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STUDY of MICROBIAL AIR CONTAMINATION IN BIOLOGICAL LABORATORIES/ COLLEGE of SCIENCE

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***Abstract**:*
Objective: A study on indoor air play important roles in human health by polluted indoor labrotory and causes some diseases such as infections skin, eyes, respiratory system , allergy and biotoxicity. Air is made up of enormous of microorganisms mainly bacteria. Their estimation is important as an indication of cleanliness of any particular environment. The presence both bacteria and fungi in indoor air is problem of health protection due to a long time period that workers and students stay indoors. Determination of level microbial pollution indoors is necessary to assess the health hazard for indoor air quality control. This study provides information on microbial contamination level of indoor air of biological labrotary was estimated. Material and Methods: Collected air samples from seven bio-laboratories and low, up corridors locations during April-June 2019. Air samples were taken three months : in the in the afternoon.
Results: The higher of percentage of isolated airborne bacteria *Staphylococcus aureus* and *Staphylococcus epidermis*, it was 58.57% and 26.84% for all biological laboratories respectively at study location, while the lowest percentage were *Klebsiella sp.* and *Protus sp.*, it was 11.98% and 4.29% for all biological laboratories, respectively.
Conclusion: It is concluded from the current study that is advisable that estimate to check microorganisms loads in Laboratories and prevent their increase and spread.

Keywords: Indoor air, Bacteria, Fungi, Contamination , Labrotaries.

INTRODUCTION

Common air environment pollutants is the presence in the atmosphere of chemicals, particulate matter or biological materials that effect on human health and other organisms [1]. In addition, air pollution comes from sources inside the building itself such as hair spray, room deodorizer, photo copiers, paints, thinners, printers, computers, and air purifiers [2].

Indoor air pollution cause harmful health effects due to life style that people spend a long time period to stay indoors at home and work places [3, 4].

There are many microbes that spread in the human environment and many of them are pathogenic and considered Universities are one of the sources of external microbes where the laboratory art, halls, etc. as well as health facilities are all contain a large number of microbes and not all these bacteria species cause infections in the normal state, but some of them may occur when they exist anywhere and these are called pathogenic bacteria and when the decline body immunity, bacterial infections are then called opportunistic bacteria [5].

These bacteria may be a major problem for university workers through the presence of bacterial contamination. In particular, bacteria where these bacteria such as *Staphyllococcus, Streptococcus* are dangerous resist disinfectants and sterilization in the absence of cleaning.

Also several fungi that are causes allergic reactions in humans belong to Ascomycota, Basidiomycota or yeast [6]. Fungi are able to break surface tension and grow into the air and on all natural and synthetic materials, are present during spring, summer, and especially autumn such as *Aspergillus , Rhizopus, Penicillium,* Cladosporium and *Alternaria* species [7, 8]. Fungi can also be present in dust in great numbers. Thus, standard fungal cultivation methods showed up to 70 million CFU/g of dust (9). Such dust associated fungi are typically related to the outside environment .The composition of these indoor microbial communities is associated with the levels of urbanization and the health of the inhabitants, in particular concerning allergic disorders, intestinal microbiome and general immune responses (10).

Airborne Viruses. Likewise, ambient air appears to not contain significant amounts of known viral pathogens However, only a small fraction of all viruses found in the environment are known, which makes it difficult to estimate potential effects of the air virome on human health, A major constituent of the airborne virome is bacteriophages that are not known to pose a risk for humans but may affect bacterial populations contributing to the spread virulence and antibiotic resistance genes (11)

Moreover, the environmental factors mainly include temperature, air exchange rate, humidity, air movement, building structures and location, poor design, ventilation system as well as interior or redesign which enhance microorganism's growth and multiplication in the indoor atmosphere [12].

This study provides information and estimation of air microbial contamination and determination of bacterial and fungal levels for bio-laboratories / College of Science at Tikrit University.

MATERIALS AND METHODS

Study area

The study was conducted in bio- laboratories of College of Science buildings of Tikrit university, Iraq. The Biology department, established in 1998. The study included seven libraries of biology department , namely, Microbiology Library, Molecular biology Library, Cell and Genetic Library, Mycology Library, Tissue and Physiology Library, Ecology and pollution Library, Ecology and pollution Library for Research. Air samples were taken in a room bio-laboratories and Underpass and Overpass of the department building, Twice a month (one day a week) from April to June 2019.

Materials

We used *laboratory* material and *equipment* are including autoclave, incubator, oven , hot plates, refrigerator, forceps, spatula , Conical flasks, Petri dishes, dropper, pipettes, cotton, distilled water, test tubes, cylinder, glass slides, cover slips, immersion oil, Xysol, , filters, gas burners, sensitive balance, Hood, inoculating loop, needle, cotton swab, and microscope.

Collection and Sampling procedure

Air sample are collected by placed the device on the bench (one sampling site in the middle of the room) or at land level (for outdoor measurements at the corridors) to simulate aspiration from the human breathing zone [13].

We used Nutrient agar (NA) for bacterial sample and cultivation. Also used Potato dextrose agar (PDA) with 10 mg/L chloramphenicol for fungi isolation. Both plates of NA and PDA were exposed to air indoor and corridor for 30 min in the afternoon between the hours 12 noon and 2.00 pm. After collection the nutrient agar plates were incubated at 37°C for 24 h to allow the growth of aerobic bacteria, while PDA plates were incubated at 25°C for 3-5days to allow the growth of fungal colonies.After incubation, total plate count was done on the basis of growth on NA plates. The colony characteristics were studied from mannitol salt agar, MacConkey agar, and EMB agar. Then, bacterial colonies were subjected to gram staining and biochemical tests such as catalase, oxidase, and coagulase testsm and identified bacterial species were performed according to standard procedures [14].

Bacterial colonies were initially characterized by morphology and microscopic appearance, and identified further by according to Sneath *et al*. [15]. Whereas fungal colonies were prepared by using Lacto phenol blue solution and examined microscopically. Identification of fungi was based mainly on growth colonial appearance, microscopic examination of the spore and hyphal characteristics of the stained preparations [16].

RESULTS AND DISCUSSION

In the present study, the percentage of air microbial contamination from bacteria and fungi was calculated in the biological laboratories of the College of Science / Tikrit University for a period of three months from April to June 2019. The results showed the growth of number of bacterial and fungal species in laboratories. For bacteria, it is shown in the Table (1) the highest microbial contamination rate in June was 82.71%, which caused contamination with *Staphylococcus aureus* in the mycology laboratory, and then *Staphylococcus epidermis* in June, at the rate of 82.28% in the microbiology laboratory, and 79.69% *Staphylococcus aureus* in the Research of Ecology & pollution laboratory. The lowest infection rate was 0.33% of the bacterial cause, *Staphylococcus aureus* in May, while no case of infection appeared for some bacterial species at the study months. Also, non-containment dishes appeared on growth of 20% of the cultured samples. Returns to the place of taking the sample that is not exposed to contamination, or the method of taking the sample may be wrong or due to the sterilizers and disinfectants used in cleaning the laboratories [17].

Table (1): Percentage of bacteria isolated from the laboratories of the Department of Biology by months during the study period 2019

Dates	Laboratory / Bacteria species	Microbiology Lab.	Molecular biology Lab.	Genetic Lab.	Mycology Lab.	Histo. & Physiology Lab.	Ecology & pollution Lab.	Research of Ecology & pollution Lab.	Corridor Low	Corridor Up
7/4	***S .aureus***	2.11%	0.36%	---	---	---	---	---	5.81%	---
	S. epidermis	---	---	---	---	---	1.40%	2.57%	---	0.57%
	Klebsiella sp.	0.84%	---	1.85%	2.25%	10.34%	1.75%	---	3.78%	1.72%
	Protus sp.	0.42%	---	---	---	---	---	---	---	---
22/4	***S .aureus***	3.16%	8.03%	7.41%	6.39%	5.73%	---	5.14%	10.17%	57.47%
	S. epidermis	---	---	---	---	---	1.05%	---	5.81%	---
	Klebsiella sp.	1.05%	---	7.41%	---	---	---	0.77%	---	---
	Protus sp.	---	---	3.70%	---	---	3	---	5.52%	15.90%
5/5	***S .aureus***	4.64%	---	11.11%	0.33%	---	4.21%	---	13.08%	0.77%
	S. epidermis	---	5.84%	---	---	1.79%	---	---	---	1.15%
	Klebsiella sp.	1.89%	---	9.26%	---	---	5.26%	---	5.81%	0.96%
	Protus sp.	---	---	---	---	---	---	---	---	---
19/5	***S .aureus***	---	3.65%	14.81%	3.01%	0.72%	3.86%	---	3.44%	4.21%
	S. epidermis	1.27%	---	---	---	---	---	3.34%	---	---
	Klebsiella sp.	---	---	---	---	---	---	---	2.90%	2.29%

	Protus sp.	---	---	---	---	---	---	---	0.87%	1.15%
10/6	***S .aureus***	---	---	---	2.25%	2.51%	1.75%	8.48%	11.63%	---
	S. epidermis	2.23%	4.02%	33.33%	---	---	---	---	---	4.78%
	Klebsiella sp.	---	---	---	1.13%	---	---	---	7.56%	3.83%
	Protus sp.	---	---	---	---	---	---	---	---	---
22/6	***S .aureus***	---	---	---	82.71%	78.85%	79.65%	79.69%	---	---
	S. epidermis	82.28%	78.10%	9.26%	---	---	---	---	---	2.87%
	Klebsiella sp.	---	---	---	---	---	---	---	23.54%	1.23%
	Protus sp.	---	---	1.85%	1.88%	---	---	---	---	---

The reason for the emergence of these types and proportions of bacteria *S. auerus* and *S. epidermis* and *Klebsiella sp* bacteria is because the bacteria contain important factors that help them to survive, spread and infect, such as having the Toxins , Enzymes ,capsule and its resistance to the antibiotics used against it and the speed of its transmission between people where it is spread through flying mist and direct contact where Coexistence is a combination of skin and oral cavity and is an optional anaerobic that has the ability to resist acidity, heat, and high salinity [18].

S. aureus and *Klebseilla sp*. are opportunistic pathogens that rarely cause disease in healthy people, but they are highly virulent in patients with weak defense methods or mechanics causing Bacteremia, eye-piercing, burn-out, skin, wounds, and other diseases [19]. *S.epidermes* are a natural flora of the skin in humans, but may turn into pathogen when it enters the body through cuts, bruises, scratches, etc. [20]. As for Proteus bacteria, which are low in the study samples, soil, water, and the human intestine, especially urinary tract infections, are their natural environments and are preferred for the growth of this type of bacteria. Which increases the chance of infection, and considered pathogens opportunistic diseases in humans. It also demonstrated their ability to live in aerobic and anaerobic environments (optional) [21]

When calculating the total percentage of isolation of bacterial etiology for all laboratories (Table 2), the highest final isolation rate for bacteria was 58.57 % for all laboratories due to *S.auerus* bacterial cause, and then the percentage of bacteria *S.epidermis* was 26.84% for all laboratories, either *Klebseilla* by 11.98 % and the lowest isolation rate Finally, the bacteria *Proteus* accounted for 4.29%.

The difference in the percentage of bacteria isolation from the samples for different laboratories may be due to the isolation places or due to the degree of interest in cleaning and the type of sterilizers and different disinfectants in cleaning the biological laboratories because the bacteria are resistant to most sterilization materials and antibiotics [17].

Table (2): Total percentages of bacteria isolated for all laboratories of the Department of Life Sciences during the study period 2019

S .aureus	***S. epidermis***	***Klebsiella sp***	***Protus sp.***
58.57%	**26.84%**	**11.98%**	**4.29%**

While fungi , it is shown in the Table (3) the highest air fungal contamination rate in April was 28.40%, which caused contamination with *Alternaria sp.* in the ecology and pollution laboratory, and then *Mocur* in May, at the rate of 21.25% in the Research of Ecology & pollution laboratory, and 18.52% of *Pencillum sp*. in the Corridor up. The lowest infection rate was 0.25% of the fungal cause, *Alternaria sp.* in May, while no case of infection appeared for some bacterial species , in most of study months. Also, non-containment dishes appeared on growth of 10% of the cultured samples. When calculating the total percentage of isolation of fungal etiology for all laboratories (Table 4), the highest final isolation rate for fungi was 28.25 % for all laboratories due to

Alternaria sp. and *Pencillum sp*. and then the percentage of fungi *Aspergillus sp.* was 19.96% for all laboratories, either *Mucor* by 16.07 % and the lowest isolation rate Finally, the *Rhizopus* and Yeast accounted for 2.74 and 4.72% respectivety.

Results of this study showed that common fungus belonging to the genera: *Penicillium*, *Aspergillus*, *Alternaria, Mucor* and *Rhizopus*. Air indoor of examined labrotary room was dominated by *Aspergillus* sp. and isolated throughout this study. This may be due to continuous input the microbes from outside via visiting people (22).
These result agree with (23)(26) , which concluded ,the main bacteria species detected in indoor and outdoor air in kindergartens were *Bacillus* spp., *Staphylococcus aureus, Micrococcus* spp., *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Enterococcus* spp., and *Streptococcus* spp. The predominant genera of the airborne fungi isolated from indoor and outdoor air in kindergartens were *Aspergillus terreus*, *Aspergillus flavus*, *Cladosporium* spp., *Penicillium* spp., *Rhodotorula* spp., *Ulocladium* spp., and *Alternaria* spp

Table (3): Percentage of fungal isolated from the Bio-laboratories by months during the study period 2019

Dates	Laboratory / Fungi species	Microbiology Lab.	Molecular biology Lab.	Genetic Lab.	Mycology Lab.	Histo. & Physiology Lab.	Ecology & pollution Lab.	Research of Ecology & pollution Lab.	Corridor Low	Corridor Up
7/4	***Alternaria sp.***	10.20%	10.18%	16.06%	8.81%	15.49%	28.40%	1.25%	13.11%	17.90%
	Pencillium sp.	12.25%	15.27%	13.76%	10.36%	14.08%	14.39%	5.63%	13.74%	18.52%
	Asperigillus niger	---	12.72%	9.17%	4.66%	7.04%	---	4.38%	11.21%	15.43%
	Mucor	---	0.51%	1.38%	0.52%	2.11%	---	---	0.63%	---
	Yeast	3.06%	0.76%	2.29%	1.04%	0.70%	1.89%	---	0.42%	1.85%
22/4	***Alternaria sp.***	10.20%	1.02%	2.29%	1.55%	4.93%	1.89%	1.25%	2.54%	6.79%
	Pencillium sp.	7.14%	0.76%	1.83%	2.07%	2.81%	2.65%	2.5%	2.11%	0.62%
	Asperigillus niger	---	0.51%	0.46%	1.55%	---	---	0.63%	1.69%	1.86%
	Mucor	10.20%	3.05%	11.93%	2.07%	11.97%	0.76%	6.25%	5.71%	6.17%
	Yeast	3.06%	1.02%	1.83%	---	1.40%	---	---	0.63%	---
5/5	***Alternaria sp.***	4.08%	13.99%	1.38%	11.39	2.11%	11.74%	12.5%	6.98%	---
	Pencillium sp.	2.04%	14.25%	1.83%	15.54%	1.40%	9.47%	15.63%	8.67%	---
	Asperigillus niger	2.04%	16.79%	0.92%	12.44%	---	15.15%	6.88%	4.23%	---
	Mucor	15.30%	0.76%	11.93%	8.29%	14.08%	3.79%	21.25%	7.82%	3.09%
	Rhizopus sp.	2.04%	---	3.67%	1.04%	---	---	0.63%	---	---
	Yeast	3.06%	2.76%	10.55%	5.69%	0.70%	4.55%	5%	1.69%	2.16%
19/5	***Alternaria sp.***	2.04%	0.25%	2.29%	1.04%	0.70%	0.38%	1.88%	2.54%	3.70%
	Pencillium sp.	4.08%	0.76%	1.83%	2.07%	1.40%	0.76%	3.13%	0.42%	4.32%
	Asperigillus niger	1.02%	0.76%	0.92%	4.15%	1.40%	1.14%	3.13%	0.42%	1.85%
	Mucor	3.06%	---	0.92%	4.15%	1.40%	1.52%	1.25%	2.11%	4.94%
	Rhizopus sp.	---	---	1.38%	---	---	0.38%	0.63%	---	0.31%
	Yeast	---	0.25%	2.75%	0.52%	---	0.38%	3.13%	0.85%	1.23%
10/6	***Alternaria sp.***	2.04%	0.51%	0.92%	---	2.11%	---	0.63%	2.54%	1.54%
	Pencillium sp.	1.02%	1.02%	0.92%	0.52%	2.81%	---	1.25%	2.96%	2.16%
	Asperigillus niger	1.02%	0.51%	0.92%	---	2.81%	0.38%	0.63%	2.11%	1.23%
	Mucor	---	0.51%	---	---	1.40%	---	---	1.27%	0.31%
	Rhizopus sp.	---	---	---	---	---	---	---	---	---
	Yeast	---	0.25%	---	---	---	---	---	0.63%	---
22/6	***Alternaria sp.***	---	0.25%	---	---	1.40%	---	---	1.06%	1.23%
	Pencillium sp.	1.02%	0.51%	0.46%	0.52%	2.81%	---	0.63%	1.27%	1.54%

Asperigillus niger	---	0.51%	---	---	2.11%	0.38%	---	0.42%	0.62%
Mucor	---	0.25%	---	---	0.70%	---	---	0.21%	0.62%
Rhizopus sp.	---	---	---	---	---	---	---	---	---
Yeast	---	---	---	---	---	---	---	---	---

Table (4): Total percentages of Fungi isolated for all laboratories of the Department of Life Sciences during the study period 2019

Alternaria sp.	*Pencillium sp.*	*Asperigillus niger*	*Mucor*	*Rhizopus sp.*	*Yeast*
28.25%	**28.25%**	**19.96%**	**16.07%**	**2.74%**	**4.70%**

There are also many factors that cause low indoor air quality, including but not limited to the design of the building inappropriately for its purpose, insufficient ventilation, as well as dust and wall painting materials (paints), cleaning products, and others (24)(25).

CONCLUSION

Therefore, through the results, attention must be paid to the quality of indoor air in manned biological laboratories of high quality, that is, to be hygienic and contain ventilation filters that prevent entry or exit of microbes from biological laboratories in addition to gases and dust atoms that may be carrying the microbe. As well as attention to laboratory cleanliness and its emphasis on students and laboratory workers periodically, especially in late spring and summer months with temperatures between 25-38 °C and the use of sterilizers and disinfectants more influenced by microbes because some of them are resistant to it.

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