IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 10, Oct 2018

www.ijasrm.com

ISSN 2455-6378

Assessment of airborne microbes in different indoor atmosphere

Amthul Azeez[1] and Chandana. Y. J.[2]

[1]Associate Professor, P.G & Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Teynampet, Chennai – 600 018.

[2]P.G & Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Teynampet, Chennai – 600 018.

Abstract

Good indoor air quality is important for human health and comfort, because people spend a substantial fraction of time within buildings. Microbial pollution is a key element of indoor air pollution. Bacteria and fungi growing indoors when sufficient moisture is available usually cause indoor air pollution. Hence a cross-sectional prospective study was conducted, examined for total viable bacterial count and fungi count at 5 sites. The bacterial count was found to be highest in site – 4 (Office Room - Morning) and lowest in site - 5 (Office Room – Evening) and fungal count was recorded to be high in site – 3 (Home Science - Lab) and low in site – 4 (Office Room - Morning) for a period of 30 minutes. The bacterial count was found to be highest in site - 4 (Office Room - Morning) and lowest in site – 5 (Office Room - Evening) and fungal count was recorded in site – 3 (Home Science - Lab) and lowest in site – 5 (Office Room - Evening) for 60 minutes. The microbial species were further identified. The results of the identification of microbial species showed the presence of *Staphylococcus aureus, Micrococcus leutus, Micrococcus roseus, Bacilli cereus and Bacilli subtilis* and the fungal species were *Rhizopus oryzae, Dermatophyte, Mucor* sp., *Aspergillus niger, Pencillium* sp., *Aspergillus fumigatus and Aspergillus flavus.* The airborne microbial contamination can cause health problems and can also compromise the normal activities in the work environment and could affect the Performance, morale and productivity of staff and students.

Keywords: *Airborne Microbes, Indoor Atmosphere, Human Health.*

1. Introduction

Air is one of the most important content in the environment (Vijaya Ramesh, 2004). Of all environments, air is the simplest one and it occurs in a single phase gas. Various layers can be recognized in the atmosphere upto a height of about 1000 km. The layer nearest to the earth is troposphere. This troposphere is characterized by a heavy load of microorganisms (Joseph Daniel, 1996).

The air inhaled by people is abundantly populated with microorganisms which form the so called bioaerosol. Bioaerosol is a colloidal suspension formed by liquid droplets and particles of solid matter in the air, whose components contain or have attached to them viruses, fungal spores and conidia, bacterial endospores, plant pollen and fragments of plant tissues (Kalwasinska *et al.*, 2012).

Majority of airborne pathogens are uniquely adapted for spreading in indoor environments. Most airborne pathogens die out rapidly in outdoor air but as individual species they depend entirely on man and his indoor environment for their propagation (Vijaya Ramesh, 2004). Possible sources of biological contamination of indoor air include people, organic dust, various materials stored in the buildings and inflowing from the ventilation and air conditioning systems (Karwowska, 2003). There is a growing evidence that exposure to biological agents in the indoor environment can have adverse health effects.

Microorganisms present in air are of no importance to man but when they come to rest, they may be beneficial or harmful (Crook and Burton,

2010). The main sources of airborne microorganisms are human beings. Pathogenic flora of the upper respiratory tract and the mouth are constantly discharged into the air by activities like coughing, sneezing, talking and laughing. The microorganisms are discharged out in three different forms which are grouped on the basis of their relative size and moisture content (Ambu *et al*., 2008).

Environmental factors that affect air microflora include atmospheric temperature, humidity and the height at which the microorganisms are found. Survival of bacteria in air has been studied experimentally, but the determinants are still rather obscure. Air is an extreme environment for bacteria, where their survival is limited by environmental stress. In indoor air, the main sources of bacterial aerosols are usually humans and animals, but bacterial aerosols may also be created by disturbing previously settled dust. Furthermore, air humidifiers are potential sources of airborne bacteria. However, there are limited reports on total viable counts of airborne microbes in the literature probably due to the inability to identify the potential problem causing microbes (Aruliah and Rajasekar, 2011).

Hence, the present study was aimed to evaluate quality and quantity of bacteria and fungi in different indoor atmosphere, to determine the humidity and temperature of different environments and to isolate and identify bacteria and fungi in such environments.

2. Materials and Methods

2.1. Sample Collection

Five different sites (Library, Laboratory - Chemistry and Home science, Office room - Morning and Evening) of the college were selected to assess and monitor the indoor air quality. Air samples were collected from these sites by using settle plates technique for a period of 30 minutes and 60 minutes, transferred to laboratory after collection and tested. Samples collection was carried for a period of 2 months from January 2017 to February 2017. Settle plate is a direct method of estimating the number of bacteria present in the air by permitting microorganism to "settle" on open petridishes (containing culture media) over a fixed duration by following the procedure of Stryjakowska *et al.* (2007). All microorganisms concentrations were expressed as Colony-Forming Unit, "CFU" per plate (Napoli *et al.*, 2012). The number of microorganisms expressed as Cfu/m^3 was estimated for the settle plate technique using Koch's sedimentation method.

Micro-organisms grown on petriplates were streaked and subcultured on Nutrient agar (bacteria) and Sabouraud's agar medium (fungi) for further identification. Hanging drop technique was used to perform motility test of a bacteria to find out whether it is motile or non-motile. Bacteria present in the air were further identified by biochemical reactions such as IMViC tests. Indole, Methyl red, Vogesproskauer, Citrate utilization test, Urease test, Nitrate test and TSI test and confirmed by catalase test by following the procedure of Collee *et al.* (1996).

Statistical analysis of the data obtained from the experiments was carried out using one way ANOVA.

3. Results and Discussion

Indoor air quality is one of the most important factors that influence our general life quality. Microorganism play an important role in air. The number of microorganism in the air greatly depends on the local area and the activities of environment. Therefore, this study was undertaken to assess the bacterial and fungal colonies of different environments and to identify the species.

The results of bacterial growth on nutrient agar medium and fungal growth on Sabouraud's agar medium were reported in Plate – 1 and 2.

3.1. Bacteria

3.1.1. Colony forming unit per meter cubic

The results of bacterial growth for 30 minutes showed that, Site - 4 has the highest number of bacteria with 275 Cfu/m^3 followed by Site - 3 with 128 Cfu/m^3, Site - 2 with 121 Cfu/m^3 and Site - 1 with 86 Cfu/m^3 of bacteria. Whereas

least number of bacteria was recorded in Site - 5 with 33 Cfu/m^3 (Table - 1 and Figure - 1).

Whereas, for 60 minutes, Site - 4 has the highest number of bacteria with 395 Cfu/m^3 followed by Site - 3 with 201 Cfu/m^3, Site - 2 with 193 Cfu/m^3, Site - 1 with 137 Cfu/m^3 of bacteria and least number of bacteria was recorded in Site - 5 with 45 Cfu/m^3 (Table - 1 and Figure - 1). The results of the above study is in accordance with the reports of Dong and Yao (2010).

One Way ANOVA analysis shows that there is no significant difference among the different timing of the sample as the calculated value is lesser than the table value 0.8751 (Table – 2).

According to Abdul Hameed *et al.* (2009) caution should be taken when the results of different studies are compared due to differences in the geographic zone, season and time of sampling, media of cultivation, type and intensity of human activity, growth cycle of organisms, and meteorological factors.

3.1.2. Isolation and identification of Microbes - Bacteria

Bacterial analysis of air samples was done using Preliminary test which confirmed the presence of gram positive cocci and gram positive rod. The gram positive cocci and gram positive rod were further subjected to biochemical tests for confirmation.

3.1.2.1. Biochemical test for Gram Positive Cocci

The results of biochemical test for Gram positive Cocci revealed that:

Staphylococcus aureus

Catalase test gave positive result and showed the presence of enzyme catalase which degrade the hydrogen peroxide. Negative result was obtained for Citrate utilization which was due to the inability to ferment citrate. Negative result was obtained for urease test. Negative result for Nitrate test was due to the inability to reduce nitrates to nitrites. Positive result for Glucose, Sucrose and Manitol test confirmed the presence of gram positive cocci (Plate – 3 and Table - 3).

Micrococcus leutus

Catalase test gave a positive result which shows the presence of enzyme catalase which degrade the hydrogen peroxide. Positive result for Citrate utilization was due to the ability to ferment citrate. Negative result was obtained for urease test. Negative result for Nitrate test was due to the inability to reduce nitrates to nitrites. Negative result for Glucose, Sucrose and Manitol test confirmed the presence of gram positive cocci (Plate – 4 and Table – 3).

Micrococcus roseus

Catalase test gave positive result which shows the presence of enzyme catalase which degrade the hydrogen peroxide. Negative result for Citrate utilization was due to the inability to ferment citrate. Negative result was obtained for urease test. Positive result for Nitrate test was due to the ability to reduce nitrates to nitrites. Positive result for Glucose, Sucrose and Manitol test confirmed the presence of gram positive cocci (Plate – 5 and Table – 3).

3.1.2.2. Biochemical test for Gram Positive Rod

The results of biochemical test for Gram positive Rod revealed that:

Bacillus cereus

Indole test gave negative result which indicated the absence of tryptophan. Positive result was obtained for Methyl Red test. Negative result was obtained for Voges – Proskauer test showed the absence of acetone. Positive result for Citrate utilization was due to the ability to ferment citrate. Negative result was obtained for urease test. Positive result for Nitrate test was due to the ability to reduce nitrates to nitrites. Triple sugar iron show alkaline slant and acid butt which confirmed the

presence of gram positive rod, *Bacillus cereus* (Plate – 6 and Table – 4).

Bacillus subtilis

Indole test gave negative result which shows the absence of tryptophan. Positive result was obtained for Methyl Red test. Negative result was obtained for Voges – Proskauer test showed the absence of acetone. Positive result for Citrate utilization was due to the inability to ferment citrate. Negative result was obtained for urease test. Negative result for Nitrate test was due to the inability to reduce nitrates to nitrites. Triple sugar iron showed acid slant and acid butt which confirmed the presence of gram positive rod, *Bacillus subtilis* (Plate – 7 and Table – 4). The mean and standard deviation were analysed (Table – 5 and Figure - 2). Similar results were obtained by Yassin and Almouqatea (2010).

Temperature

The temperature was higher in Site - 3 at 27° C followed by Site – 4 at 26.7° C, Site - 5 at 26.1° C, Site – 2 at 26.2°C and least temperature was recorded in Site – 1 at 26° C (Table – 8 and Figure – 3).

Humidity

The humidity was higher in Site - 4 at 38.2% followed by Site - 3 at 37.2%, Site - 5 at 36%, Site - 2 at 35.2% and least humidity was recorded in Site – 1 with 35% (Table - 6 and Figure – 4).

3.2. Fungi

3.2.1. Colony forming unit per meter cubic

The results of fungal growth for 30 minutes showed that, Site - 3 has the highest number of fungi with 41 Cfu/m^3 followed by Site - 4 with 40 Cfu/m^3 and least number of fungi was recorded in Site - 1, Site - 2 and Site -5 with 20 Cfu/m^3 (Table – 7 and Figure - 5).

Whereas, for 60 minutes, Site - 3 has the highest number of fungi with 102 Cfu/m^3 followed by Site - 4 with 82 Cfu/m^3 and least number of fungi was recorded in Site - 1, Site - 2 and Site - 5 with 61 Cfu/m^3 of fungi (Table – 7and Figure - 5).

One Way Anova analysis shows that there is no significant difference among the different timing of the sample as the calculated value is lesser than the table value 0.105 (Table – 8).

3.2.2. Isolation and Identification of microbes - Fungi

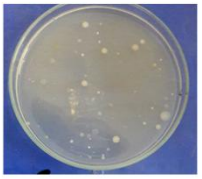
The fungal analysis from air samples was done by colony morphology and further subjected to lactophenol cotton blue test to confirm the microscopic morphology. The results of isolation and identification of fungi from air samples showed the presence of *Rhizopus oryzae, Dermatophyte, Mucor* sp., *Aspergillus niger, Pencillium* sp., *Aspergillus fumigatus and Aspergillus flavus.* Statistical analysis such as Mean and standard deviation were carried out (Table – 9 and Figure - 6). Similar fungal species were obtained by Jacob *et al.* (2016).

According to Jain (2000) Fungal spores also have the ability to cause allergies as well as other respiratory diseases and hypersensitivity reactions not only in immune suppressed patients but also in healthy individuals.

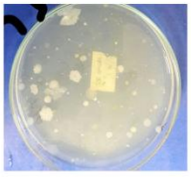
In order to safeguard the health of students, staff and workers, proper control measures has under taken over the environmental factors which favour the growth and proliferation of different bacteria and fungi in indoor environment of the college building (Kavita Naruka and Jyothi Gaur, 2013). Hence, more attention should be given to safeguard indoor environments otherwise the growth of pathogenic microorganisms can cause toxigenic health hazards.

Plate – 1. Enumeration of Bacterial colonies

30 Minutes



60 Minutes



IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 10, Oct 2018

www.ijasrm.com

ISSN 2455-6378

Plate – 2. Enumeration of Fungal colonies

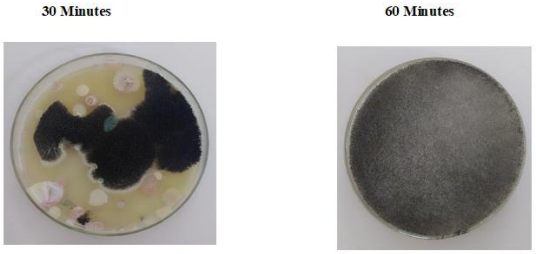


Table 1. Enumeration of total bacterial colonies of air samples from various sites of the college.

Location	Mean of Cfu/m^3 for 30 minutes	Mean of Cfu/m^3 for 60 minutes
Site – 1 (Library)	86	137
Site -2(Chemistry lab)	121	193
Site-3 (Home science lab)	128	201
Site - 4(Office Room - Morning)	275	395
Site – 5(Office Room – Evening)	33	45

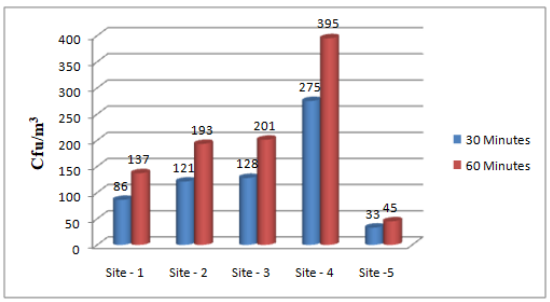


Figure 1. Enumeration of total bacterial colonies of air samples from various sites of the college.

Table 2. ANOVA: Single factor

Groups	Count	Sum	Average	Variance
Bacteria (30 Minutes)	5	643	128.6	8111.3
Bacteria (60 Minutes)	5	971	194.2	16475.2

ANOVA

Source of variation	SS	df	MS	F-value
Between Groups	10758.4	1	10758.4	0.8751
Within Groups	98346	8	12293.2	

One Way Anova analysis shows that there is no significant difference among the different timing of the sample as the calculated value is lesser than the table value 0.8751

Table 3. Biochemical test of Gram-Positive Cocci

Test	*Staphylococcus aurens*	*Micrococcus leutus*	*Micrococcus roseus*
Catalase	+	+	+
Citrate	-	+	-
Urease	-	-	-
Nitrate	-	-	+
Glucose	+	-	+
Surcose	+	-	+
Mannitol	+	-	+

\+ = Present
\- = Absent

Plate – 3. Biochemical test for *Staphylococcus aureus*

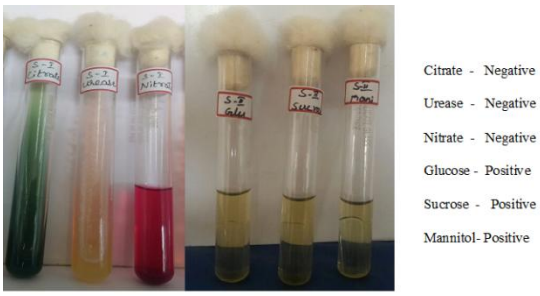


Plate – 4 Biochemical test for *Micrococcus leutus*

Plate – 5 Biochemical test for *Micrococcus roseus*

IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 10, Oct 2018

www.ijasrm.com

ISSN 2455-6378

Table 4. Biochemical tests of Gram- Positive Rod

Test	*Bacillus cereus*	*Bacillus subtilis*
Indole	-	-
MR	+	+
VP	-	-
Citrate	+	+
Urease	-	-
Nitrate	+	-
TSI	K/A	A/A

Plate – 6 Biochemical test for *Bacillus cereus*

Plate – 7 Biochemical test for *Bacilli subtilis*

Table 5. Number and percentage of bacterial species isolated from various sites.

Organism	Number	Percentage	Mean ± S.D
Micrococcus leutus	62	28%	76 ± 24.041
Bacillus cereus	40	18%	58 ± 15.556
Staphylococcus aureus	36	16%	44± 14.142
Bacillus alvei	26	12%	32± 9.899
Micrococcus roseus	55	25 %	67.5±21.22

±Standard deviation

Figure 2: Percentage of bacterial species in various sites.

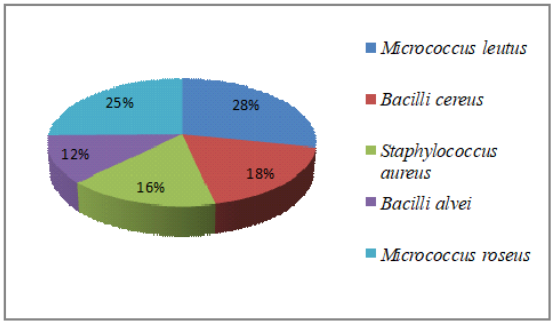


Table – 6: Temperature and Humidity of various sites of college**.**

Location	Temperature	Humidity
Site – 1(Library)	26° C	35%
Site -2(Chemistry lab)	26.2° C	35.20%
Site-3(Home science lab)	27° C	37.20%
Site – 4(Office Room - Morning)	26.7° C	38.20%
Site – 5(Office Room – Evening)	26.1° C	36%

Figure 3: Temperature of various sites of college.

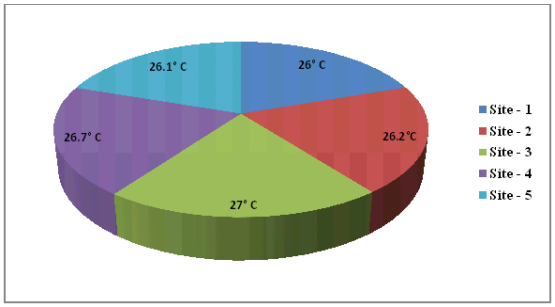
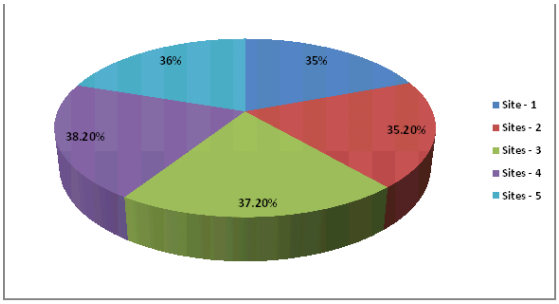


Figure 4: Humidity at various sites in college.



IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 10, Oct 2018

www.ijasrm.com

ISSN 2455-6378

Table 7: Enumeration of total fungal colonies of air samples from various sites.

Location	Mean of Cfu/m^3 for 30 minutes	Mean of Cfu/m^3 for 60 minutes
Site – 1 (Library)	20	61
Site -2 (Chemistry lab)	20	61
Site-3 (Home science lab)	41	102
Site – 4 (Office Room - Morning)	40	82
Site – 5 (Office Room – Evening)	20	61

Figure 5: Enumeration of total fungal colonies from various sites of air samples

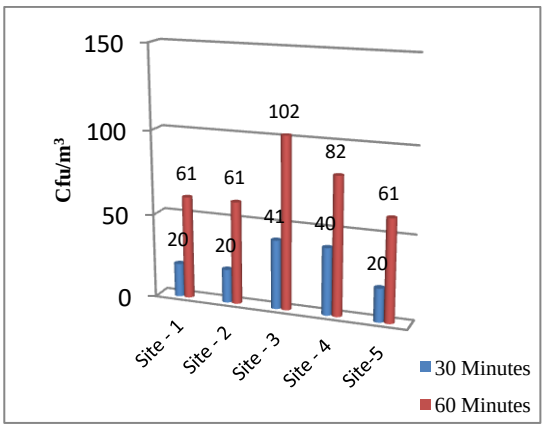


Table 8: ANOVA: Single factor

Groups	Count	Sum	Average	Variance
Fungi (30 Minutes)	5	15	2.87	1.2725
Fungi (60 Minutes)	5	17	3.09	6.3642

ANOVA

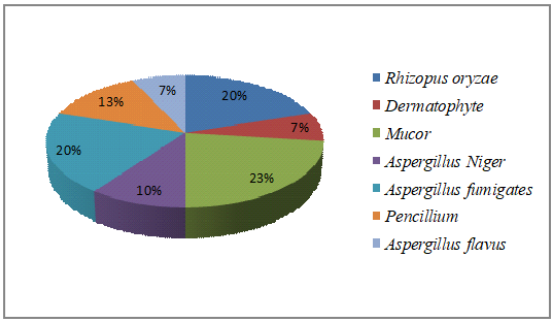
Source of variation	SS	Df	MS	F Value
Between Groups	1425	1	1425	0.105
Within Groups	11.45	8	1.431	

One Way Anova variance analysis shows that there is no significant difference among the different timing of the sample as the calculated value is lesser than the table value 0.105.

Table 9: Number and percentage of fungal species isolated from various sites of college.

Organism	Number	Percentage (%)	Mean ± S.D
Rhizopus oryzae	6	20%	16 ± 9.899
Dermatophyte	2	7%	5.5 ± 3.5355
Mucor sp.	7	23%	18.5 ± 11.313
Aspergillus niger	3	10%	15 ± 4.949
Pencillium sp.	4	13%	10.5 ± 6.363
Aspergillus fumigatus	6	20%	16 ± 9.899
Aspergillus flavus	2	7%	5.5± 3.5355

Figure 6: Percentage of fungal species in various sites of college.



4. Conclusion

Thus it can be inferred that microbial pollution is a key element of indoor air pollution. The airborne microbial contamination can cause health problems and can also compromise the normal activities in the work environment and could affect the performance, morale and productivity of staff and students. Therefore the findings of epidemiological research indicate that exposure to high concentrations of microbes in the air frequently leads to allergies, asthma, hay fever, pneumonia and many other health side-effects, including infections.

5. References

[1] Abdul Hameed, A., Khoder, M.I., Yuosra, S., Osman, A.M. and Ghanem, S., 2009. Diurnal distribution of airborne bacteria and fungi in the atmosphere of Helwan area, Egypt. *Sci. Total Environ.*, 407: 6217-6222.

[2] Ambu, S., WahMak, J., FungWong. 2008. Environmental Health and Building Related Illnesses, *Int. E. J. Sci. Med. Edu.* (*IeJSME*).11-15.

[3] Aruliah Rajasekar and Rajasekhar Balasubramanian, 2011. Assessment of airborne bacteria and fungi in food courts. *Aerobiologia*, 46(10): 2081-2087.

[4] Collee, G.J., Marimon, P.B., Fraser, G.A. and A. Simmons. 1996. Mackie and McCartney Medical Microbiology. *Pearson Professional Limited*. 14th edition 245-413.

[5] Crook, B. and N. Burton. 2010. Indoor molds, Sick Building Syndrome and building related illness, *Fung. Biol. Rev.*, 24: 106- 113.

[6] Dong S and Yao M. 2010. Exposure assessment in Beijing, China: biological agents, ultrafine particles and lead. *Environ. Mon. Assess.*, 170: 331–343.

[7] Jacob H. Jacob, Fawzi I. Irshaid and Mohamed A. Alhalib, 2016. Estimation and identification of airborne bacteria and fungi in the outdoor atmosphere of Al-Mafraq Area, Jordon. *Jordon Journal of Biological Sciences*, 9(1): 3-10.

[8] Jain A.K., 2000. Survey of bioaerosol in different indoor working environments in central India, *Aerobiologia*, 16: 221 – 225.

[9] Joseph C. Daniel, 1996. Environmental Aspects of Microbiology, *Bright sun publications*, 5 – 13.

[10] Kalwasinska, A., Burkowska, A. and Wilk, I., 2012. Microbial air contamination in indoor environment of the University library. *Ann. Agr.Env. Med.*, 19 (1): 25-29.

[11] Karwowska E., 2003. Microbiological air aontamination in some educational settings. *Pol. J. Env. Stud.* 12(2):181-185.

[12] Kavita Naruka and Jyothi Gaur V., 2013. Microbial air contamination in a school. *In. J. Curr. Microbiol. App. Sci.*, 2(12) : 404-410.

[13] Napoli, C., Marcotrigiano V. and M.T. Montagna. 2012. Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres. *BMC Public Health*, 12:594.

[14] Stryjakowska, S., Piotraszewska, P., Szyszka, M., Nowicki, M., and Filipiak, 2007. Microbiological quality of indoor air in University rooms, *Pol. J. Env. Studies*, 16 (4):623-632.

[15] Vijaya Ramesh, K., 2004. Environmental Microbiology, MJP Publishers, 56 -60.

[16] Yassin, M.F. and Almouqatea, S., 2010. Assessment of airborne bacteria and fungi in an indoor and outdoor environment. *Int. J. Env. Sci. Tech.*, 7(3): 535-544.