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Evaluation of Negative Air Ions in Bioaerosol Removal: Indoor Concentration of Airborne Bacterial and Fungal in Residential Building in Qom City, Iran

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Abstract**—**The present investigation was conducted to detect the type and concentrations of bacterial and fungal bioaerosols in one room (bedroom) of each selected residential building located in different regions of Qom during February 2015 (n=9) to July 2016 (n=11). Moreover, we evaluated the efficiency of negative air ions (NAIs) in bioaerosol reduction in indoor air in residential buildings. In the first step, the mean concentrations of bacterial and fungal in nine sampling sites evaluated in winter were 744 and 579 colony forming units (CFU)/m^3, while these values were 1628.6 and 231 CFU/m^3 in the 11 sampling sites evaluated in summer, respectively. The most predominant genera between bacterial and fungal in all sampling sites were detected as *Micrococcus* spp. and *Staphylococcus* spp. and also, *Aspergillus* spp. and *Penicillium* spp., respectively. The 95% and 45% of sampling sites have bacterial and fungal concentrations over the recommended levels, respectively. In the removal step, we achieved a reduction with a range of 38% to 93% for bacterial genera and 25% to 100% for fungal genera by using NAIs. The results suggested that NAI is a highly effective, simple and efficient technique in reducing the bacterial and fungal concentration in the indoor air of residential buildings.

Keywords**—**Bacterial, fungal, negative air ions, indoor air, Iran.

I. Introduction

IN last decades, exposure to various indoor air pollutants has been received public attention all over the world due to its effects on human health. This increasing concern is due to people who usually spend almost 90% of their time at indoor spaces [1], [2]. Among the different enclosed spaces, residential home is the most vital space because people spent most of their time in this space [3]. Among the indoor air pollutants, bioaerosol is one of the most important pollutants so that its contribution is approximately 5-35% of indoor air pollutants [4]. Bioaerosols are present in the atmosphere in size of larger than 0.2 μm comprising of living organisms (bacterial, viruses, fungal, protozoa) or compounds released from living organisms origin (pollen, spores, cell debris and biofilms) that may be attached to particulate matter or not [5]-[8]. Bacterial and fungal are the most dominant bioaerosols in indoor environments. High concentration of airborne bacterial is related to various adverse health effects such as allergies, respiratory, and dermatological infections and diseases [9]. Moreover, there are many studies on fungal separated from indoor environments which show that being in damp and moldy indoor environments caused some health problems such as respiratory symptoms, wheeze, cough, etc. [10], [11]. Therefore, it is important to study the concentration of bacterial and fungal in indoors air. The fungal concentrations suggested by the American Conference of Government Industrial Hygienists were between 100 and 1000 CFUs/m^3, while the count for total bacterial should not exceed 500 CFUs/m^3 [12], [13]. The distribution and concentration of indoor bioaerosols can be affected by some factors such as air conditioning systems, ventilation, plants, resuspension of particulate matter, indoor sources, temperature, and relative humidity [3], [14].

With respect to the mentioned reasons, various researches aimed at eliminating bacterial and fungal from indoor environment such as restrooms have been carried out. These techniques are spore infiltration using window air conditioner [15], inhibition of growth by avoiding available moisture [16], catalyst technology [17], portable vacuum systems [18], plasma ion [19], disinfect indoor environments using UV lights [20], etc. Furthermore, NAIs are one of the newest and strong oxidants that can destruct the fungal and bacterial. Tyagi and Malik [21] reported that NAIs generator in a 93.75-L chamber killed 45.5% in the first 4h and 58.6% after 12h of *Pseudomonas fluoresens.* Arnold et al. [22] conducted a laboratory reactor with a negative ionizer 7.6 cm above a stainless steel surface against bacterial including *Campylobacter jejuni*, *Esterichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes*, and *Staphylococcus* and reached to 99.8% removal efficiency.

Nevertheless, up to now, all the studies that conducted on removing of airborne bacterial and fungal with NAIs were laboratory and small scale and no previously published research could be found applying NAIs directly to removal of indoor airborne bacterial and fungal in the real indoor spaces. To cover this knowledge gap, we conducted a research to survey the effect of NAIs in destructing of indoor airborne bacterial and fungal in the residential buildings. Due to the difficulty of the study, we limited the territory of research to

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the city of Qom, Iran. Thus, the main objectives of this research were the following: (1) to evaluate the concentration of indoor fungal and bacterial bioaerosol in the residential building of Qom (2) to determine the predominant indoor fungal spores and bacterial genera in the residential building of Qom (3) to investigate the effect of characteristics of buildings and some parameters of indoor air on concentration of bacterial and fungal and (4) to determine the efficiency of NAIs in removal of indoor bacterial and fungal in the residential buildings.

II. Material and Methods

A. Site Description and Sample Collection

The present study was conducted in Qom city, the 8th largest city in Iran. Qom (50.88oN 34.64oE) is the capital of Qom Province and located in center of Iran. It lies on the 125 km by road southwest of Tehran. Qom is one of the industrialized and densely populated cities in Iran, with urban population of over 1 million. Due to distance from the sea and being situated in the vicinity of desert, this city has a dry and warm climate. Summer season is extremely hot with maximum temperature of 39.4 °C in July during daytime. Winter season is cold with minimum temperature of -1.6 °C in January. Annual rainfall averages are 125 mm which happen between October to May, also, the precipitation varies 28 mm between the driest month and the wettest month. Monsoons create steady strong winds from December to April, but calm winds from June to October [23].

Airborne viable bacterial and fungal were sampled before and after the NAIs release from indoor air. All bioaerosol samples (n=20) were collected from one room (bedroom) of randomly selected houses located in different regions of Qom during February 2015 (n=9) to July 2016 (n=11). Fig. 1 describes the study region. Samples were taken in one room of each house, with an area of approximately 12-m^2. All selected houses were assessed in terms of age of building and mode of ventilation. Also, humidity, temperature and concentration of Particulate Matters (PM_{10}, $PM_{2.5}$ and PM_1) of indoor air were evaluated during the sampling. During the experiments, all windows and doors were closed. More details about each sampling location are given in Table I.

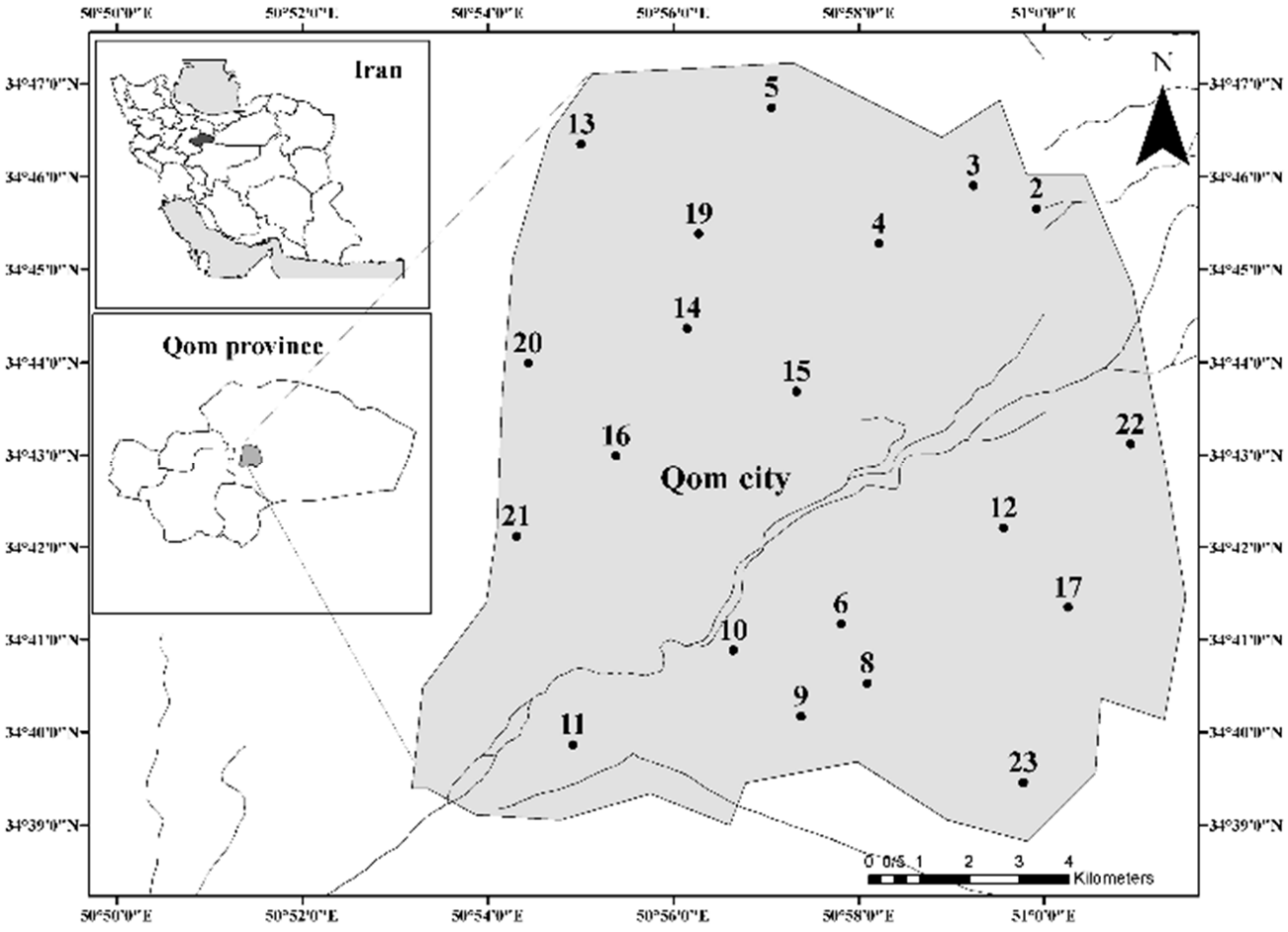

Fig. 1 Location of sampling sites in Qom City

TABLE I
MAIN CHARACTERISTICS AND CONCENTRATION OF PARTICULATE MATTERS (PM) IN TWENTY SAMPLE SITES

Number of houses	Season1	Age of building (Year)	PM1 (μg/m^3)	PM2.5 (μg/m^3)	PM10 (μg/m^3)	Relative Humidity (%)	Temperature (^{o}C)	Mode of ventilation[2]
1	S	10	7.5	33	49	67	21	W
2	S	10	2.3	35	52	40	22	W
3	S	10	2.7	10	15	20	28	N
4	S	10	6.4	23	33	68	26	N
5	S	22	6.4	29	41	60	20	A
6	W	30	9.5	62	88	66	22	W
7	W	22	9.4	53	76	45	22	W
8	S	12	16.9	40	56	60	18	A
9	W	20	5.3	66	94	58	25	W
10	S	22	3.5	32	46	59	27	W
11	S	15	41.8	67	111	45	26	W
12	W	25	6.5	36	60	31	24	W
13	W	10	7.1	40	70	70	24	W
14	W	10	4.7	22	31	50	23	N
15	W	3	11.7	84	120	65	21	W
16	S	10	3	13	17	65	28	W
17	W	10	2.4	28	37	51	20	A
18	W	10	6.3	38	54	65	21	W
19	S	10	7.2	50	72	40	24	N
20	S	10	7.5	45	64	61	26	N

1. S: Summer, W: Winter
2. N: Natural (window opening), W: Water cooler, A: Air-Conditioning

B. Sampling Methods

Sampling was done by active method to collect indoor bioaerosols. In the active method, we used QuickTake 30 sample pump equipped with the Bio Stage single-stage cascade impactor (SKC, USA) including 400 holes. The pump was set at flow rate of 28.3 L/min for 2 min [14] in the center of each room at a height of 1.5 m above the floor and sterilized with 70 % ethanol before the sampling [24]. During the sampling period, temperature, relative humidity (RH) and PM concentrations were also measured using portable GRIMM dust monitors-models 1.108 (GRIMM Aerosol Technik GmbH, Ainring, Germany) using light-scattering laser method by the flow rate of 1.2 L/min [25].

Air culturable bioaerosol samples were collected onto Petri dishes with 90 mm diameter. Tryptic soy agar (TSA) culture media was used for airborne bacterial and Sabouraud's dextrose agar (SDA) for airborne fungal [26]. In addition, cycloheximide (500 mg/L) was used as an antibiotic to prevent bacterial growth in the fungal culture media and chloramphenicol (100 mg/L) to inhibit fungal growth in the bacterial media [24]. All collected samples were placed in zip kips and transferred to the laboratory in cool box.

C. Determination of Bacterial and Fungal Bioaerosols Concentration

To identify bacterial and fungal, plates incubated at 37 ^{o}C for 2 days and 25 ^{o}C for 4-7 days, respectively [27], [28]. The counts of bacterial and fungal in air sample plates were corrected for multiple impactions using the positive hole conversion method [29] and expressed as colony forming unit per cubic meter of air in (CFU/m^3). The bacterial genera were recognized according to Bergey's manual and biochemical tests, while the fungal species were identified by using optical microscopes at 100×400 magnification [30].

The bioaerosol's CFUs enumerated are related to the bioaerosol level in the indoor air in the active method by [30]:

$$\mathrm{CFU/M^3} = \frac{\mathrm{C} \times 1000}{\mathrm{t} \times \mathrm{F}}$$

where C is the number of bacterial or fungal colonies, t is the duration of sampling (min), and F is the flow of the pump (L/min).

D. The Effect of NAIs

For studying the antimicrobial efficiency of NAI in removal of bioaerosols, we applied the NAI generator (Neotec, XJ-2100) with several ionizing pins to provide the negative charge to the air ions. Before using the NAI generator, UV lamp was covered with aluminum foil to eliminate its effect. The NAI generators were positioned at a height of 1.2 m above the floor, and its independently-controlled fan was turned on to increased air circulation. The removal experiments were carried out in closed rooms (12 m^2 floor areas), and ventilation was turned off. The continuous concentration of NAI was maintained at about 10^6 NAI/cm^3. The portable ion counters (Air Ion Counter IC 1000, Ion Trading, Tokyo, Japan) were used to record the ion concentration in the room air. This can be explained through the facts that the NAIs have short live with a typical lifetime of 100–1000 s in clean air, so concentration remained almost constant and their average concentration does not change [31]. Relative humidity and air temperature were recorded during the experiments. Ozone concentrations were monitored with a portable O_3 detector (PortaSensil, Ati, Collegevile, PA, USA)

and the concentrations of ozone were detected in the ranged of 0.01-0.07 ppm when the NAIs generator was operating.

After sampling of the initial concentration of bacterial and fungal in indoor air, NAI generator was switched on for 5 hours. After each particular time period (1, 2, 3, 4, and 5 h) sampling of bioaerosols was carried out. The relative humidity and air temperature were recorded after each hour. The results of removal were demonstrated as percentage reduction incolony in treated plates in relation to the untreated initial plates.

E. Statistical Analysis

SPSS software version 20 was utilized for analysing the experimental data. The normality of the data was evaluated by Kolmogorov-Smirnov test. The concentrations of bacterial and fungal were supposed as independent variable and were described by minimum, maximum, mean, standard deviation (SD), and median. On the other hand, the factors affecting bioaerosol levels such as season (winter: W and summer: S), temperature, relative humidity, particulate matter (PM) concentrations, age of building and mode of ventilation (Natural: N, Water cooler: W, Air-Conditioning: A) were assumed as dependent variables. Linear regression was used to assess the correlation between concentration of bioaerosols and particulate matters. Then, Pearson correlation coefficient was performed to determine the relationship among the bacterial, fungal detected and dependent variables. P-values of 0.05 or less are accepted as statistically significant.

III. Result and Discussion

A. Environmental and Building Parameters

Environmental parameters such as relative humidity, temperature, season, and particulate matters (PM) concentration in 20 houses were measured during microbial measurement. Also, some characteristics of building such as age of building and mode of ventilation were recorded during the sampling to estimate the influence of these parameters on bioaerosol concentration. The result of these parameters was summarized in Table I. Also, the results of the correlation analysis are presented in Table II. It is apparent from this table that the indoor concentration of bacterial and fungal were influenced by season ($P<0.0001$) and relative humidity ($p=0.03$), respectively. There was a positive correlation between relative humidity and fungal concentrations ($p=0.03$), while no significant correlation was found between relative humidity and bacterial concentrations ($p=0.72$). The relative humidity measured in the indoor air ranged from 20 to 70%, which was upper the recommended comfort range (30–60%) in 40% of houses [32]. Accordingly, it is suggested that the concentration of fungal bioaerosols is closely associated with relative humidity, and the relative humidity more than 70% is optimal condition for fungal growth [33].

Although significant correlation was found between bacterial concentration and season, but there was no correlation between season and fungal concentration ($p>0.05$). These results agree with the findings of other studies [34]. However, these results differ from the findings of Lee et al., in which there was no difference in the bacterial concentrations between summer and winter but total fungal concentration was higher in the summer than in the winter [26]. Our results also demonstrated that indoor temperature had no effect on the bacterial and fungal concentration ($p>0.05$). This could be attributed to more variation in the temperature and climate during the year through the warm and cold seasons. It causes to close the door and window in the most days of the year and almost the temperature of indoor air is constant (23-25 ^{o}C) over the year. These findings support the idea of Hussin et al. who found that the indoor temperature had no effect on airborne bacterial and fungal [35]. At the same time, bioaerosol concentration was not affected by age of building and ventilation ($p>0.05$).

TABLE II
Correlation between Environmental and Building Parameters and Total Bacterial and Fungal Concentration in the Sample Sites

	Temperature	Humidity	Season	PM_{10}	$PM_{2.5}$	PM_1	Age of building	Ventilation	Total Bacterial	Total Fungal
Temperature	1									
Humidity	0.32	1								
Season	0.18	0.69	1							
PM10	0.42	0.51	0.12	1						
PM2.5	0.30	0.43	0.11	0.0001	1					
PM1	0.93	0.94	0.51	0.0007	0.02	1				
Age of building	0.98	0.68	0.39	0.51	0.53	0.84	1			
Ventilation	0.05	0.32	0.18	0.35	0.32	0.92	0.12	1		
Total Bacterial	0.50	0.70	<0.0001	0.33	0.22	0.17	0.75	0.4718	1	
Total Fungal	0.82	0.03	0.11	0.58	0.71	0.72	0.48	0.97	0.53	1

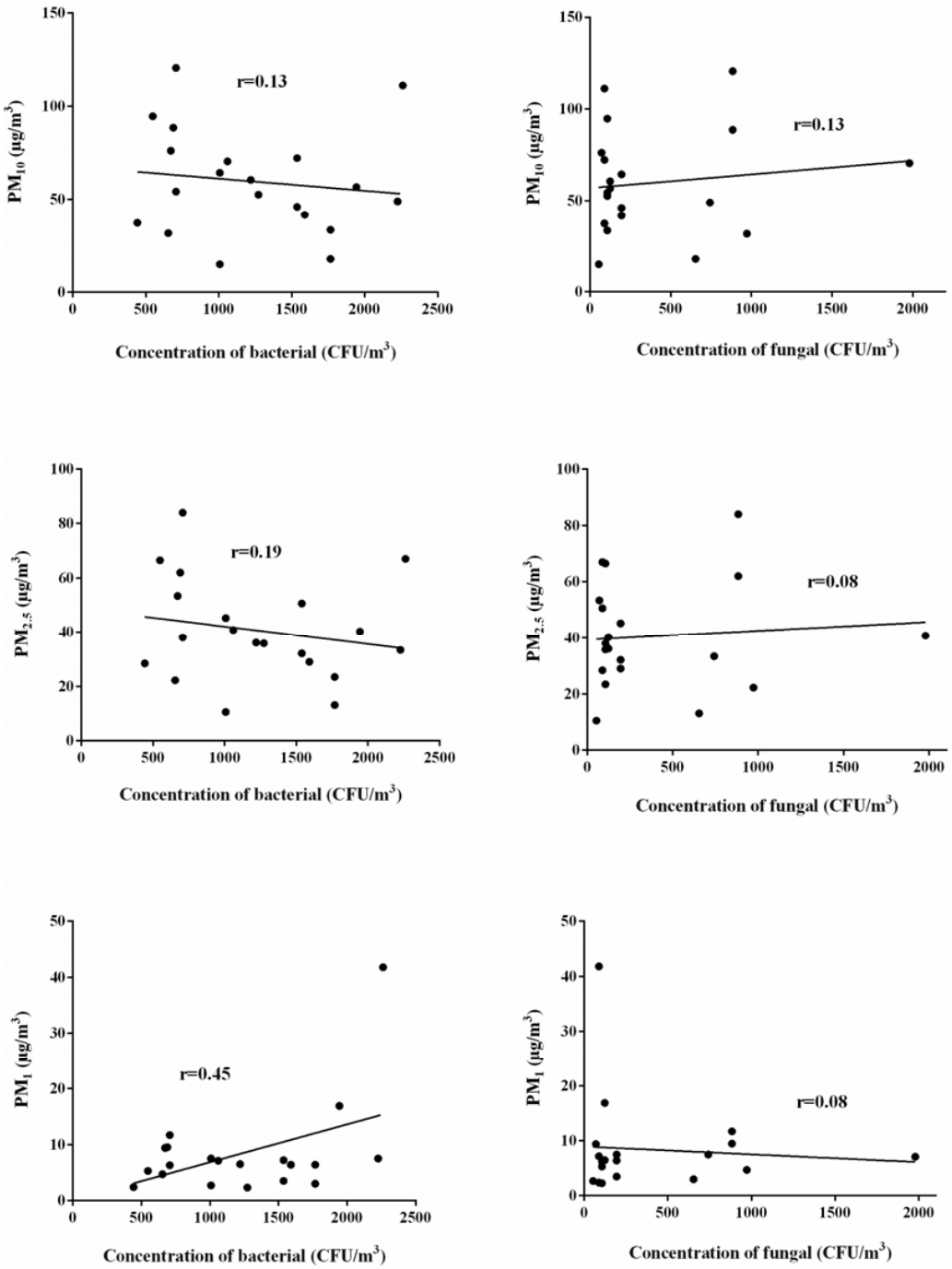

Fig. 2 Linear regression plots of concentration of bioaerosols and particulate matters (PM)

As shown in Table I, PM_{10}, $PM_{2.5}$ and PM_1 concentrations ranged from 15-120.6 µg/m^3, 10.5-84 µg/m^3 and 2.3-41.8 µg/m^3, respectively. These maximum levels of PM were recorded in the site samples of 15 and 11 which could attribute to heavy tobacco smoking and opium abuse in these houses. It may be the reason for a rise in particulate matter counts especially for smaller than 0.5 µm and also for larger diameter particulate matters, furthermore, weak ventilation could be responsible for high concentrations of particles [36]. To examine the correlation among bacterial and fungal concentration and PM concentration, statistical linear regression analysis was performed. Fig. 2 showed that regression coefficients were 0.13, 0.19, and 0.45 for PM_{10}, $PM_{2.5}$ and PM_1 concentration and bacterial concentration, respectively. In addition, the regression coefficients for PM_{10}, $PM_{2.5}$ and PM_1 concentration and fungal concentration were

0.13, 0.08 and 0.08, respectively. Except for PM_1 and bacterial concentrations, all regression coefficients were lower than 0.4 and there was no relationship between indoor bioaerosols and particulate matters (PM). Indoor air may contain bioaerosols (fungal, bacterial and allergens) and non-biological particles such as dust, smoke, organic and inorganic gases and cooking-generated particles. Therefore, according to the results of Hsu et al., the low percentage of bioaerosols could be the reason of low relationship between bioaerosols and particulate matters [37]. However, bacterial concentrations and PM_1 were linked by a significant linear relationship and a moderate correlation which are consistent with the results of Paratet al.. On the other hand, PM concentration should not substitute for bacterial measurements because there is no definite relationship between two parameters, and also, bacterial concentration cannot predict with PM concentration in the different sites [36].

B. Concentration of Bacterial and Fungal Bioaerosols

Table III shows the indoor concentration levels of bacterial and fungal and their genus and species. All concentration levels are based on colony forming unit per cubic meter (CFU/m^3). The numbers of bioaerosol detected in 20 rooms (only bedrooms) of selected houses and reported as median, mean±SD, minimum and maximum values. Number of the samples is summarized as two groups of winter and summer seasons.

The mean concentrations of detected bacterial and fungal in the nine sampling sites evaluated in winter were 744 and 579 CFU/m^3 and in the 11 sampling sites evaluated in summer were 1628.6, 231 CFU/m^3, respectively. The concentration levels of bacterial in samples of winter ranged from 441 to 1219 CFU/m^3 and in samples of summer ranged from 1007 to 2261 CFU/m^3, respectively. These values for fungal bioaerosols were found from 70 to 1978 (579.1) CFU/m^3 in nine samples of winter and 53 to 742 (231.3) CFU/m^3 in 11 samples of summer. Comparable concentrations in a wide range of concentrations found by other researchers, including 47-12341 CFU/m^3 for bacterial concentration in residential environments in Beijing, China [38], 103 CFU/m^3 for bacterial concentration in homes and 10-104 CFU/m^3 in healthy and moldy buildings for fungal concentration in Upper Silesia, Poland [39] and 10-103 CFU/m^3 for bacterial values and 10-103 CFU/m^3 for fungal values in Korean [26]. The maximum concentrations of indoor bacterial recommended by Kowalski [13] and American Conference of Governmental Industrial Hygienists (ACGIH) [12] were 500 CFU/m^3. Moreover, indoor air concentrations of fungal recommended level established by Kowalski and ACGIH were 150 and 200 CFU/m^3, respectively [12], [13]. According to these values, bacterial concentration was exceeded the recommended concentrations in 95% of the houses, and fungal concentration in 45% of the houses was more than recommended concentrations (according to Kowalski recommendation). It seems to be possible that these results are taken due to high density occupancy, insufficient ventilation and air exchange rates and also, high relative humidity and temperature [40]. The concentrations of bacterial and fungal in the indoor samples in the different houses are shown in Fig. 3. In accordance with the present results, previous studies have demonstrated that the fungal concentration was lower than the total bacterial concentration in all the indoor samples [14], [30] which can be attributed to faster sedimentation of fungal due to longer aerodynamic diameter and also the abundant number of bacterial in natural resources such as soil and vegetation [30]. On the other hand, the concentration of bacterial in the sampling sites given in summer is significantly higher than the ones in winter. The highest level of bacterial concentration was observed in sampling site 1 and 11 (2226.1 CFU/m^3) which could be due to the denser population, heavy smoking, inadequate ventilation and high concentration of PM_1 (41.8 $\mu g/m^3$) (in sampling site 11). Results also indicated that the total bacterial concentration in all sampling sites was over the recommended level except sampling site 17 (441.7 CFU/m^3). It may be due to correctly operated air-conditioning system, limited activity of persons, low concentration of PM_1 (2.4 $\mu g/m^3$) and low temperature (20 ^oC) in this house than the other houses. In case of fungal, the highest level of fungal concentration was seen in sampling site 13 which can be attributed to high relative humidity (70%) and existence of many plant and flower pots in this sampling site. As you seen in Fig. 3, in some sampling sites, the concentration of fungal was exceeded the recommended level by Kowalski (150 CFU/m^3).

TABLE III
THE CONCENTRATIONS AND SPECIES/GENUS OF BACTERIAL AND FUNGAL BIOAEROSOLS (CFU/M^3)

	Winter (n=9)			Summer (n=11)		
	Min-Max	Mean±SD	Median	Min-Max	Mean±SD	Median
Total Bacterial	441.7-1219.1	744±243.6	689.04	1007-2261.5	1628.6±425.8	1590.1
*Micrococcus*spp.	53.0-865.7	344.5±237	371.0	441.7-1766.8	1026.3±527.2	812.7
*Staphylococcus*spp.	176.7-759.7	335.7±182.5	282.7	53-1166	547.7±368	459.4
Bacillus spp.	0-35.3	19.6±13.8	17.7	0-70.7	40.1±25	53
Diphtheroids	0-88.3	11.8±29.3	0	0-88.3	14.4±29.4	0
Total Fungal	70.7-1978.8	579.1±657	123.7	53-742	231.3±236.4	123.7
Aspergillus spp.	35.5-1766.8	451.5±590.7	106	35.5-706.7	184.7±247.7	70.7
Penicillium spp.	0-636	112±209	17.66	0-88.3	27.3±29.9	17.6
Cladosporium spp.	0-53	13.7±21.2	0	0-35.3	14.4±15.4	17.6
Syncephalostrum spp.	0	0	0	0-17.7	1.6±5.3	0
Rhizopus spp.	0-17.7	2±5.8	0	0-17.7	3.2±7.1	0

C. Identification of Bacterial and Fungal Bioaerosols

Table II summarized the genus/species of bacterial and fungal bioaerosols in sampling sites. The results showed that the bacterial species and genus identified from the sampling sites were *Micrococcus* spp., *Staphylococcus* spp., *Bacillus* spp. and *Diphtheroid* where two genera of *Micrococcus* spp. and *Staphylococcus* spp. have the most prevalent with almost 60% and 37% of the total detected bacterial, respectively (Fig. 4) and also, they were detected in all sampling sites. They were consistent with those reported in Upper Silesia in Poland and Ankara in Turkey [14], [39]. Furthermore, the third and fourth dominant bacterial genera were *Bacillus* spp. (2.5% of total bacterial) and *Diphtheroid* (1.1% of total bacterial) in indoor environments in sampling sites. There is no difference between contributions of the bacterial genera in 11 sampling sites evaluated in summer with nine sampling sites evaluated in winter. In other studies, the same contribution of the bacterial indoors was found, however, in different indoor environments, the percentage of dominant bacterial was difference. Fang et al. reported that the dominant indoor bacterial in residential environments were *Micrococcus* (26.74%), *Bacillus* (14.56%), Kocuria (12.66%), and *Staphylococcus* (12.03%) [38] while Aydogduet al. detected *Staphylococcus* (58.67%), *Micrococcus* (10.88%), Corynebacterium (6.90%), and *Bacillus* (6.61%) as the dominant indoor bacterial in child day-care centers in Turkey [9].

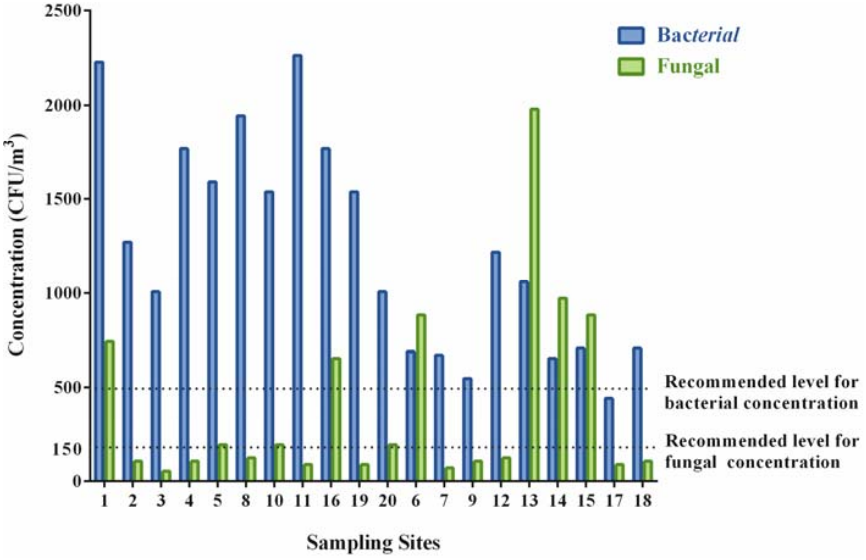

Fig. 3 Concentration of total bacterial and fungal bioaerosols in the indoor samples in the different houses

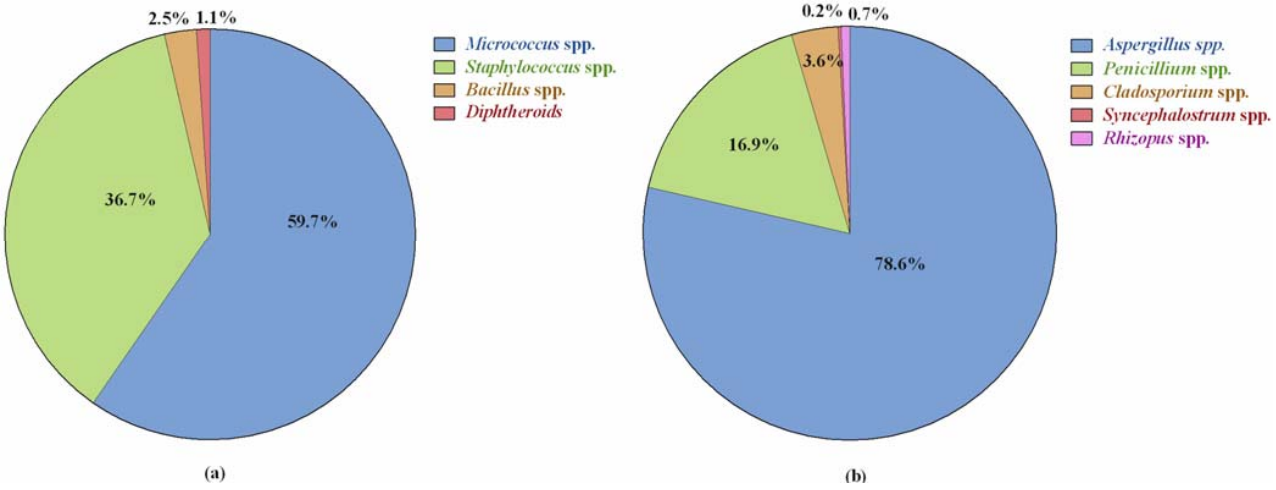

Fig. 4 Distribution of (a) bacterial and (b) fungal genera in indoor air in sampling sites

The occurrence of seven viable fungal genera was recognized from the indoor air of sampling sites. There were consist of *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Syncephalostrum* spp. and *Rhizopus* spp. which two most

prevalent fungal genera were *Aspergillus* spp.and *Penicillium*Spp.. The combined portion of *Aspergillus* spp.and *Penicillium* Spp. was almost 95.5% of the total fungal in indoor air of sampling sites. The individual portions of other genus include of *Cladosporium* spp., *Syncephalostrum* spp. And *Rhizopus* spp. were 3.6, 0.2, and 0.7% of the total fungal, respectively. In accordance with the present results, *Aspergillus* spp. were also the most predominant fungal genera in the previously conducted study with a portion (of the total) of 18.42% [35]. However, according to the study of Menteseet al. and Bonettaet al., *Penicillium* spp. with the range of 23.8-43.4% and 60-68% were predominant fungal genera in indoor air [14], [34]. The *Penicillium*, *Aspergillus* and *Cladosporium* identified in indoor air are recognised as possible causes of many allergic and respiratory symptoms in humans who live in contaminated buildings, and also, sick building syndrome may attributed to *Penicillium* species [34].

D.Effect of NAIs on Indoor Bacterial and Fungal Removal

Tables IV and V present the concentrations of bacterial and fungal under NAIs application through 5 hours. As can be seen, NAIs resulted in a reduction in concentrations of bacterial with an average of 70% after 5 h. The NAIs removal ranged from 38% in sample site 20 to 93 % in sample site 4. Average removal of fungal concentration in indoor air in sample sites was estimated 76.6% and ranged from 25% in sample site 7 to 100% in sample sites 4, 11, 12, and 17. Fig. 5 shows reduction in colony number of bacterial and fungal during 5 hours. Our results were similar to other studies of air ion effects on bacterial. Tyagi et al. studied the effect of NAIs against *Pseudomonas fluorescens* and saw the reduction of 45.5% in the first 4h and 58.6% after 12h in viability of bacterial cells [21]. Furthermore, Arnold et al. applied a negative ionizer 7.6 cm above a stainless steel surface and reported that the *Campylobacter jejuni*, *Esterichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes*, and *Staphylococcus* were reduced with 99.9% and also, *Bacillusstearothermophilus* was removed in 99.8% in 3 h with NAIs [22]. In the fungal case, Shargawi et al. assessed the effect of NAIs on *Candida albicans* growth by measuring the area of the zone of inhibition generated around the electrode of the ionizer and reported a significant increase in growth inhibition [41]. Pratt et al. described that expositor to NAIs caused about 80% reduction in the yield of penicillin in cultures after seven day [42].

Several mechanisms were described for action of NAIs which included the disruption of plasma membrane by production of a strong electrical field, attraction of charged airborne particulates by physical effect to grounded surfaces such as walls and floors and electroporation acting as a secondary role via generation of radicals such as ozone (O_3), hydrogen peroxide (H_2O_2), atomic oxygen (O or O•–), superoxide (O_2•–), and hydroxyl radicals (OH•) [43], [44]. These reactive species dissolve to the moisture of cell surface and diffuse into it, as well as, chemistry reaction with various biomacromolecules could create the secondary radicals which promote the reactive oxygen species level in cell and make damage effects. The hydroxyl radicals OH• have most reactive among reactive species which could damage to biological molecules significantly. Relative humidity in the air also could increase the production of hydroxyl radicals and hydrogen peroxide and reduce ozone concentration [44]. Yu et al. showed that production of hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•) was increased with relative humidity elevated that leads to higher bacterial cell membrane damage [45]. However, Fletcher et al. reported that principal cause of cell death in the bacterial could be attributed to exposure to ozone and Fan et al. revealed a high efficiency in combination of ozone and NAIs on bacterial cell death [46], [47]. In the present study, concentration of ozone was detected in ranged of 0.01-0.07 ppm where we did not consider the effect of ozone or combination of ozone and NAIs in reduction efficiency of indoor bioaerosols, which might be a topic for future study.

E. Distribution of Indoor Bacterial and Fungal after Using NAIs

Fig. 6 shows the distributions of bacterial (A) and fungal (B) genera in indoor air in the different sample sites before and after using NAIs. In bacterial genera, the *Micrococcus* spp. and *Staphylococcus* spp. were predominant in all sample sites after using NAIs just like before. There was no significant difference in the air bacterial distribution between before and after using NAIs. However, *Bacillus* spp. was removed completely in some sampling sites. Concerning distribution of fungal in indoor air, *Aspergillus* spp. had the most isolated airborne fungal after using NAIs and detected as the only genus of fungal in sampling sites of 1, 2, 3, 7, 9, 18, and 19. There were detected no fungal bioaerosols in sampling sites of 4, 11, 12, and 17. Furthermore, *Cladosporium* spp. and *Syncephalostrum* spp. were removed totally in all sampling sites except in site 6 for *Cladosporium* spp.

IV. Conclusion

The present study was among the very few studies which evaluates bacterial and fungal bioaerosol in indoor air in 20 sampling sites. Moreover, we assessed the efficiency of NAIs in removal of bacterial and fungal bioaerosols in these sampling sites. In the first step, bioaerosols detected from all sampling sites, indicating that bacterial concentration was strongly influenced by season ($P<0.0001$) and fungi correlated with relative humidity ($p=0.03$). Because of using ventilation in the most days of the year and constant temperature in buildings (23-25 ^oC) over the year, there was no correlation between indoor temperature bacterial and fungal concentration ($p>0.05$). According the regression coefficients, no relationship was find between indoor bioaerosols and particulate matters (PM) (<0.4) except for PM_1 and bacterial. The concentrations of indoor bacterial and fungal were higher than recommended levels in 95% and 45% of the sampling sites, respectively. It could be related to high density occupancy, insufficient ventilation and air exchange rates, high relative humidity and temperature. In all sampling sites, *Micrococcus* spp. and *Staphylococcus* spp. and also,

Aspergillus spp.and *Penicillium* Spp. have the most predominant genera between bacterial and fungal, respectively. In the second step, NAIs resulted in a maximum reduction in concentrations of bacterial and fungal with 93% and 100% after 5h. The reactive species generated by NAIs (ozone (O_3), hydrogen peroxide (H_2O_2), atomic oxygen (O or O•–), superoxide (O_2•–), and hydroxyl radicals (OH•)) could promote the reactive oxygen species level in cell and make damage effects. On the basis of present results, it can be stated that NAIs is highly effective method and could be used as a simple and more efficient technique in reducing the bacterial and fungal concentration in the indoor air of buildings. Future research can investigate about the other mechanisms of action of NAIs and also, effect of ozone or combination of ozone and NAIs in reduction of representative bioaerosols in residential buildings.

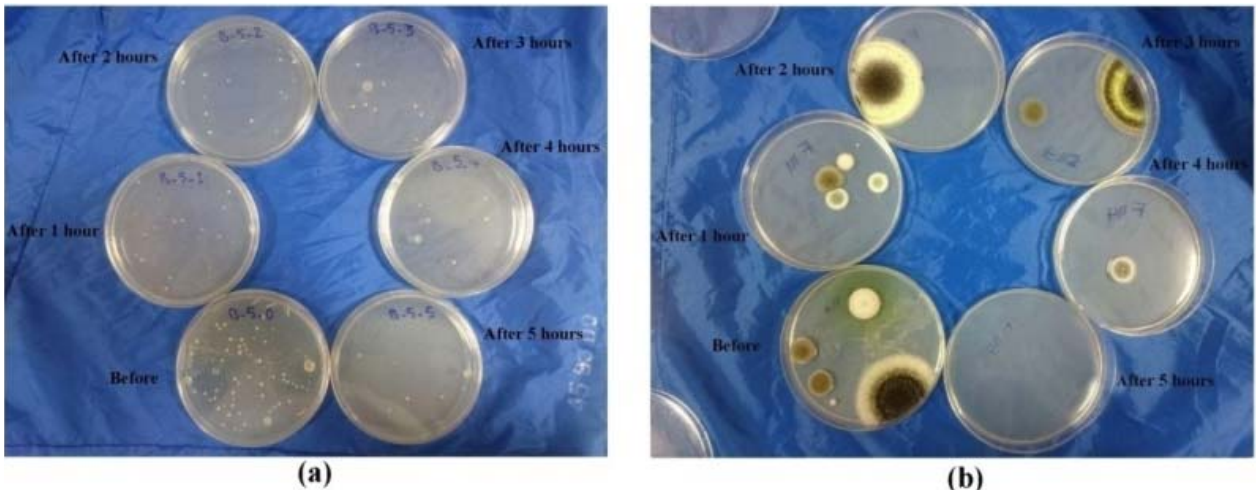

Fig. 5 Reduction in (a) bacterial and (b) fungal colony number after using NAIs during 5 hours

TABLE IV
THE CONCENTRATION OF BACTERIAL (CFU /M^3) AFTER USING NAIS THROUGH 5 HOURS IN THE SAMPLE SITES

Number of houses	Time (hours)						Removal (%)
	0	1	2	3	4	5	
1	2226.1	759.7	1201.4	742.0	989.4	865.7	61
2	1272.0	1007.0	759.7	583.0	583.0	371.0	70
3	1007.0	830.4	477.0	424.0	353.3	300.3	70
4	1766.8	530.0	353.3	176.7	176.7	123.7	93
5	1590.1	530.0	282.7	265.0	141.3	123.7	92
6	689.0	812.7	653.7	477.0	247.3	265.0	61
7	671.4	636.0	371.2	318.0	406.3	318.0	52
8	1943.4	1060.0	1007.0	229.7	477.0	335.7	82
9	547.8	441.7	300.3	247.3	212.0	159.0	71
10	1537.1	477.0	335.7	282.7	194.3	159.0	89
11	2226.1	618.4	1289.7	795.5	1007.6	883.4	60
12	1219.0	936.4	706.7	530.0	530.0	318.0	73
13	1060.0	883.4	477.3	530.0	406.3	353.3	66
14	653.7	777.4	618.4	441.7	212.0	229.7	64
15	706.7	618.4	406.3	300.3	371.0	318.0	55
16	1766.8	989.4	936.4	176.7	512.3	424.0	76
17	441.7	335.7	194.3	141.3	106.0	53.0	88
18	706.7	530.0	406.4	406.3	282.7	247.3	65
19	1537.1	1219/1	1272.8	1007.0	812.7	477.0	68
20	1007.0	989.4	636.0	742.0	742.0	618.3	38
Mean							70

TABLE V
THE CONCENTRATION OF FUNGAL (CFU/M^3) AFTER USING NAIS THROUGH 5 HOURS IN THE SAMPLE SITES

Number of houses	Time (hours) 0	1	2	3	4	5	Removal (%)
1	742.0	159.0	106.0	123.7	300.3	424.0	43
2	106.0	17.7	53.0	53.0	35.3	35.3	66
3	53.0	17.7	0	17.7	0	17.7	66
4	106.0	35.3	17.7	17.7	17.7	0	100
5	194.3	194.3	282.7	88.3	17.7	17.7	91
6	883.4	106.0	53.0	53.0	106.0	141.3	84
7	70.7	53.0	35.3	17.7	0	53.0	25
8	123.7	53.0	53.0	35.3	35.3	35.3	71
9	106.0	0	35.3	35.3	70.7	35.3	66
10	194.3	17.7	17.7	35.3	0	53.0	72
11	88.3	88.3	35.3	35.3	17.7	0	100
12	123.7	17.7	17.7	35.3	17.7	0	100
13	1766.7	547.7	618.4	848.0	618.4	795.0	55
14	971.7	406.7	371.0	194.3	123.7	123.7	87
15	883.4	318.0	424.0	406.4	265.0	212.0	76
16	653.7	742.0	335.7	335.7	88.3	88.3	86
17	88.3	0	0	35.3	17.7	0	100
18	106.0	88.3	35.3	35.3	17.7	17.7	83
19	88.3	70.7	70.7	35.3	17.7	17.7	80
20	194.3	123.7	106.0	106.0	53.0	35.3	82
Mean							76.6

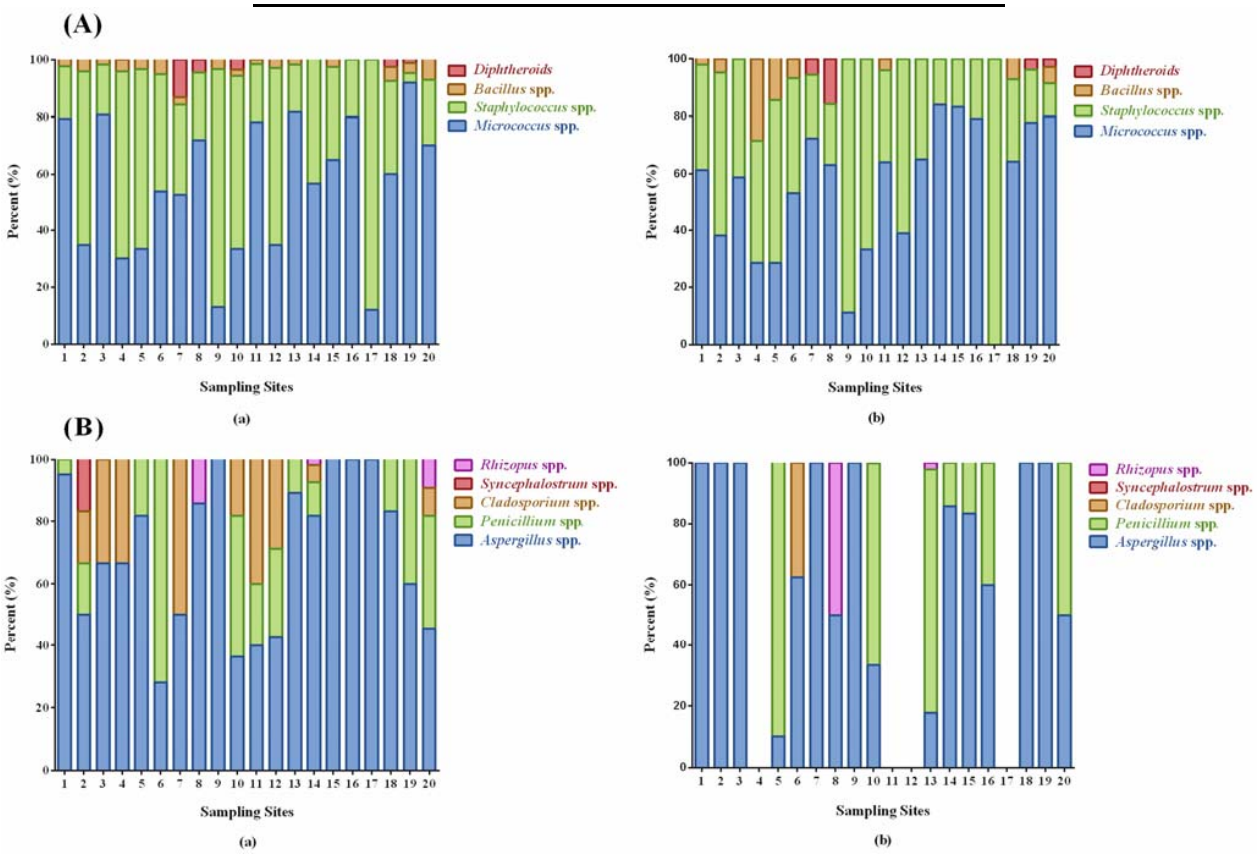

Fig. 6 Distribution of (A) bacterial and (B) fungal genera in indoor air in the different sample sites (a) before and (b) after using NAIs

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REFERENCES

[1] Crawford JA, Rosenbaum PF, Anagnost SE, Hunt A, Abraham JL. Indicators of airborne fungal concentrations in urban homes: understanding the conditions that affect indoor fungal exposures. Science of the total environment. 2015;517:113-24.
[2] Sarkhosh M, Mahvi AH, Zare MR, Fakhri Y, Shamsolahi HR. Indoor contaminants from hardcopy devices: characteristics of VOCs in photocopy centers. Atmospheric environment. 2012;63:307-12.
[3] Nasir ZA, Colbeck I, Sultan S, Ahmed S. Bioaerosols in residential micro-environments in low income countries: a case study from Pakistan. Environmental pollution. 2012;168:15-22.
[4] Srikanth P, Sudharsanam S, Steinberg R. Bio-aerosols in indoor environment: composition, health effects and analysis. Indian journal of medical microbiology. 2008;26(4):302.
[5] Gilbert Y, Duchaine C. Bioaerosols in industrial environments: a review. Journal of Environmental Engineering and Science. 2014;9(1):4-19.
[6] Després VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, et al. Primary biological aerosol particles in the atmosphere: a review. Tellus B. 2012;64.
[7] Van Leuken J, Swart A, Havelaar A, Van Pul A, Van der Hoek W, Heederik D. Atmospheric dispersion modelling of bioaerosols that are pathogenic to humans and livestock–A review to inform risk assessment studies. Microbial Risk Analysis. 2015.
[8] Wéry N. Bioaerosols from composting facilities—a review. Frontiers in cellular and infection microbiology. 2014;4.
[9] Aydogdu H, Asan A, Otkun MT. Indoor and outdoor airborne bacteria in child day-care centers in Edirne City (Turkey), seasonal distribution and influence of meteorological factors. Environmental monitoring and assessment. 2010;164(1-4):53-66.
[10] Bornehag C, Blomquist G, Gyntelberg F, Järvholm B, Malmberg P, Nordvall L, et al. Nordic interdisciplinary review of the scientific evidence on associations between exposure to'Dampness' in buildings and health effects. Indoor Air. 2001;13:72-86.
[11] Mendell MJ, Mirer AG, Cheung K, Tong M, Douwes J. Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. Environ Health Perspect. 2011;119(6):748-56.
[12] Burge H, Feeley J, Kreiss K, Milton D, Morey P, Otten J, et al., editors. Guidelines for the Assessment of Bioaerosols in the Indoor Environment. American Conference of Governmental Industrial Hygienists, Cincinnati, OH; 1989.
[13] Kowalski W. Aerobiological engineering handbook: airborne disease and control technologies: McGraw Hill Professional; 2005.
[14] Mentese S, Rad AY, Arısoy M, Güllü G. Seasonal and spatial variations of bioaerosols in indoor urban environments, Ankara, Turkey. Indoor and Built Environment. 2012;21(6):797-810.
[15] Ayoko GA, Morawska L, Kokot S, Gilbert D. Application of multicriteria decision making methods to air quality in the microenvironments of residential houses in Brisbane, Australia. Environmental science & technology. 2004;38(9):2609-16.
[16] Barnes CS, Dowling P, Van Osdol T, Portnoy J. Comparison of indoor fungal spore levels before and after professional home remediation. Annals of Allergy, Asthma & Immunology. 2007;98(3):262-8.
[17] Yun SJ, Seo Y. Removal of bacteria and odor gas by an alumina support catalyst and negative air ions. Journal of Aerosol Science. 2013;58:33-40.
[18] Oren I, Haddad N, Finkelstein R, Rowe JM. Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: before and after chemoprophylaxis and institution of HEPA filters. American journal of hematology. 2001;66(4):257-62.
[19] Cho MS, Ko HJ, Kim D, Kim KY. On-site application of air cleaner emitting plasma ion to reduce airborne contaminants in pig building. Atmospheric environment. 2012;63:276-81.
[20] Levetin E, Shaughnessy R, Rogers CA, Scheir R. Effectiveness of germicidal UV radiation for reducing fungal contamination within air-handling units. Applied and environmental microbiology. 2001;67(8):3712-5.
[21] Tyagi A, Malik A. Antimicrobial action of essential oil vapours and negative air ions against Pseudomonas fluorescens. International journal of food microbiology. 2010;143(3):205-10.
[22] Arnold L, Boothe D, Mitchell B. Use of negative air ionization for reducing bacterial pathogens and spores on stainless steel surfaces. The Journal of Applied Poultry Research. 2004;13(2):200-6.
[23] Climate-Data.Org. Available from: https://en.climate-data.org/location/956725/.
[24] Niazi S, Hassanvand MS, Mahvi AH, Nabizadeh R, Alimohammadi M, Nabavi S, et al. Assessment of bioaerosol contamination (bacteria and fungi) in the largest urban wastewater treatment plant in the Middle East. Environmental Science and Pollution Research. 2015;22(20):16014-21.
[25] Hassanvand MS, Naddafi K, Faridi S, Arhami M, Nabizadeh R, Sowlat MH, et al. Indoor/outdoor relationships of PM 10, PM 2.5, and PM 1 mass concentrations and their water-soluble ions in a retirement home and a school dormitory. Atmospheric Environment. 2014;82:375-82.
[26] Lee J-H, Jo W-K. Characteristics of indoor and outdoor bioaerosols at Korean high-rise apartment buildings. Environmental research. 2006;101(1):11-7.
[27] Hoseini M, Jabbari H, Naddafi K, Nabizadeh R, Rahbar M, Yunesian M, et al. Concentration and distribution characteristics of airborne fungi in indoor and outdoor air of Tehran subway stations. Aerobiologia. 2013;29(3):355-63.
[28] Naddafi K, Jabbari H, Hoseini M, Nabizadeh R, Rahbar M, Yunesian M. Investigation Of Indoor And Outdoor Air Bacterial Density In Tehran Subway System. Iranian Journal of Environmental Health Science & Engineering (IJEHSE). 2011;8(4).
[29] Andersen AA. New sampler for the collection, sizing, and enumeration of viable airborne particles. Journal of Bacteriology. 1958;76(5):471.
[30] Faridi S, Hassanvand MS, Naddafi K, Yunesian M, Nabizadeh R, Sowlat MH, et al. Indoor/outdoor relationships of bioaerosol concentrations in a retirement home and a school dormitory. Environmental Science and Pollution Research. 2015;22(11):8190-200.
[31] Gaunt L, Higgins S, Hughes J. Interaction of air ions and bactericidal vapours to control micro-organisms. Journal of applied microbiology. 2005;99(6):1324-9.
[32] Standard A. Ventilation for acceptable indoor air quality. Atlanta. 1989;62:1989.
[33] Burge H, Chew G, Muilenberg M, Gold D, editors. Role of fungi in-house dust ecosystems. Journal of Allergy and Clinical Immunology; 1995: Mosby-Year Book INC 11830 Westline Industrial DR, ST Louis, MO 63146-3318.
[34] Bonetta S, Bonetta S, Mosso S, Sampò S, Carraro E. Assessment of microbiological indoor air quality in an Italian office building equipped with an HVAC system. Environmental monitoring and assessment. 2010;161(1):473-83.
[35] Hussin NHM, Sann LM, Shamsudin MN, Hashim Z. Characterization of bacteria and fungi bioaerosol in the indoor air of selected primary schools in Malaysia. Indoor and Built Environment. 2011;20(6):607-17.
[36] Parat S, Perdrix A, Mann S, Baconnier P. Contribution of particle counting in assessment of exposure to airborne microorganisms. Atmospheric Environment. 1999;33(6):951-9.
[37] Hsu Y-C, Kung P-Y, Wu T-N, Shen Y-H. Characterization of indoor-air bioaerosols in Southern Taiwan. Aerosol Air Qual Res. 2012;12:651-61.
[38] Fang Z, Gong C, Ouyang Z, Liu P, Sun L, Wang X. Characteristic and concentration distribution of culturable airborne bacteria in residential environments in Beijing, China. Aerosol Air Qual Res. 2014;14(3):943-53.
[39] Pastuszka JS, Paw UKT, Lis DO, Wlazło A, Ulfig K. Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. Atmospheric Environment. 2000;34(22):3833-42.
[40] Mandal J, Brandl H. Bioaerosols in indoor environment-a review with special reference to residential and occupational locations. The Open Environmental & Biological Monitoring Journal. 2011;4(1).
[41] Shargawi J, Theaker E, Drucker D, MacFarlane T, Duxbury A. Sensitivity of Candida albicans to negative air ion streams. Journal of applied microbiology. 1999;87(6):889-97.
[42] Pratt R, Barnard RW. Some effects of ionized air on Penicillium notatum. Journal of Pharmaceutical Sciences. 1960;49(10):643-6.
[43] Tyagi AK, Malik A. Bactericidal action of lemon grass oil vapors and negative air ions. Innovative Food Science & Emerging Technologies. 2012;13:169-77.
[44] Cai K, Liu X, Xu Y, Ren C, Chen H, Xu J, et al. Damage effects induced by electrically generated negative air ions in Caenorhabditis elegans. Science of the total environment. 2008;401(1):176-83.
[45] Yu K-P, Lee GW-M, Lin S-Y, Huang CP. Removal of bioaerosols by the combination of a photocatalytic filter and negative air ions. Journal of Aerosol Science. 2008;39(5):377-92.

[46] Fletcher LA, Gaunt LF, Beggs CB, Shepherd SJ, Sleigh PA, Noakes CJ, et al. Bactericidal action of positive and negative ions in air. BMC microbiology. 2007;7(1):32.
[47] Fan L, Song J, Hildebrand P, Forney C. Interaction of ozone and negative air ions to control micro-organisms. Journal of applied microbiology. 2002;93(1):144-8.