Identification of Bacteria and Fungi Contamination of Some Classrooms at Srinakharinwirot University, Ongkharak Campus

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ABSTRACT— ***The air quality of air inside the classroom is an important factor effect to health of people. The objective of the study was to estimation the air quality and identification of bacteria and fungi contamination in the air inside the classroom at Srinakharinwirot University Ongkharak Campus. The open plate technique was used to collect samples in the morning at 8.30 a.m. (before students enter to the classroom) and in the afternoon at 4.30 p.m. (after students left the classroom) between November 2018 to April 2019. The result showed that the average amounts of bacteria and fungi were 67.60 to 352.50 and 69.60 to 370.80 CFU/dm^2/h, respectively. The evaluations of the index of microbial air contamination (IMA) were fair to very poor. However, the concentrations of airborne bacteria and fungi were not higher than the proposed air quality index. Therefore, the air quality of classrooms had a good hygienic standard. The most isolated bacteria were Enterobacter spp., Escherichia coli, Staphylococcus spp., Bacillus spp., and Pseudomonas spp. and fungi were found to include Cladosporium spp., Aspergillus spp., Penicillium citrinum and Neurospora crassa. So, the air quality of air inside classroom control management is appropriate to be used to reduce bacteria and fungi contamination in the classroom.***

Keywords— microorganism in the air, indoor air quality, classroom, contamination

1. INTRODUCTION

Indoor air quality is an important factor affect the health of people concern as most people spend more than 90% of their life indoors, in houses, offices buildings and classrooms [1, 2]. Indoor air pollution is associated with inadequate building environments, including building materials, air conditioning systems, ventilation rates, and human factors, such as overcrowding in constrained spaces [3, 4, 5].

One of the factors to the effect of indoor air quality is the contamination of microorganism which includes bacteria, fungi and viruses. Moreover, A high concentration of microorganisms in the air can be the allergenic disease. The most isolated airborne bacteria and fungi found the contamination in laboratory rooms, libraries, hospitals and schools such as *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Serratia* spp., *Penicillium* spp., *Aspergillus* spp., *Rhizopus* spp., *Cladosporium* spp., *Fusarium* spp. and *Curvularia* spp. which these microorganisms associate with allergy and asthma [6, 7, 8, 9].

The objective of the study was to estimation the air quality and identification of bacteria and fungi contamination in the air inside the classroom at Srinakharinwirot University, Ongkharak Campus.

2. METHODOLOGY

Sample Collections

Airborne bacteria and fungi were taken in the classroom at Srinakharinwirot University Ongkharak Campus. This study included a total of five hundred three seventy samples of 38 classrooms were collected including 5 faculty using the open plate technique. In this technique, standard 90 mm diameter Petri dishes containing 20 ml. of plate count agar (PCA) and Potato dextrose agar (PDA) to collect bacteria and fungi, respectively. The both plate were left open to air and placed at two positions in each classroom. The sampling height was 1 m above the floor and 1 m away from the wall for 60 min. The samples were collected twice a day at in the morning at 8.30 a.m. (before students enter to the classroom) and in the afternoon at 4.30 p.m. (after students left the classroom) between November 2018 to April 2019. After exposure, the sample was taken to the laboratory (Department of Health Promotion, Faculty of Physical Therapy, Srinakharinwirot University).

Microbiological Assessed

The samples were incubated at 37ºC at 48 h for bacteria and at 25ºC at 5 to 7 days for fungi. The amount of bacteria and fungi colonies were counted and calculated the number of colony forming unit (CFU)/plate/h. and calculated the number of CFU/dm^2/h to compare with the index of microbial air contamination classes (IMA class) [11] (Table 1).

Table 1: The index of microbial air contamination

IMA value	CFU/dm^2/h	Class
0-5	0-9	very good
6-25	10-39	good
26-50	40-84	fair
51-75	85-124	poor
≥76	≥125	very poor

Isolation and identification

Bacterial isolates were characterized and identified using cultural, morphological and microscopic examinations. Different biochemical tests such as Gram staining, Catalase, Coagulase, DNase, Methyl-red, Oxidase, Voges-proskauer and sugar fermentation test were employed to differentiate the bacterial isolates [12].

Fungi colonies were identified using standard microbiological procedures based on their colony appearance, microscopic examination of their spores and hypal characteristics using lactophenol cotton blue preparation [13].

Statistical analysis

Simple percentage was used to express the frequency of occurrence of bacterial and fungi isolates where necessary.

3. RESULTS AND DISCUSSION

The average amounts of airborne bacteria and fungi were 67.6-352.5 CFU/dm^2/h (Table 2) and 69.6-370.8 CFU/dm^2/h (Table 3), respectively. The evaluations of the Index of IMA of airborne bacteria and fungi in all laboratories were fair to very poor. Those concentrations of airborne bacteria and fungi compare with air quality index of Malaysia [14] were not exceed 500 CFU/m^3 for bacteria and 1000 CFU/m^3 for fungi which as the good quality air. The comparison of the number of airborne bacteria and fungi in during time and between the faculty was no statistically significant difference (p>0.05) (Table 2 and 3).

Table 2: Amount of airborne bacteria of classroom at Srinakharinwirot University, Ongkharak Campus.

	Airborne bacteria						
Investigated room	Sampling time	Mean (colony)	CFU/dm^2/h	IMA	Air quality index (≥500 CFU/m^3)*	During time (p-value)	Between faculty (p-value)
PT	8.30 a.m.	57.70	90.70	poor	Pass	0.667	0.29
	4.30 p.m.	43.30	68.00	fair	Pass		0.217
NS	8.30 a.m.	171.30	340.50	vary poor	Pass	0.499	0.29
	4.30 p.m.	180.90	194.40	vary poor	Pass		0.217
PE	8.30 a.m.	43.10	67.60	fair	Pass	0.655	0.29
	4.30 p.m.	73.30	114.70	poor	Pass		0.217
EN	8.30 a.m.	175.70	276.10	vary poor	Pass	0.926	0.29
	4.30 p.m.	184.30	289.60	vary poor	Pass		0.217
LT	8.30 a.m.	216.70	352.50	vary poor	Pass	0.499	0.29
	4.30 p.m.	123.70	194.40	vary poor	Pass		0.217

*air pollution index of Malaysia (industry code of practice on indoor air quality 2010)

Table 3: Amount of airborne fungi of classroom at Srinakharinwirot University, Ongkharak Campus.

Airborne fungi							
Sampling locations	**Sampling time**	**Mean (colony)**	CFU/dm^2/h	IMA	Air quality index (≥1000 CFU/m^3)*	During time (p-value)	Between faculty (p-value)
PT	8.30 a.m.	236.0	370.80	vary poor	Pass	0.232	0.721
	4.30 p.m.	44.30	69.60	fair	Pass		0.410
NS	8.30 a.m.	151.30	238.40	vary poor	Pass	0.635	0.721
	4.30 p.m.	118.30	185.90	vary poor	Pass		0.410
PE	8.30 a.m.	179.00	281.30	vary poor	Pass	0.121	0.721
	4.30 p.m.	62.30	97.90	poor	Pass		0.410
EN	8.30 a.m.	125.70	197.50	vary poor	Pass	0.377	0.721
	4.30 p.m.	60.30	94.80	poor	Pass		0.410
LT	8.30 a.m.	210.70	340.50	vary poor	Pass	0.499	0.721
	4.30 p.m.	123.70	194.40	vary poor	Pass		0.410

*air pollution index of Malaysia (industry code of practice on indoor air quality 2010)

Attempts were performed to identify the bacteria from the colonies more often isolated from sampling sites in the third seasons are represented on Table 4. Over four five hundred and seventy-six isolated colonies were selected at random and biochemically characterized to establish an overall taxonomic classification of the airborne bacteria at the level of genus. The most isolated bacteria were *Enterobacter* spp., *Escherichia coli*, *Staphylococcus* spp., *Bacillus* spp., and *Pseudomonas* spp. to a lesser extent to *Stenotrophomonas* spp., *Proteus penneri*, *Acinetobacter* spp. and *Micrococcus* spp. *Enterobacter* was also occasionally isolated as a representative member of the enterobacteriaceae family. The above results reveal that the bacteria most often isolated are gram-positive cocci belonging to saprophytic microflora generally associated to human skin and mucosa, thereby suggesting that the main bacterial contamination suspended into the indoor air derives from human presence.

The frequency of fungi species isolated from the sampled classroom. The most isolated of the fungal is as follows; *Cladosporium* spp., *Aspergillus* spp., *Penicillium citrinum* and *Neurospora crassa*. to a lesser extent to *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Curvularia shahidchamranensis*, *Curvularia lunata*, *Fusarium* spp., *Penicillium citrinum*, *Penicillium roqueforti*, *Penicillium* spp. and *Rhizopus* spp. As shown in Table 4, *Cladosporium* spp. (17.65%), *Aspergillus* spp. (16.10%), *Penicillium citrinum* (13.10%) and *Neurospora crassa*. (12.07%) were the most abundant fungi genera in the third seasons.

The concentrations of airborne bacteria and fungi in the contamination in the air inside classroom at Srinakharinwirot University Ongkharak campus. were not higher than proposed air quality index (≥500 CFU/m^3 for bacteria and ≥1000 CFU/m^3 for fungi) [14]. The result showed that all classrooms had good hygienic standard. Moreover, the amount of airborne bacteria and fungi were not depending on the period of time and type of classrooms. The microbial isolates included three bacteria (*Micrococcus* spp., Gram negative bacilli (non-pathogenic bacteria) and *Bacillus* spp.) [1, 15] and four fungi (*Penicillium* spp., *Curvularia* spp., *Rhizopus* spp. and *Cladosporium* spp.) [16]. The data were according to several reports that demonstrated these microbial were isolated in laboratory and indoor environment. The most isolated bacteria were *Enterobacter* spp. that was also occasionally isolated as a representative member of the enterobacteriaceae family. The *Cladosporium* spp. and *Aspergillus* spp. were recognized as opportunistic pathogens for humans and often associated with allergy and asthma [17, 18].

Table 4: Observation frequency of bioaerosols (%).

Bacteria specie/genus	Rainy season		Cool season		Hot season		Total	Frequency (%)
	8.30 a.m.	4.30 p.m.	8.30 a.m.	4.30 p.m.	8.30 a.m.	4.30 p.m.		
1.Enterobacter spp.	43	22	17	8	13	8	111	19.27
2.Escherichia coli	35	24	12	8	8	6	93	16.15
3.Staphylococcus spp.	28	17	12	6	18	8	89	15.45
4.Bacillus spp.	22	13	18	12	10	4	79	13.72
5.Pseudomonas spp.	17	15	9	6	17	9	73	12.67
6.Stenotrophomonas spp.	7	4	12	19	1	1	44	7.64
7.Proteus penneri	6	6	8	15	2	1	38	6.60
8.Acinetobacter spp.	7	4	3	2	5	6	27	4.69
9.Micrococcus spp.	6	4	4	5	2	1	22	3.82
Fungi specie/genus	Rainy season		Cool season		Hot season		Total	Frequency (%)
	8.30 a.m.	4.30 p.m.	8.30 a.m.	4.30 p.m.	8.30 a.m.	4.30 p.m.		
1.Aspergillus spp.	9	3	6	7	19	8	52	16.10
2.Aspergillus flavus	4	1	2	2	13	4	26	8.05
3.Aspergillus niger	2	5	9	4	2	-	22	6.81
4.Candida albicans	1	2	2	3	2	1	11	3.41
5.Cladosporium spp.	4	2	18	13	13	7	57	17.65
6.Curvularia shahidchamranensis	2	2	6	4	2	2	18	5.57
7.Curvularia lunata	2	2	3	1	7	2	17	5.26
8.Fusarium spp.	2	1	1	3	6	4	17	5.26
9.Neurospora crassa	3	4	9	6	8	9	39	12.07
10.Penicillium citrinum	3	1	8	5	16	9	42	13.00
11.Penicillium roqueforti	2	1	4	2	7	5	21	6.50
12.Penicillium spp.	3	1	3	1	1	5	14	4.33
13.Rhizopus spp.	4	2	1	1	5	-	13	4.02

4. CONCLUSION

The present work was among the very few studies evaluating of bacteria and fungi contamination in the air inside classroom at Srinakharinwirot University. Moreover, we assessed the hourly and season variations of the air inside classroom. Almost all the classroom at Srinakharinwirot University were heavily contaminated with bacteria and fungi. Thus, attention must be given to control those environmental factors which favor the growth and multiplication of microbes in indoor environment of classroom to safeguard health of users and workers. However, Air conditioner should be maintained regularly, cleaning and disinfected daily.

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