mass concentrations of $PM_{2.5}$ and PM_{10} were calculated and expressed as microgram per cubic meter of air (μg/m^3).

2.3. Measurement of gaseous pollutants

Gaseous pollutants were continuously monitored using a UV-absorption ozone analyzer (model 400E, Teledyne Technologies Company, San Diego) for ozone; a chemiluminescence $NO/NO_2/NO_x$ analyzer (model 200E, Teledyne Technologies Company, San Diego) for NO_2; and a UV fluorescence analyzer (model M100E, Teledyne Technologies Company, San Diego) for SO_2. The detection limits of gas analyzers are in the range of 0–10 ppm for O_3, and 0–20 ppm for NO_2 and SO_2. The gas analyzers provide update-readings every 1 min. These readings were calculated over an hour and 24 h average. PM samplers were operated in conjunction with gas analyzers. Quality control procedures were performed every week, including inspection of the instruments and zero/span checks.

2.4. Meteorological parameters

Temperature, relative humidity and wind speed were continuously measured using Lufft WS600-UMB Compact weather station. Hourly readings were averaged over a 24 h period (10 AM–10 AM). During this study, temperature ranged within 24–33 °C with a mean value of 27.14 °C. Relative humidity ranged within 46–67% with a mean value of 54.47%. Wind speed ranged between 1.38 and 6.21 m/s with a mean value of 2.94 m/s (Table 1). The prevailing wind directions were from west to north-west.

2.5. Microorganisms associated PM

Half of the glass fiber filters were suspended in 50 ml buffer phosphate solution containing 0.05% w/v Tween 80 (Sigma-Aldrich, USA) and shaken for 30–60 min. Serial dilutions up to 10^{-3} were prepared. Aliquots, 0.5 ml, of the original sample and its serial dilutions were spread-plated, in triplicate, onto the surface of trypticase soy agar supplemented with 50 ppm cycloheximide, malt extract agar supplemented with 50 ppm chloramphenicol, and starch casein agar media (BD, Sparks, USA), for counting of bacteria, fungi and actinobacteria, respectively.

Table 1
Concentrations of PM, gaseous pollutants (μg/m^3), microorganisms associated PM (CFU/m^3), temperature (T°C), relative humidity (RH%), and wind speed (m/s) during the measurement period.

Variable	Parameter				
	Min	Max	Mean	SD	Median
PM_{10} (μg/m^3)	61.23	216.3	159.94	56.67	147.25
$PM_{2.5}$ (μg/m^3)	13.61	211.4	60.03	42.36	50.0
Bacteria associated PM_{10} (CFU/m^3)	100	591	248.3	155.3	220
Fungi associated PM_{10} (CFU/m^3)	11.0	28.0	18.30	5.37	17.0
Actinobacteria associated PM_{10} (CFU/m^3)	2.0	16.0	5.30	3.90	4.0
Bacteria associated $PM_{2.5}$ (CFU/m^3)	45.0	590	170	139.8	117
Fungi associated $PM_{2.5}$ (CFU/m^3)	4.0	15.0	9.21	2.01	10.0
Actinobacteria associated $PM_{2.5}$ (CFU/m^3)	1.0	5.0	2.76	1.13	3.0
O_3 (μg/m^3)	12.0	63.34	35.73	16.76	35.3
SO_2 (μg/m^3)	9.0	178.2	38.11	48.0	20.0
NO_2 (μg/m^3)	31.73	94.34	52.52	14.96	53.0
T°C	24.0	33.0	27.14	2.83	27.0
RH%	46.0	67.0	54.47	6.52	54.0
Wind speed (m/s)	1.38	6.21	2.94	1.02	2.77

Fungal and actinobacteria Petri plates were incubated at 28 °C for 5–7 and 7–15 days, respectively. Bacterial plates were incubated at 28 °C for 48 h. The growing colonies were counted and the mean count was calculated, and concentration expressed as colony forming units per cubic meter of air (CFU/m^3).

Fungal isolates were purified and identified by direct observation on the basis of micro- and macro-morphological features, reverse and surface coloration of colonies on different media (Raper and Fennell, 1973; Pitt, 1979; Barnett and Hunter, 1999; Klich, 2002).

2.6. Aerodynamic diameter (d_{ae}) of fungal spores

Physical diameter of fungal spores was measured by light microscopy (x = 400) using ocular "May Graticule". It consists of a series of lines and circles of graduated size set on a glass disc. The aerodynamic diameter (d_{ae}) was calculated from the density (1 g/m^3), shape (hypothetical sphere) and physical diameter (Hinds, 1999).

2.7. Statistical analysis

Nonparametric Spearman's rank correlation test was used to determine the relationships between concentrations of airborne microorganisms-associated PM with air pollutant concentrations and meteorological parameters. Nonparametric parameter method was used because the data were not normally distributed. Multiple regression analysis was performed to explain the change of the dependent variables (microorganisms) in relation to independent variables (air pollutants and meteorological parameters). Statistical analysis was performed using SPSS 18 (PASW Statistics 18). $P \leq 0.05$ was considered as significant.

3. Results and discussion

3.1. PM

The 24 h of PM_{10} and $PM_{2.5}$ concentrations ranged between 61.3 and 216.3 μg/m^3 and between 13.6 and 211 μg/m^3, respectively (Table 1). The ratio of $PM_{2.5}/PM_{10}$ was ~0.4. PM_{10} concentrations highly fluctuated due to the contributions of the natural sources (windblown dust). PM_{10} and $PM_{2.5}$ mass concentrations were significantly correlated ($r = 0.92$, $P \leq 0.05$). The highest PM concentrations were found in 15 March, and the lowest in 14 December (Fig. 2).

The mean concentration of PM_{10} (159.9 μg/m^3) and $PM_{2.5}$ (60 μg/m^3) exceeded the European Union air quality limit values of 50 μg/m^3 for PM_{10}, and 25 μg/m^3 for $PM_{2.5}$ (WHO, 2006). PM_{10} concentration exceeded the US air quality standard of 150 μg/m^3 and $PM_{2.5}$ did not exceed the US-standard of 65 μg/m^3 (USEPA, 2004). In spite of the mean concentration of PM_{10} was below the Saudi Arabia limit value of 340 μg/m^3 (PME, 2013) but it had a significant contribution to Jeddah's air quality.

3.2. Gaseous pollutants

O_3, SO_2 and NO_2 concentrations averaged 35.73 μg/m^3, 38.11 μg/m^3 and 52.52 μg/m^3, respectively (Table 1). The daily mean concentrations of O_3, NO_2 and SO_2 are illustrated in Fig. 3. The highest O_3 concentration was found during spring (18 March), because tropospheric O_3 is produced by the reaction of solar radiation on NO_x. The lowest NO_2 concentration was found in 28 December and the highest in 26 February. NO_x emitted in cities reduces local O_3 concentrations because NO reacts with O_3 to form NO_2. This means that O_3 precursors generated in countries with large traffic and industrial emissions may affect less polluted countries (Geyh et al., 2000).

SO_2 concentrations highly varied, i.e.: standard deviation exceeded the mean value (Table 1). Higher concentrations of SO_2 and NO_2 in the winter months are attributed to the increase in amount of consuming

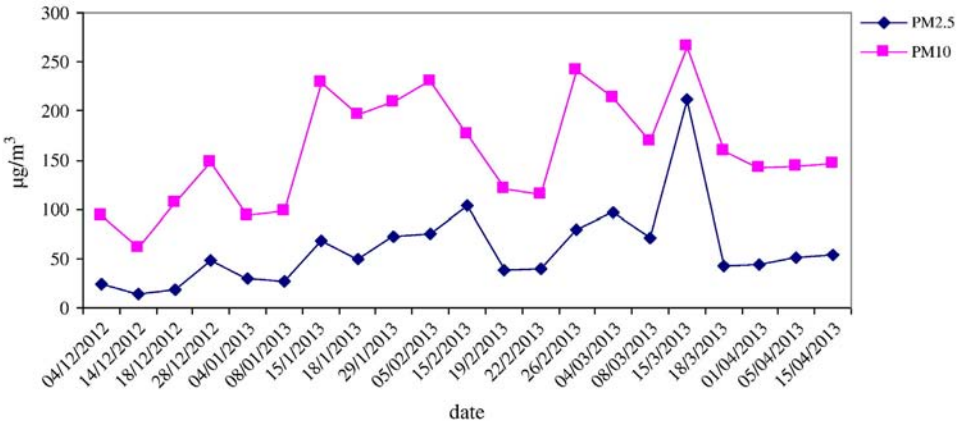

Fig. 2. Daily mass concentrations of PM_{10} and $PM_{2.5}$ during the period of study.

fuels, stability of weather conditions, and formation of low inversion layer (Afif et al., 2008). The daily SO_2 mean concentration exceeds the allowable limit value of 20 µg/m^3 that was given by WHO (2006), but did exceed the Saudi Arabia's limit value of 360 µg/m^3 (PME, 2013). The daily O_3 and NO_2 concentrations, respectively, were below the US ambient air quality standard of 100 µg/m^3 (USEPA, 2004), and Kuwait limit value of 100 µg/m^3 (Abdel Hameed, 2002). In the present study, the gaseous pollutant mean concentrations were found to be similar/or below those found in other countries. NO_2 concentrations were 73 µg/m^3 in Athens (Chaloulakou et al., 2008), and 22.27 µg/m^3 in Turkey (Özden et al., 2008). SO_2 concentrations were 50 µg/m^3 in China (Chan and Yao, 2008), and 49 µg/m^3 in Egypt (Abdel Hameed et al., 2012).

3.3. Microorganisms associated PM

Table 1 shows the mean concentrations of airborne culturable bacteria, fungi and actinobacteria associated PM_{10} and $PM_{2.5}$. The daily variations of microorganisms associated PM_{10} and $PM_{2.5}$ are illustrated in Figs. 4 and 5, respectively. Microorganisms associated PM concentrations were low, because outdoor microbial sources such as: soil, plant litter, phylloplane, composts, wastewater treatment plants, and animal feces are rare (Bowers et al., 2011). The highest bacterial and fungal concentrations were found in February and January, respectively. The concentrations of bacteria associated PM (45–591 CFU/m^3) were higher than fungi (4–28 CFU/m^3) and actinobacteria (1–16 CFU/m^3). Non-significant positive ($r = 0.23$) and negative ($r = -0.2$) correlations were found between bacterial and fungal concentrations associated PM_{10} and $PM_{2.5}$, respectively.

The sum of microorganisms loading PM_{10} was higher than microorganisms loading $PM_{2.5}$ (Fig. 6). However a significant correlation ($r = 0.57$, $P \leq 0.05$) was found between airborne microorganisms associated PM fractions, because particles may partially have the same sources. The coarse particles often contain mineral species from soil, and particles of biological origin (Layton and Beamer, 2009). However, fine particles contain soot, metals, secondary inorganic components and a variety of organic compounds of both natural and anthropogenic origins (Sillanpää et al., 2005).

Bacteria associated PM concentrations were highly variable. This may be attributed to the influence of anthropogenic activities and atmospheric changes (Kellogg et al., 2004), and a large portion of bacteria tend to be associated with dust particles (Lighthart, 1997). Airborne terrestrial and marine bacteria were mainly distributed in coarse particles >7 µm (Li et al., 2011). The results in the present study correspond with those detected by Chihara and Someya (1989) and Mouli et al. (2005) who found airborne bacteria in the range of 1–32 CFU/m^3 and 10–100 CFU/m^3, respectively at semi-arid urban region.

The low concentrations of fungi and actinobacteria seem to be a characteristic of the geographical area, i.e. the absence of biotic sources, and arid and barren environments. Actinobacteria and fungi are ubiquitous in soil and dust, and are known to be important air bio-pollutant in occupational environments (Nielsen et al., 1997). In hot weather conditions a significant decrease in airborne fungi was reported (Fröhlich-Nowoisky et al., 2011). The mass contributions of *Aspergillus*/*Penicillium* and *Cladosporium* were estimated at 0.17 ± 0.13% and 0.95

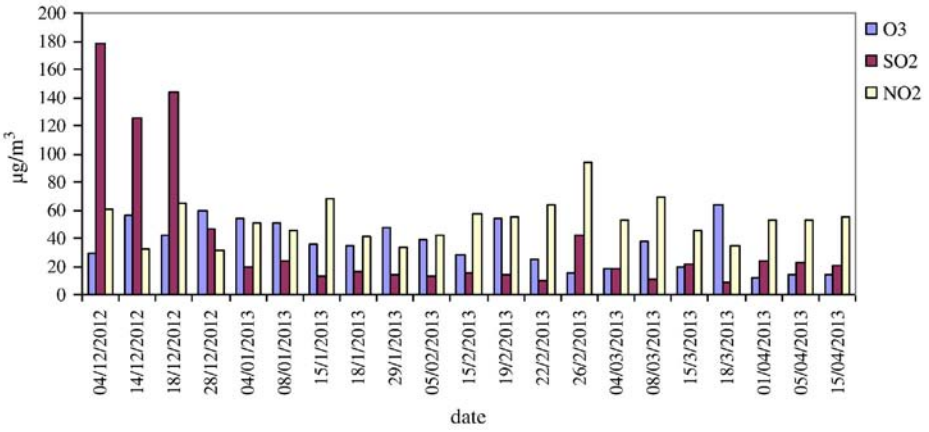

Fig. 3. Daily mean concentrations of O_3, SO_2 and NO_2 during the period of study.

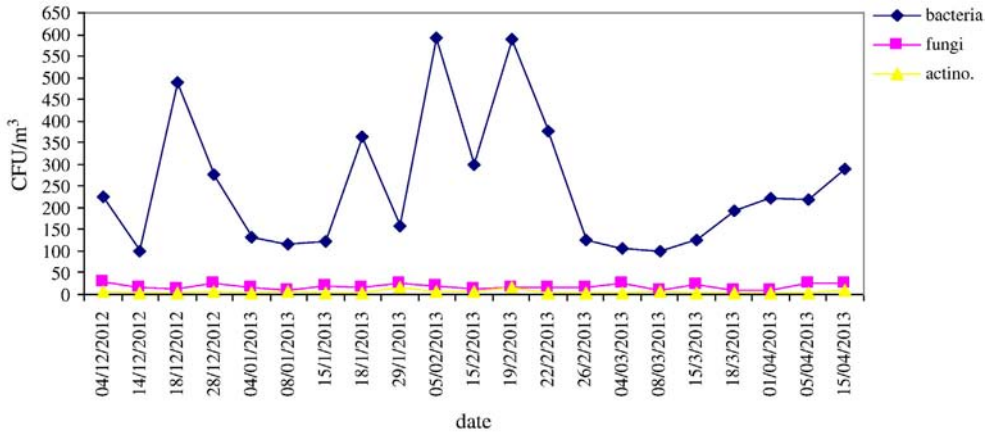

Fig. 4. Concentration of airborne microorganisms associated-PM_{10}.

$\pm$ 1.63%, respectively of the total PM_{10} mass concentration (Adhikari et al., 2006).

3.4. Identification of fungi-associated PM

Table 2 shows the numbers, percentages, frequency of occurrence, and aerodynamic diameters of the identified fungi associated PM_{10} and $PM_{2.5}$. *Aspergillus* and *Penicillium* were the common fungal genera. *Aspergillus* and its telemorphic (*Eurotium* and *Emericella*) constituted 88.25% and 72.12% of the total fungal counts associated $PM_{2.5}$ and PM_{10}, respectively. The frequency of occurrence (number of isolation out of 21 samples) was categorized into 4 groups, 1) high occurrence fungi (recorded 21–15 times out of 21 samples) represented by *Aspergillus fumigatus* and *Aspergillus niger* in both PM fractions, 2) medium occurrence fungi (recorded 14–10 times out of 21 samples) represented by *Alternaria* and sterial hyphae associated PM_{10}, 3) low occurrence fungi (recorded 9–5 times out of 21 samples) represented by *Emericella*, *Aspergillus ochraceus*, *Aspergillus sydowii*, *Epicoccum*, and *Fusarium*, depending on PM size fraction, and 4) rare occurrence fungi (recorded 4–1 times out of 21 samples) represented by: *Trichoderma*, *Trichothecium*, *Mucor*, and *Rhizopus* depending on PM size fraction.

The aerodynamic diameters (d_{ae}) of the identified fungal spores ranged between 2 and 14 μm. The aerodynamic diameter of the common fungi ranged between 1.7 and 3 μm (Table 2). The largest number of fungal colonies was found at the size fraction with aerodynamic diameter ranging between 2.1 and 3.3 μm, in aerobiological studies in coastal areas (Li et al., 2011), these results correspond with our findings.

3.5. The common fungal genera

Aspergillus, *Alternaria*, *Penicillium*, *Rhizopus* and *Mucor* were the common fungal types, with higher concentrations associated PM_{10} (Table 3). *Aspergillus* averaged 7.9 CFU/m^3 in $PM_{2.5}$ and 13.1 CFU/m^3 in PM_{10}. *Aspergillus* positively ($r = 0.26$) and negatively ($r = -0.28$) correlated with concentrations of PM_{10} and $PM_{2.5}$, respectively.

The dominance of airborne *Aspergillus*, *Penicillium*, and *Alternaria* is attributed to their ability to grow in various substrata in all regions under different weather conditions, and high capacity to produce and release high spore numbers into the air (Abdel Hameed et al., 2009; Lima and Gadelha, 1983). Airborne *Fusarium* has been reported with low incidence in many cities (0.015–0.3.1%) (Cavalo et al., 1980; Takahashi, 1997). In this study the absence of *Cladosporium* is an indicator of hot weather, and barren region, because it is sensitive to T°C (Pyrri and Kapsanaki-Gotsi, 2007) and lives on dead herbaceous plants (Cventic' and Pepeljnjak, 1997).

Aspergillus, *Mucor*, and *Rhizopus* can pose a threat to vulnerable individuals. *Aspergillus* is the common invasive mold infection worldwide (Soleimani et al., 2013). The risk of aspergillosis increased when mean concentration of *Aspergillus* was close to 0.9 CFU/m^3 (Perdelli et al., 2006). The allergens and microbial mediated respiratory diseases can coincide with elevated microorganisms associated particles, as may be enhanced when chemical and biological constituents of PM are combined. The synergetic effect of microorganisms and PM can aggravate respiratory allergy and other pulmonary diseases (Adhikari et al., 2006). Dust, soot and hydrocarbons are found besides pollen grains

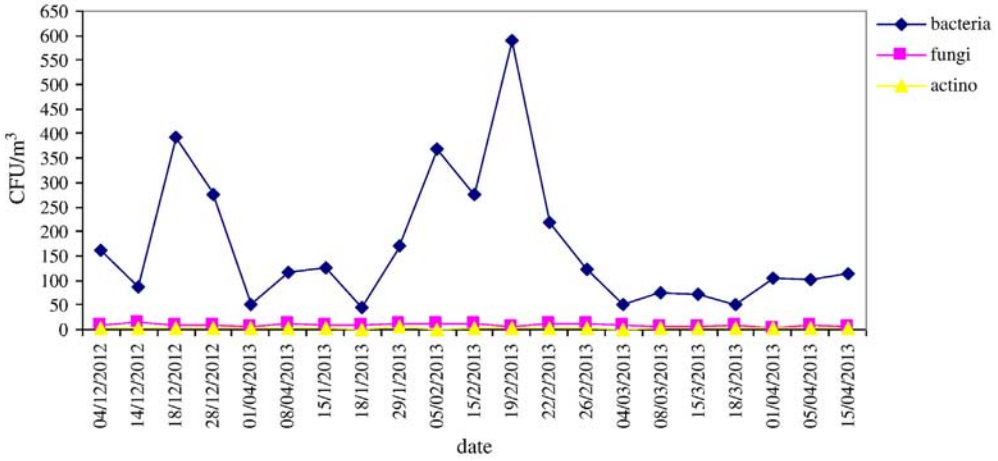

Fig. 5. Concentration of airborne microorganisms associated-$PM_{2.5}$.

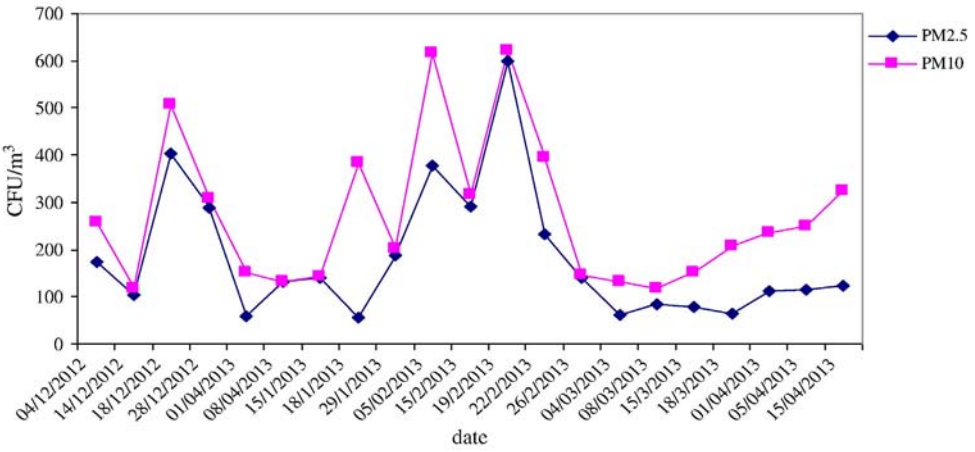

Fig. 6. The sum of airborne microorganisms loading PM_{10} and $PM_{2.5}$.

and fungal spores and contributed to increase respiratory tract problems, either as agents that cause illness themselves (D'Amato et al., 1994) or adjuvant effect that is provoked in people suffering from respiratory allergies (Santra et al., 1991). The daily exposure to air pollution may impair mucociliary clearance, depresses immune system, and increases airway responsiveness to aeroallergens. Therefore people who live in urban areas tend to become more affected by respiratory problems, at low aeroallergen concentrations, than those living in rural areas (Abdel Hameed, 2003).

3.6. Correlations between microorganisms associated PM with air pollutants and meteorological conditions

Table 4 shows the Spearman's correlation coefficients between microorganisms associated PM with air pollutants and meteorological parameters. Bacteria associated PM seemed to be independent from PM mass concentrations. Positive and negative correlations were found between both O_3 and NO_2 with microorganisms associated $PM_{2.5}$ and PM_{10}, respectively.

Ozone is known as a phototoxic oxidant (Tiedemann and Firsching, 2000). However, positive correlation was found between microorganisms associated $PM_{2.5}$ and O_3. This can be attributed to low retention time between O_3 and $PM_{2.5}$ to kill microorganisms or react with $PM_{2.5}$ compounds, as $PM_{2.5}$ mainly emitted from traffic activity or formed by chemical reaction near the sampling site. We hypothesized that a considerable amount of PM_{10} was transferred from other far sources and microorganisms might have time (days) to be affected by O_3.

Wind speed positively correlated with microorganisms associated PM_{10} and $PM_{2.5}$. Temperature showed significant positive correlations with mass concentration of PM_{10} ($r = 0.44$) and $PM_{2.5}$ ($r = 0.5$). Significant negative correlations were found between PM_{10} and SO_2, and between $PM_{2.5}$ and O_3.

In this study microorganisms associated PM were regressed against meteorological factors and air pollutants. The results of the multiple regression analysis indicated that the main predication variable of fungi associated PM was the inverse mass concentration of $PM_{2.5}$ ($1/PM_{2.5}$) ($P = 0.036$).

The effects of air pollutants and meteorological factors on microorganisms associated PM are complex. The low viable biological fraction associated PM may be attributed to many factors such as: PM composition, meteorological parameters, air pollution, physical and chemical transformation, and geographical characteristics. $PM_{2.5}$ may contain toxic compounds which kill or affect microbial viability (Hood, 1973; Handley and Webster, 1995). Toxic gases emitted by human activities

Table 2
Identification of fungi types associated $PM_{2.5}$ and PM_{10}.

Type	$PM_{2.5}$			PM_{10}			d_{ae} μm
	Number	%	Isolation out of 21 trials	Number	%	Isolation out of 21 trials	
Alternaria	10	2.62	8 (L)	32	6.81	16 (H)	6–13[a]
Aspergillus	321	84.03	21 (H)	308	65.53	21 (H)	1.7–4.5
A. fumigatus	241	63.1	21 (H)	96	20.42	21 (H)	1.7–2.2
A. flavus	22	5.76	11 (L)	59	12.55	17 (H)	3–4
A. niger	44	11.52	17 (H)	96	20.42	20 (H)	2.6–3
A. ochraceus	2	0.52	01 (R)	11	2.34	7 (L)	3–3.5
A. sydowii	–	–	–	6	1.28	5 (L)	2.6–3
Other *Aspergillus*	12	3.14	06 (L)	40	8.51	18 (H)	–
Emericella nidulans	12	3.14	05 (L)	4	0.85	4 (L)	3.5–4.0
Eurotium	4	1.05	06 (L)	27	5.74	14 (M)	3.5–4.5
Epicoccum	–	–	–	7	1.49	4 (L)	13–15[a]
Fusarium	1	0.26	01 (R)	7	1.49	6 (L)	2.2–3.6[a]
Mucor	1	0.26	01 (R)	7	1.49	5 (L)	4.5–7
Penicillium	10	2.62	06 (L)	36	7.66	18 (H)	1.7–3.4
Rhizopus	4	1.05	03 (R)	11	2.34	9 (L)	4–6
Sterile hyphae	15	3.92	08 (L)	28	5.96	12 (M)	–
Trichoderma	–	–	–	3	0.64	3 (R)	3–3.5
Trichothecium	2	0.52	02 (R)	–	–	–	8–10
Yeast	2	0.52	01 (R)	–	–	–	4.0
Total	382			470			

H: 21–15; M: 14–10; L: 9–4; R: 3–1; – not detected; d_{ae}: aerodynamic diameter.
[a] Short axis.

Table 3
Concentration of the predominant fungal genera-associated $PM_{2.5}$ and PM_{10}.

Genus	CFU/m^3					
	$PM_{2.5}$			PM_{10}		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Aspergillus	3.4	15.48	7.89 ± 2.93	7.12	24.52	13.1 ± 4.88
Alternaria	0.0	1.74	0.34 ± 0.47	0	3.38	1.26 ± 0.99
Penicillium	0.0	1.74	0.38 ± 0.65	0.0	3.8	1.44 ± 0.96
Rhizopus	0.0	1.63	0.14 ± 0.39	0.0	1.69	0.55 ± 0.54
Mucor	0.0	1	0.046 ± 0.21	0	1.7	0.277 ± 0.53
Sterial hyphae	0	2.31	0.46 ± 0.73	0	3.68	1.1 ± 1.05

reached levels conceivably deleterious to the survival of microorganisms (Lighthart et al., 1971). Cadman et al. (1997) found fungi spores in low counts during peak season of air pollution. Airborne bacterial and fungal concentrations decreased with increasing PM concentration (Raisi et al., 2010). PM had positive correlation with total fungi and *Aspergillus* (Adhikari et al., 2006).

The positive correlation between fungi and RH% confirmed the importance of humidity for release of fungi either by active or passive modes. However RH% may cause clumping of biological and non-biological particles, and consequently increases survivability of biological particles or helps fast settling and removing of particles from the air. Di Giorgio et al. (1996) found that various meteorological factors affected the type and concentration of airborne fungi, and relative humidity had no significant effect on viable particles.

Interestingly, temperature had negative effect on microorganisms associated $PM_{2.5}$. This is because $PM_{2.5}$ is mainly emitted from traffic activity and containing hydrocarbons and other chemical compounds. Temperature helps enhance chemical reaction on $PM_{2.5}$ surfaces to form more toxic compounds. It is clear that temperature had more deleterious effect on microorganisms associated $PM_{2.5}$ than ozone.

Wind speed positively correlated with microorganisms associated PM, and negatively correlated with PM mass concentrations (Table 4). Wind speed is a dilution factor (Lighthart and Kim, 1989), and there is a direct relationship between wind speed and libration of spores (Smith, 1966). The decay rate of airborne microorganisms increases as the aerosol age increase, i.e. decrease of wind speed, because wind speed helps transport of bioaerosols from source to the sampling site, and at the same time it decreases the net concentration of aerosols due to diffusion. Moreover aged particles have undergone physical and chemical transformations in the atmosphere such as coagulation, structural rearrangement, evaporation, adsorption and absorption which may decrease or increase survivability of microorganisms.

It should be mentioned that, the main limitations in the present study were: 1) low number of samples that may not be representative or accurately reflect concentrations, 2) explanation regarding microorganisms associated PM was mostly speculation and not supported by other previously local measurements, and 3) sampling method was considered as one limitation.

Regarding sampling method, the advantages and disadvantages of different air sampling methods were previously discussed (Jensen et al., 1994). Filtration (non-inertia) technique is inexpensive, simple, and samples can be taken continuously for long period of time. However filtration technique has three disadvantages: 1) dehydration effect is caused by large volume of air passing over microbial particle, 2) difficulty of removing deposited materials from the filters, and 3) inconsistency in recovery of microorganisms trapped in fibrous matrix. Finally in aerobiological field, many studies have been conducted to determine the relationships between biological particles with meteorological parameters, and air pollution. These studies are concerned with collecting biological particles using samplers based on inertia forces and nutrient media, and are not concerned with microorganisms associated PM, using high volume samplers.

4. Conclusion

The concentrations of microorganisms associated PM were low, with no significant correlations with PM mass concentrations. Fungi and actinobacteria are typically autochthonous organisms and probably derived from sources near the sampling area. Low numbers of fungi and actinobacteria are indicators of the barren and arid environments. *Aspergillus* was the common fungal genera associated PM. Concentrations of microorganisms associated PM varied under influence of the complex dynamics of weather conditions and air pollutants. Temperature positively correlated with PM mass concentrations; O_3 negatively affected $PM_{2.5}$ concentration, and SO_2 negatively correlated with PM_{10} and $PM_{2.5}$. Wind speed helps survival of airborne microorganisms, and helps dilution of PM. $1/PM_{2.5}$ concentration was the significant determinant of fungal concentration. This study is a contribution to understand airborne microorganisms associated PM and factors affecting their survivability.

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Table 4
Spearman's correlation coefficients between airborne microorganisms associated PM with air pollutants and meteorological parameters.

Variable	Variable							
	$PM_{2.5}$	PM_{10}	O_3	SO_2	NO_2	T°C	RH%	WS
Microorganisms associated $PM_{2.5}$								
Bacteria	−0.10		0.15	0.08	0.10	−0.22	−0.2	0.25
Fungi	0.03		0.18	0.28	−0.11	−0.31	0.14	0.2
Actinobacteria	0.10		0.11	0.18	0.12	−0.03	−0.19	0.42*
Microorganisms associated PM_{10}								
Bacteria		−0.10	−0.03	−0.04	−0.08	0.17	0.05	0.06
Fungi		0.25	−0.16	0.22	−0.06	0.35	0.1	0.14
Actinobacteria		0.11	−0.07	−0.06	−0.12	0.14	−0.25	0.28
$PM_{2.5}$	1	0.92	−0.47	−0.37	0.14	0.5	−0.02	−0.22
PM_{10}		1	−0.3	−0.48	−0.002	0.44	0.05	−0.17

* $P \leq 0.05$.

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