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EFFICACY ASSESSMENT OF PORTABLE AIR-PURIFIER FOR REGULATING MICROBIAL CONTAMINATION IN TWO KINDERGARTENS

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Abstract

The microbial contamination in the indoor-air is a potential source of infection from the health point of view. Thus, accurate assessment of microbe present indoor is needed for estimating associated health risk and to establish standards for indoor air quality. Thus, it is essential to investigate the indoor-air of kindergartens. In the current study, the microbial contamination level was estimated under specific conditions in the two kindergartens. In which, the concentration of bacteria and fungi in both the kindergartens were in the range of 1178-1307 CFU/m^3 and 136-345 CFU/m^3 (without air purifier) respectively. Whereas, on the exposure of air purifier value decreases to the range, 316-417 CFU/m^3 and 93-269 CFU/m^3 for bacteria and fungi respectively. The distinct colonies of bacteria and fungi were identified by amplifying it with universal primers (8F and 1492R for bacteria and ITS1 and ITS for fungi). The species identified were *Bacillus cereus, Bacillus licheniformis, Bacillus pumilus, Bacillus safensis, Exiguobacterium aurantiacum*, *Staphylococcus hominis*, *Aspergillus flavus, Aspergillus oryzae* and *Curvularia hawaiiensis.* Single sample t-Test reveals the ($p \leq 0.05$) significant difference between the concentration of microbes before and after the exposure portable air purifier.

***Keywords*:** Indoor-Air Microbial Contamination, Bacteria, Fungi, Kindergarten, 16S rRNA

Introduction

Daily humans spend their 90% of the time inside the building due to which indoor-air quality assessment has emerged as a matter of concern worldwide (Nguyen *et al.,* 2014). The pollutant of chemical and biological origin retaining in the air is the potential source of infection, allergen and toxin that has the potential of health hazard (Li *et al.,* 2003; Matyasovszky *et al.,* 2011). Bacterial cells have been reported to synthesize endotoxins which act as a potent allergen which results in respiratory infection, whereas its toxic effects result in complications like asthma, enteric fever and malaise (Deng *et al.,* 2016). The few reasons to assess the indoor-air of kindergarten are stated as follow: children spend the 24hr/week of their time in kindergarten, so it is prominent to assess the quality of the air (Madureira *et al.,* 2015). The environmental conditions play a crucial role in their growth and development as they are insensitive age group and have an immature immune system. Thus, this matter is of great importance in the educational system (Mainka and Zajusz-Zubek, 2015).

The current study was conducted to assess the efficacy of the Ozone air purifier in regulating the microbial contamination in the indoor-air of kindergartens. We also made an attempt the species persisting in the air.

Material and Methods

Experimental design and area of study: The experiment was conducted to evaluate the role of ozone air-purifier on the microbial contamination in the two kindergartens which were on the outskirt of the Moga (K1) and Ferozepur (K2) district, Punjab. This assessment was done twice (1st and 4th Week) in March, 2019. General characterization of two kindergartens, the age of the students was 2-3 years; the number of students was 17(K1) and 22(K2). The size of the K1 room was 20 x 14 x 12 feet and K2was 10 x 10 x 10 feet. In which K2 was having the air-conditioner as well as heater facility, on the other hand, K1 was having the ceiling fan and air-cooler installed. For maintaining the hygienic condition, K2 uses the vacuum cleaner as they have synthetic carpet flooring whereas K1 uses the moping with cleaning agent because of tiled flooring. The two students from K2 were found to have the allergy from dust, perfume, smoke and iron according to information provided by kindergarten authority.

Sample collection: The sampling was done four times a day, at 6:30-7:30 AM (Opening time, without gathering & KENT ozone air purifier), 8:30-9:30 AM (After the exposure of 1h of KENT ozone air purifier without gathering), 10:30-11:30 AM (After gathering & without KENT ozone air purifier) and 12:30-1:30 PM (After the exposure of 1h of KENT ozone air purifier with gathering). For evaluating, the bacterial and fungal load in both the kindergartens, the sediment techniques using 1/1/1 scheme of sampling (in

duplicate) was applied using nutrient agar (HiMedia) and potato dextrose agar (HiMedia) (Pasquarella *et al.,* 2000). After the collection of the sample, plates were inoculated at 37°C and 28°C for 24h and 96 h respectively. After incubation, the isolated colonies of bacteria and fungus were counted and characterized on the basis of morphology and staining. The total numbers of colonies on both plates were counted, and the following formula was applied for calculating the CFU/m^3 (Fekadu and Getachewu, 2015):

$$N = \frac{5a \times 10^{-4}}{bt}$$

Where, N-microbial CFU/m^3 of indoor air; a-number of colonies per Petri dish; b-dish surface (cm^2); and t-exposure time.

Molecular characterization of the pure culture of bacteria and fungi: The distinct bacterial and fungal colonies were selected based on their morphology and were cultured to obtain pure cultures. The pure bacterial cultures were cultured in nutrient broth and fungal cultures in potato dextrose broth. After measuring, the viable count of bacterial and fungal pure culture. The DNA of each sample was extracted according to the instruction manual provided with the kit (HiPurA™ Bacterial (MB505) and Fungal DNA (MB543) Purification Kit). After the isolation of the genomic DNA, the purity of the DNA was assessed by examining the absorbance at 230, 260, and 280. After assessing the purity of genomic DNA, it was amplified using Hi-PCR Kit (MBT075). The 16s rRNA, forward primer 8F (AGAGTTTGATCCTGGCTCAG) and reverse primer 1492R (GGTTACCTTGTTACGACTT) for bacteria whereas 18s rRNA primers, forward primer ITS1 (TCCGTAGGTGAACCTGCGG) and reverse primer ITS4 (TCCTCCGCTTATTGATATGC) for the fungal sample. The PCR reaction was optimized, and the reaction was programmed as follows: Initial denaturation at 95°C for 10min, 30 cycles of denaturation at 95°C for 30sec, annealing at 60°C for 30sec, elongation at 72°C for 45sec, with final elongation at 72°C for 10min. After that, the amplified DNA bands were visualized on the 1% agarose gel. The amplified samples were then sent to the third party (Gujarat Genomics Initiative) along with the 10µl of each bacterial primer and 5 µl of each fungal primer, for sequencing of the amplified DNA.

Statistical analysis: The statistical analysis was done on the data obtained such as mean, standard deviation, geometric mean, and to interpret the significance of the data. One sample t-test was applied using 500 CFU/m^3 for bacteria and 300 CFU/m^3 for fungi as a standard (Pitt *et al.,* 2007). These standards were taken because no standard value is available for indoor-air microbial contamination.

Results and Discussion

On analysis, the range of CFU/m^3 of both bacteria and fungi distributed among the two kindergartens with/without the use air-purifier at different time interval was estimated and illustrated in Fig. 1. The concentration of the bacteria was found to be high as compared to fungi. As the sampling was done in March (summer), low count of fungus due to unfavourable conditions for proliferation as the temperature was ranging 30-37°C and humidity was ranging 15-18%. The selected pure culture of six bacteria and four fungal strains which were amplified using universal primers (8F and 1492R for bacteria and ITS1 and ITS for fungi) and run on 1% agarose gel are illustrated in Fig. 2. The 16s rRNA amplified bacterial and fungal DNA was not separated to show species difference on the 1% agarose gel due to the unavailability of DGGE Unit. The 16S rRNA sequence results obtained were analysed using NCBI BLASTn Tool for identifying the similar sequence. The BLASTn result obtained revealed that bacterial strains to be *Bacillus cereus, Bacillus licheniformis, Bacillus pumilus, Bacillus safensis, Exiguobacterium aurantiacum* and *Staphylococcus hominis* and fungal strains to be *Aspergillus flavus* (2 strains)*, Aspergillus oryzae* and *Curvularia hawaiiensis*.

The mean, standard deviation, geometric mean value in CFU/m^3 of both bacteria and fungi distributed among the two kindergartens with/without the use air-purifier at different time interval was estimated and illustrated in Table 1. One-Sample t-Test revealed the significant difference ($p \leq 0.05$) after the use of purifier for regulating the bacterial concentration.

Presence of humans is also considered to be one of the factors which contribute as the source of air-borne bacteria. Human activity and population density were found to be more in K2 as compared to K1. Thus, it confirms that the concentration of bacteria has a direct correlation with human activity and population density. Many pathogenic bacteria are air-borne which posed a high threat to humans, especially children's. On observing the result, ozone air-purifier was found to be effective in regulating the bacterial concentration as promised by the manufacturer. However, it was ineffective in regulating fungi, which is also a significant concern and needs to be controlled. The bacterial species identified by 16s rRNA are found to be associated most commonly with food poisoning, nosocomial infection, bacteraemia in immunocompromised individuals and most importantly they are documented to be drug-resistant (Kosikowska *et al.,* 2014; Organji *et al.,* 2015; Parvathi *et al.,* 2009). Whereas, the fungal strains that are identified are associated with respiratory, neurological and gastrointestinal infection and also synthesize the mycotoxin which is harmful to humans (Pattron, 2006; Kumar *et al*., 2018). So, this clears that proper regulation and standard protocol are need for prevention.

Further, the commercially available air-purifier can be useful in controlling the concentration of these microbes in the air. Nevertheless, there is a need for improvisation to these techniques to control the fungal concentration also. Even there is a need for the optimum standards that can be used for knowing the permissible limits of the microbes. The statistical analysis also favoured that commercially available air-purifier should be installed in the social gathering area to regulate the microbial contamination. Further, modification is also needed in these air-purifiers to reduce the number of fungi effectively. Due to limited resources, only two kindergartens were assessed, and passive air sampling was done because active air-sampling needs a specific instrument. Moreover, financial constrain restricted us to conduct the Multiple Drug Resistance (MDR) pattern analysis of the isolates. Additionally, there is a fervent recommendation to regularly assess the microbial contamination in the kindergartens, frame the guidelines, standards and permissible values of the microbial contamination in indoor-air of kindergartens.

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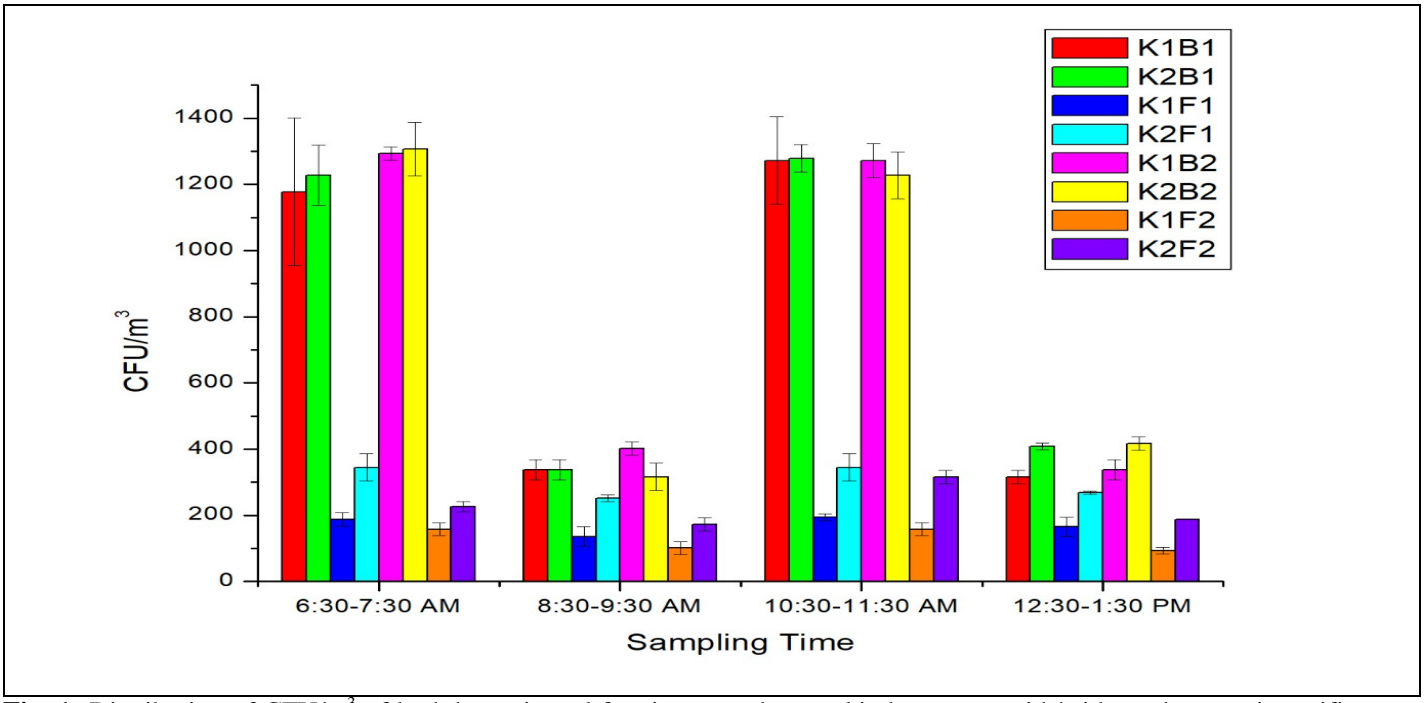

Fig. 1: Distribution of CFU/m^3 of both bacteria and fungi among the two kindergartens with/without the use air-purifier at a different time interval (K1- Kindergarten at Moga outskirt, K2- Kindergarten at Ferozepur outskirt, B- Bacterial, F- Fungal, 1- 1st-week sampling and 2- 4th-week sampling)

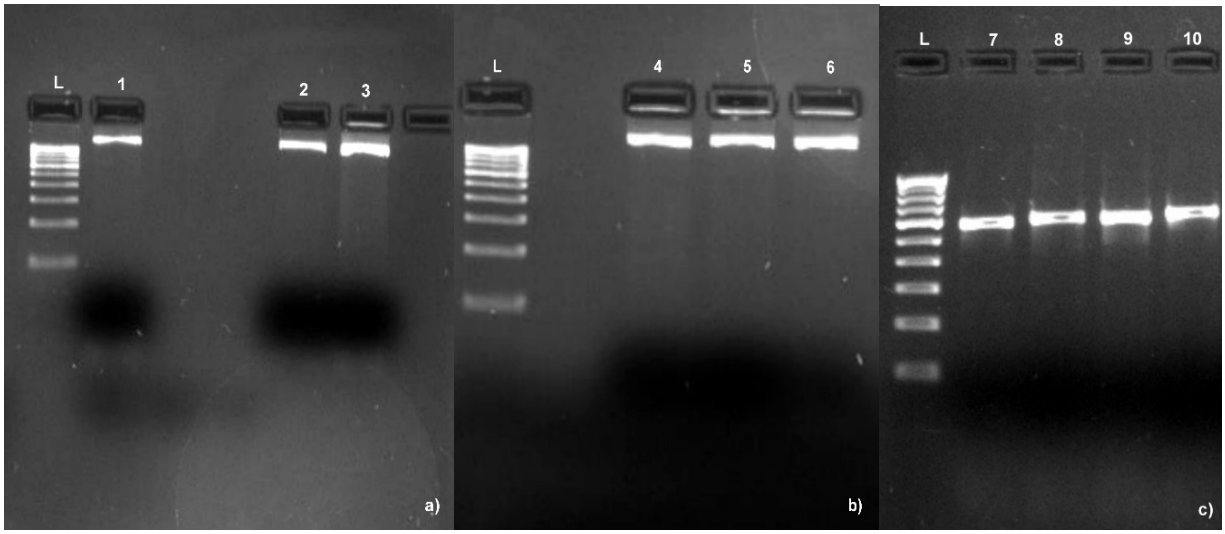

Fig. 2: 1% Agarose gel images of the Amplified DNA, L is 1kb Ladder, well 1-6 contains bacterial amplified DNA and well 7-10 contains fungal DNA.

Table 1: Descriptive statistical analysis of the Indoor-air of two kindergartens

		Timing	**6:30-7:30 AM**		**8:30-9:30 AM**		**10:30-11:30 AM**		**12:30-1:30 PM**	
			1st Week	**4th Week**	**1st Week**	**4th Week**	**1st Week**	**4th Week**	**1st Week**	**4th Week**
K1	Bacteria	Mean ± SD	1178± 244	1293 ± 20	338 ± 30	402 ± 20	1272± 132	1272 ± 51	316 ± 20	338 ± 30
		GM	1165	1293	337	402	1268	1271	316	337
		T test (α=0.05)	0.92	0.99	0.04*	0.05*	0.96	0.99	0.02*	0.04*
	Fungi	Mean ± SD	187 ± 20	158 ± 20	136 ± 30	101 ± 20	194 ± 10	158 ± 20	165 ± 30	93 ± 10
		GM	186	157	135	100	194	157	164	93
		T test (α=0.05)	0.04*	0.03*	0.04*	0.02*	0.02*	0.03*	0.05*	0.01*
K2	Bacteria	Mean ± SD	1228 ± 91	1307 ± 81	338 ± 30	316 ± 41	1279 ± 41	1228 ± 71	409 ± 10	417 ± 20
		GM	1227	1306	337	315	1278	1227	409	416
		T test (α=0.05)	0.97	0.98	0.04*	0.05*	0.98	0.98	0.03*	0.05*
	Fungi	Mean ± SD	345 ± 41	226 ± 15	251 ± 10	172 ± 20	345 ± 41	316 ± 20	269 ± 5	187 ± 20
		GM	344	226	251	172	344	316	269	186
		T test (α=0.05)	0.82	0.05*	0.05*	0.04*	0.82	0.77	0.04*	0.04*

***-** Significant value, **α**- Confidence level, SD- Standard Deviation and GM- Geometric Mean