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(RESEARCH ARTICLE)

Microbial pollution of indoor air in Riyadh city government schools

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Abstract

The presence of microorganisms in indoor air represents a dangerous issue with a major impact in the field of health protection and environmental engineering. Low indoor air quality is a major trouble in government schools because of the massive density of students per classroom, inadequate supply of air from outside, as well as weak construction and poor maintenance of school buildings. Evaluation of microbial contamination within the indoor air is important to assess health dangers and establish requirements for tracking indoor air quality. Evaluation of microbial contamination is of notable significance in intensively populated centers as government schools. A study was carried out among a hundred and twenty randomly classrooms of twenty government primary schools of Riyadh city in the academic year 2018/2019. For the determination of microbial pollution, passive air sampling settle plate technique was utilized by exposing a Petri dish of appropriate agar media for one hour. Results showed that the counts of bacteria and fungi were higher in government schools in low socioeconomic districts as compared to those of high socioeconomic districts. The indoor analyses reveal that the concentration of bacteria was higher than counts of fungi. In general, Gram positive microorganisms were the dominant organisms. Gram positive bacteria, *Bacillus*, *Micrococci* and *Staphylococci* were the predominant bacterial strains in the schools under study. Attention has to be paid for controlling the physical factors that assist the growth and reproduction of microbes in indoor air of classrooms to keep the students and teachers healthy.

Keywords: Air quality; Microbial contamination; Primary schools; Socioeconomic districts

1. Introduction

The quality of air in indoors has come to be a significant public health challenge as the general public spends more than ninety percent of their time in interior such as homes, workplaces and educational facilities. Indoor air may be polluted with one or more of the most important microorganism like bacteria and fungi. It was found that about 33% of the indoor air quality claims occurred by the microbial contamination [1] which cause allergy, respiratory and immune-toxic diseases [2]. A large number of studies were carried out on the microbial contaminants in varied indoors of educational establishments [3]. Millions of students are going every day to the educational facilities as schools. The high levels of student's activities led to indoor air pollution with bacteria and fungi. Children are more susceptible to indoor air pollutants than adults as they are exposed to unidentified amount of indoor air pollutants in school environments [4]. Schools are more danger than the other different buildings. This is attributed to the high density of students per classroom, bad sanitation and inadequate outdoor air supply. This danger is aggravated through frequent construction and upkeep of school buildings [5]. The concentration of microbial contamination of schools is a crucial factor because of its effect on the human mentality, physical up growth and students' performance.

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A study was carried out among a hundred and twenty randomly classrooms of twenty government primary schools of Riyadh city, Saudi Arabia in the academic year 2018/2019.There are 1883 public schools in Riyadh city, of which 713 are public schools (413 primary schools, 206 middle schools, and 94 secondary schools) and the rest are private schools. Therefore, the aim of the present investigation is to estimate the indoor air pollution in some government primary schools of Riyadh city in two different socioeconomic districts to upgrade knowledge and offer recommendations for better understanding of bacterial content of indoor air quality issues in such type of schools.

2. Material and methods

A study was carried out to evaluate indoor microbial pollutants and its effect on indoor air quality of government primary schools in Riyadh, capital of Saudi Arabia.

2.1. Sample size and sampling procedures

The study was carried out in randomly one hundred twenty classroom of 20 primary government schools of Riyadh city in the academic year 2018/2019, out of those ten schools are located in low socioeconomic districts (there is no ventilation or air condition) and other ten schools are located in high socioeconomic districts (there are 2 air condition in each classroom). The schools located in low socioeconomic districts were built since 30 years ago while, the schools in the high socioeconomic districts were built since only 12 years ago. In each school six classes were used to collect samples during April 2019. Samples were taken five days a week during the studied month. Saudi Arabia has an arid continental climate. The temperature in April ranged between 20.4-33.4°C and the relative humidity in the same month was 28%.

Total counts of bacteria and fungi were determined using the passive air sampling technique; the settle plate method using open Petri-dishes containing different culture media. Microbial contamination assay based totally on the quantity and type of microbial fall directly on Petri dishes left open to the air. The scheme of 1/1/1 (for 1 h, 1m faraway from the ground, at the least 1m far from partitions or any impediment) was used according to [6].

To detect the microbial count with take in consideration the environmental variation, the medium containing petri plates have been exposed at sampling classrooms an hour after the school day ends at 15:00 in the afternoon (Sunday via Thursday). To minimize dilution of air contaminants, doorways and class windows had been closed. Also, during sampling the human motion was restrained to let the air steady and prevent newly fall organisms. Nutrient agar (NA) and Potato dextrose agar (PDA) provided with 10 mg/L chloramphenicol were used for the cultivation of bacteria and fungi, respectively. Three replicates of each of nutrient agar and potato dextrose agar have been used. Nutrient agar plates were left at 37°C for forty-eight hours to let aerobic mesophilic bacteria to grow. Potato dextrose agar dishes have been incubated for 5 days at 25°C to allow the growth of fungal colonies. The mean of colony forming units (CFU) of each of bacteria and fungi turned into calculated and converted to organisms per cubic meter of air (CFU m^{-3}) applying the equation of. [7].

$$N = a * 10000/b * t * 0.2$$

Where N: Microbial CFU/m^3 of indoor air; a: Number of colonies per Petri dish; b: Dish surface area (cm^2); t: Exposure time.

Bacterial colonies had been characterized at the beginning with the aid of morphology and microscopic look, and recognized further via biochemical assessments as mentioned before by Bergey's Manual of Systematic Bacteriology [8]. Each fungal colony was moistly prepared using Lacto phenol cotton- blue solution and examined microscopically. Identification of fungi was done depending on growth colonial appearance, microscopic examination of the spore and hypha traits of the stained preparations [9]. Along with microbial sample collection, indoor temperature, and relative humidity had been measured.

2.2. Statistical analysis

The received data have been analyzed statistically applying the general linear version technique of [10]. Mean comparisons had been made with an F-protected LSD at $P < 0.05$.

3. Results and discussion

Results in Table 1 showed that most of the government schools under study have high indoor microbial population counts. The total bacterial counts ranged from 396-3589 and 712-5760 CFU m^{-3} in the schools of high and low socioeconomic districts, respectively. The overall indoor air bacterial loads were 1881.2 and 3073.6 CFU m^{-3} in the schools of high and low socioeconomic districts, respectively (Table 1). Potato dextrose agar (PDA) petri plates showed heavy fungal growth when exposed to indoor air in government primary schools of both high and low socioeconomic districts of all sampled schools. The fungal counts in primary government schools were 127-978 and 198-1326 CFU m^{-3} in high and low socioeconomic districts, in respective order (Table 1). The overall indoor air fungal loads were 534.7 and 791.4 CFU m^{-3} in the schools of high and low socioeconomic districts, respectively (Table 1).

It was obvious that the indoor air bacterial counts in the schools of low standard level districts were significantly higher than indoor air bacterial counts recorded in the schools of high socioeconomic districts Table 1. This is may be due to the absence of ventilation in such types of schools beside they were built since more than 30 years ago, while the schools of high socioeconomic districts have good ventilation and 2 air conditions per classroom and it were built since only 12 years. The variations of the fungal concentration between schools and the level of districts were followed the same trend of the indoor air bacterial concentration. The bacterial concentration was high in all primary schools than fungi regardless the school level and the other environmental factors.

Table 1 Average concentration of total indoor airborne bacteria and fungi (CFU m^{-3}) in the different government primary schools of high and low socioeconomic districts

Schools	**Total airborne bacteria**		**Total airborne fungi**	
	High socioeconomic district	**Low socioeconomic district**	**High socioeconomic district**	**Low socioeconomic district**
1	615	912	187	275
2	997	2115	312	598
3	396	712	127	198
4	2840	5760	826	1215
5	3015	4120	865	1045
6	3589	5085	978	1326
7	1180	2190	322	622
8	2145	3520	605	904
9	2670	3612	760	966
10	1365	2710	365	765
Mean	1881.2	3073.6	534.7	791.4
LSD at 5% for different schools	2321.5		617.5	
LSD at 5% for different districts	612.7		134.8	

CFU colony-forming unit

One of the most vital investigations to assess indoor air pollution with different microbes is the microbiological quality assessment. Information on the indoor air content of bacteria and fungi is necessary to assess health risks and to establish standards for controlling indoor air quality. The total airborne bacteria and fungi of indoor air environments of government primary schools in Riyadh city was found in the range between 396-3589 and 712-5760 CFU m^{-3} in the schools of high and low socioeconomic districts, respectively. These results were higher than the findings of other

studies [11]. Bacterial load of indoor air might be correlated with indoor temperature. This means that the density of aerosols will increase when the temperature increase. These results are in line with those recorded by [12, 13, 14]. The difference among the varied government schools on high and low socioeconomic districts might be because of the reality that different environmental factors increase the microbial contamination in school rooms and also the density of school students might also bring about a major biodiversity of high indoor air bacterial load. [15] got the same finding of the present study. The indoor air microbial loads of different buildings are related to the surrounding environmental conditions. Humidity is the health risk indicator for indoor air pollutants as reported by [16]. Therefore, to improve indoor air quality, congestion should be avoided and good ventilation systems designed. The most vital approach for heading off adverse health outcomes is the prevention (or minimization) of persistent humidity and microbial growth on interior surfaces and in schools constructing systems.

Facilities such as good ventilation, daily cleaning of classrooms, and increased student density in classes are responsible for increasing the microbial content of indoor air of schools [17] and [18]. The movement of outdoor air leads to the accumulation of gaseous emissions that enter the classrooms depending on the direction of the wind [19]. This leads to a decrease in the quality of indoor air of the classrooms.

During physical parameter measurement, it was found that all examined classrooms did not have an HVAC (heating, ventilation, and air conditioning) system in the government schools of low socioeconomic districts. However, there were ventilation system and air conditions in each classroom in the schools of high socioeconomic districts. The temperature in April ranged between 20.4-33.4°C and the relative humidity in the same month was 28%. Previous studies have shown that the environmental temperature affects the survival of microorganisms in the air [20] The survivability pattern of airborne bacteria in present study shows that temperature and humidity had effect on survival of most of these airborne bacteria. Since the group of Gram Positive Bacilli and cocci which have mechanism to resist the desiccation factors they were prevalent in all schools under study.

3.1. Identification of bacteria and fungi species

Three bacterial species were isolated and identified. They were *Bacillus* species, *Micrococcus* species and *Staphylococcus* species. *Bacillus* species was predominant in most government primary schools represented 85% of total bacteria presented in indoor air of schools under study (Table 2). Identification of microorganisms is important issue from the ecological and health point of view. Three bacterial species were isolated and identified. They were *Bacillus* species, *Micrococcus* species and *Staphylococcus* species. *Bacillus* species was predominant in most government primary schools. The predominant bacterial flora was Gram positive bacteria. Bacillus species are the most dominant Gram positive bacteria. Bacillus is aerobic saprophytic, endospore formers and extensively disbursed within the atmosphere [21]. However, cocci bacteria are normal flora of skin and mucous membranes in human and mammals [22].

In the present study about 85 % of the bacteria isolated were Bacillus species which caused many of human diseases. On the hand, about 60 % of bacteria isolated were Gram positive cocci belonging to saprophytic micro flora generally associated to human skin and mucosa which can be dispersed through droplets or skin peeling and maintained in air [7] observed a significant increased concentration of bacteria in afternoon as compared to morning in various rooms of a university [23] found the same result, concluding that bacterial contamination into indoor air derives from human presence. The association of airborne microbes and human activity has also been reported by many studies. [17] recorded highest level of bacteriological contamination in corridor and rooms during lessons in a school.

Gram positive bacteria, despite their abundance in the environment are less harmful; however, they have potential to be opportunistic pathogens [24-25]. The finding of isolated bacterial species of the present study partly agrees with the work by [26-27]. It was also harmonized in a study conducted in India [28].

Fungal flora isolated from indoor air of sampled schools showed dominant species like *Aspergillus* species, *Penicillium* species and *Alternaria* species (Table 2). The prevailing species *Aspergillus* found in about 60% of total fungal count and detected in indoor air of sampled schools followed by *Penicillium* species (about 50%) and *Alternaria* species (about 30%), respectively (Table 2). Fungal flora isolated from indoor air of sampled schools showed dominant species like *Aspergillus* species, *Penicillium* species and *Alternaria* species. The prevailing species was *Aspergillus* followed by *Penicillium* and *Alternaria*, respectively (Table 2). *Aspergillus, Penicillium, Alternaria,* and *Cladosporium* are the dominant fungal flora all over the world; because they can grow in numerous habitats in diverse ways [29]. According to some previous studies the microbiological quality of indoor air is highly influenced by the microbiological composition of outdoor air, which very much influenced by environment, season, the weather and even daytime. In the present study *Aspergillus* sp. and *Alternaria* sp. as outdoor molds occurred in a large number in schools under study. This result provided the evidence that high concentration of fungi in atmosphere can influence microbiological indoor

air contamination. [30] also noticed that most commonly occurred molds in Norwich schools belonging to the genera: *Penicillium*, *Aspergillus*, *Cladosporium* and *Mucor*. Fungal flora of the indoor air of school room under study was dominated by *Aspergillus* sp. The present results are in agreement with those of [31] who found the same results in other indoor setting.

Table 2 Types of microorganism isolated from each school in high and low socioeconomic district in Riyadh city

Schools	**Bacteria**			**Fungi**		
	***Bacillus* sp.**	***Staphylococcus* sp.**	***Micrococcus* sp.**	***Aspergillus* sp.**	***Penicillium* sp.**	***Alternaria* sp.**
High socioeconomic district						
School 1	−	−	+	+	−	−
School 2	+	−	+	−	+	
School 3	+	−	−	+	−	+
School 4	+	+	+	−	−	−
School 5	+	+	+	+	+	−
School 6	+	−	+	+	+	−
School 7	+	−	−	+	−	+
School 8	−	+	+	−	−	+
School 9	+	−	−	−	+	−
School 10	+	+	−	+	+	−
Total	**8**	**4**	**6**	**6**	**5**	**3**
Low socioeconomic district						
School 1	+	−	−	+	+	−
School 2	+	+	+	−	−	−
School 3	+	−	−	+	+	+
School 4	+	+	+	+	+	+
School 5	+	+	+	+	−	−
School 6	+	−	+	−	−	−
School 7	−	+	+	+	+	+
School 8	+	−	−	+	+	+
School 9	+	−	−	+	−	−
School 10	+	+	+	−	+	−
Total	**9**	**5**	**6**	**7**	**6**	**4**

CFU colony-forming unit

3.2. Indoor air quality of government schools

Based on the European sanitary requirements for non- industrial premises, the degree of air pollution via bacteria population in the studied schools ranges among high to extremely high (Table 3). There are not any regularly values regarding concentrations of the air of indoor bacteria, and the obtain results from the present study may be as compared simplest with the values endorsed with the aid of numerous authors or scientific establishments.

Table 3 Bacterial air quality assessments in selected government primary schools in high and low socioeconomic districts in Riyadh, according to the health requirements of non-industrial buildings

Degree of Air Pollution and Range of values (CFU m^{-3})	**Schools**									
	1	2	3	4	5	6	7	8	9	10
High socioeconomic district										
Very low (< 50)	—	—	—	—	—	—	—	—	—	—
Low (50 – 100)	—	—	—	—	—	—	—	—	—	—
Intermediate (100–500)	—	—	▲	—	—	—	—	—	—	—
High (500 -2000)	▲	▲	—	—	—	—	▲	—	—	▲
Very High (>2000)	—	—	—	▲	▲	▲	—	▲	▲	—
Low socioeconomic district										
Very low (< 50)	—	—	—	—	—	—	—	—	—	—
Low (50 – 100)	—	—	—	—	—	—	—	—	—	—
Intermediate (100–500)	—	—	—	—	—	—	—	—	—	—
High (500 -2000)	▲	—	▲	—	—	—	—	—	—	—
Very High (>2000)	—	▲	—	▲	▲	▲	▲	▲	▲	▲

CFU colony-forming unit

The work conducted by a WHO expert group on assessment of health risks of biological agents in indoor environments suggested that total microbial concentration should not exceed 1000 CFU m^{-3} [32], whereas other scholars considered that 750 CFU m^{-3} should be the limit for bacteria [33]. Airborne microbial concentrations ranging from 4500 to 10,000 CFU m^{-3} also have been suggested as the upper limit for ubiquitous bacterial aerosols [34]. Based on the European sanitary requirements for non- industrial premises, the degree of air pollution via bacteria population in the primary schools under study ranges among high to extremely high polluted. The permissible limits of bacterial load were recorded as ≤ 500 CFU m^{-3} based on the sanitary standards of the European Commission for non- industrial premises [35]. The bacterial load in indoor education establishments is varied according to environmental factors such as temperature, humidity, and particulate matter concentration. In the present study, however the concentration of bacteria and fungi, had been surpassed the mentioned above value. Therefore, it is of importance to address microbial air pollution in these educational facilities as well as to expand related standards. It was found that more than 14 m^3 of air is daily inhaled by a human adult [36] leads to the conclusion that the airborne microbial intake per day of the occupants of the analyzed building might likely exceed by at least 14-fold the average number of microorganisms expressed above. The control of the microbial load of the surrounding air is thus important to establish the quality and health conditions of the services rendered by any public institution.

4. Conclusion

An excessive indoor air bacterial load was recorded in government primary schools in Riyadh metropolis in comparison with various indoor air biological requirements specification. *Bacillus, Micrococcus* and *Staphylococcus* had been the dominant isolated bacterial species. It should be taken in consideration to controlling the physical factors that support the growth and reproduction of bacteria in the internal environment of the classrooms to maintain the health of students and teachers. The microbial air quality of public facilities is a crucial factor in determining the extent of risk to human health. Undoubtedly, bacterial and fungal content varied with regard to the pupil activity, occupancy density, and ventilation. Government primary schools in Riyadh city recorded the worst quality of the microbiological air, as these

educational facilities depend on natural ventilation in addition to the high student density. The indoor air microbial concentration is higher in the government schools, indicating suspected indoor microbial sources. In public instructional facilities, students, teachers and all labors are exposed to excessive microbial contamination. There is an urgent need to establish rules governing the high limits of the different types of airborne microorganisms for all public educational institutions.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have read and approved to submit it to World Journal of Advanced Research and Reviews. There is no conflict of interest of any author in relation to the submission.

Statement of informed consent

This study was carried out in some government schools in Riyadh city according to a permission given by schools' managers from ministry of Education.

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