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Air Microbial Contamination and Factors Affecting Its Occurrence in Certain Book Libraries In Egypt.

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BACTERIAL and fungal contamination in the air and settled dust were studied in old and new book libraries located in the National Research Center (NRC), Dokki, Giza, Egypt. The investigated libraries differ in age, design, size, ventilation type, and a number of occupants in relation to microclimatic parameters and particulate matter (PM) load. Airborne microorganisms were collected using an Andersen two stage impactor sampler. Indoor airborne bacteria and fungi ranged from 0-1060.4 CFU/m^3 and 11.8-315.6 CFU/m^3, respectively. Outdoor airborne bacteria and fungi ranged within 11.7-2514.7 CFU/m^3 and 0-713.7 CFU/m^3, respectively. Bacteria and fungi associated surface settled. The dust ranged from 0.4-10x10^6 CFU/gm and 0-73x10^4 CFU/gm, respectively. Fine microbial fraction (particles ≤ 8 μm in size) constituted 2-24.94% and 68.35-94.15% of the total airborne bacterial and fungal concentrations, respectively. Indoor/outdoor (I/O) ratios of airborne microorganisms were less than 1 at both libraries, indicating no indoor microbial sources. Gram positive cocci (14.3-47%) and bacilli (52.9-85.7%) were the dominant bacterial isolates in the air state, while bacilli represented 100% of the total isolates in the surface settled dust. *Bacillus pseudomycoides* and *B. subtilis* dominated indoors while *B. subtilis* and *Staphylococcus* outdoors. *Aspergillus* and *Penicillium*, were the common fungal species in both libraries under investigation. Many of the isolated fungal taxa had enzymatic activities (lipase, protease and cellulase), with *A. flavus*, *Curvularia pallescens*, *Fusarium oxysporium*, *P. notatum* and *Trichoderma viride* presented all enzymatic activities. Complex correlations and no-clear patterns were found between the airborne microorganisms and the environmental factors.

Keywords: Library, Microbial contamination, Particle Size, Identification, Enzymatic Activities, Microclimatic Factor.

Libraries are institutions for preserving books and cultural heritage. Library collections undergo process of aging, alternation and deterioration by physical, chemical and biological agents (Gallo, 1993 and Maggi *et al.*, 2000 & Cappitelli *et al.,* 2010). Organic matter (vegetable, animal and synthetic origins), relative humidity and dust are suitable conditions for microbial growth in libraries (Ciferri *et al.*, 2003, Karbowska-Berent *et al.,* 2011). Microorganisms may pose a danger to books and archival collections (Harkawy *et al.,* 2011 and Tao *et al.,* 2014) and to staff and visitors (Mesquita *et al.,* 2009, Skóra *et al.,* 2012).

Microbial aerosols in the library may exist as a result of infiltration of ambient air, human activity, air ventilation systems, presence of indoor reservoirs (ACGIH, 1999). Microorganisms are always attached dust particles (Alghamdi *et al.,* 2014). Dust serves as a source of nutrients and forms a proper microenvironment on surfaces for microorganisms (Urzi and Realini, 1998). Water and suitable microclimatic conditions, *e.g.,* temperature and relative humidity, are essential conditions for microbial growth indoors (Ritchkoff *et al.,* 2000).

Fungi and bacteria may cause biological deterioration of materials, and there are many microbial taxa responsible for biodeterioration. Fungi cause great damage to cultural heritage because they possess high biodeteriogenic capacity of organic matter (Borrego and Perdomo, 2012). May *et al.* (1993) listed dematiaceae fungi among the major agents of biodeterioration of surfaces and dark spots. Urzi *et al.* (2001) concluded that once fungi settle on and colonize surfaces, they are responsible for a great variety of alternation like black paints, intergranular growth, sugaring and biopitting. Species of *Trichoderma*, *Penicillium*, *Botrytis*, *Trichothecium*, *Phoma*, *Chaetomium*, *Aspergillus*, *Cladosporium*, *Stemphylium*, *Alternaria*, *Hormodendrum*, *Aureobasidium*, *Papularia*, *Bacillus*, *Cellulomonas*, *Cellfalciculata*, *Cellvibrio*, *Sporocytophaga* and *Streptomyces* have celluolytic properties, *Aureobasidium*, *Chaetomium*, *Cladosporium*, *Botrytis*, *Trichoderma*, *Verticillium*, *Mucor*, *Epicoccum*, *Gymnoascus* and *Actinomycetes* have proteolytic properties, all the proteolytic fungi listed above and *Paecilomyces* have lipolytic properties (Borrego *et al.,* 2010 and Berent *et al.,* 2011).

Inhalation of microorganisms may cause health threats. Wiszniewska *et al.* (2009) identified allergy to fungi in 31% of staff working at the national museum in Warsaw. Mycotoxins producing fungi, *e.g*. *Aspergillus*, *Penicillium*, and *Stachybotrytis* have been isolated from museum, libraries and archive settings (Eduard, 2009 and Skóra *et al.,* 2015).

The evaluation of microorganisms in air and surface dust is the first step to control library's environment and maintains deterioration. The present study aims to evaluate microbiological indoor air quality at two libraries and their relationships with microclimatic factors and particulate matter (PM). Moreover the potentiality of fungi, particularly biodegradable taxa, with cellulolytic, proteolytic and lipolytic enzymatic activities was evaluated.

Materials and Methods

Sampling sites and strategy

The sampling was performed at two different libraries differ in age, size, design, height, location, ventilation, and number of books and users. The libraries are located at the main campus of the National Research Centre (NRC), Dokki, Giza governorate, Egypt. This is an urban area characterized by heavy traffic, parking, playgrounds, small workshops, hospitals, educational settings and hostels. A variety of vegetation is present in the area but there is no predominant ground cover.

The old is library of (National Center for Information and Documentation (NIDOC); affiliated to the Academy of Scientific Research & Technology (ASRT) located at the 2nd floor in the main building of the NRC. It was established in 1956 and characterized by fans and natural ventilation, with no air conditioning systems, and all windows are faced to the busy- traffic street (Fig. 1). On the other hand, the new library was built in 2009. It is located on the ground floor ~75 m inside the main campus of the NRC away from the busy traffic street. It is fully air-conditioned library with low opening windows. The old library is a duplex floor, and it is ~3 times bigger in size than the new one; however the users and readers are higher in the new library than the old one. The two libraries had no historic indoor air quality issues.

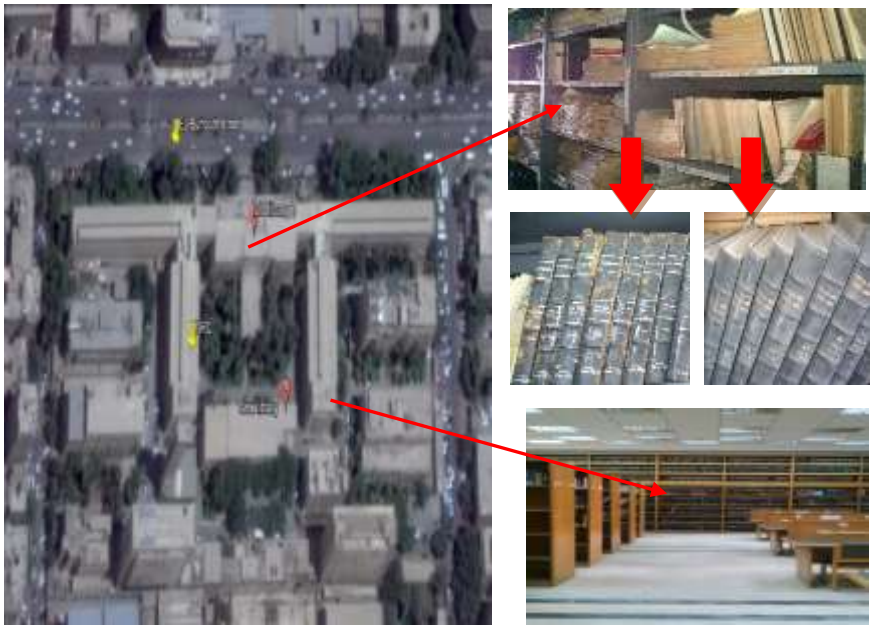

Fig. 1. Map showing locations of the both libraries at the NRC.

Air samples were taken between January to December 2013 during the normal working days and human activities. Sampling was conducted between 9 am and 2 pm, during 10^{th} – 15^{th} and 25^{th} -30^{th} days per month. Each library was scheduled for sampling twice per month, a total of 6 sampling events were conducted per season. The air samples were positioned at a height of ~ 1.5 m above the floor level in the middle of the library. The comparison samples were taken 3-5 m outside the main front door of the new library and 50-75 cm of the window's ledge at the old library, facing to the main street.

A portable weather station (SATO, PC- 5000 TRH-II sampler) was used to determine the indoor and outdoor temperatures and relative humidity (microclimatic parameters) at the time of the microbial sample collection. Sampling was not take place during or within 48 hr of a rain event. The ranges of libraries average temperatures were 17-29°C indoors and 16-32°C outdoors, while the ranges of libraries average relative humidity were 37-66% indoors and 28-55% outdoors. Temperature and relative humidity records were slightly higher in the old library than the new one. No significant differences were found between the indoor temperature and relative humidity at both libraries. However significant difference ($t= 2.53$, $P \leq 0.05$) was observed between the outdoor relative humidity at both libraries.

Microbiological sampling and analysis

Airborne microorganisms including environmental bacteria, mesophilic bacteria and fungi were collected using an Andersen two-stage viable cascade impactor sampler (TE-10-160, Tisch Environmental Cleves, OH, USA). It separates particles into fine (≤ 8 μm) and coarse (≥8 μm) size ranges. Particle diameter determines where particles are likely to deposit in the respiratory tract (Thomas, 2013). Nutrient agar supplemented with cycloheximide and rose-bengal streptomycin agar media were used to count bacteria and fungi, respectively (Mouli *et al.,* 2005 and Sarica *et al.,* 2005). The sampler was operated at a manufacturer recommended flow rate of 28.3 l/min, for 5 min. Because of the short sampling time two consecutive samples were taken (1 hr rest between sampling) during every sampling event (4 plates /indicator/location, a total of 24 plates/ sampling event).

The bacterial plates were incubated at 25°C for 48 hrs for growing environmental bacteria and at 37 °C for 48 hrs for growing mesophilic bacteria. Fungal plates were incubated at 28°C for 5-7 days and checked daily. Positive hole correction (Andersen, 1958) was applied to the raw colony forming unit (CFU) recorded on each plate and by using the CFU with sampling time and flow rate, the concentrations were calculated and expressed as the colony forming unit per cubic meter of the air (CFU/m^3).

Surface dust accumulated over time on floor, books, shelves and furniture were collected, twice every month, using a brush and sterilized plastic bags, and microbiologically analyzed. The dust sample was thoroughly mixed and sieved using a sieve with pore diameter ≤ 45 μm (Rumo shaker model 2200, U.K) to

remove particles >45µm. A weight of 0.05-0.1 gm of dust ≤ 45 µm was suspended in 25 ml sterile distilled water and shaken well for 45-60 min. Aliquots (0.5 ml) of the original sample and its serial dilutions (10^{-2}) were spread plated, in duplicate, onto the surface of the nutrient agar and rose-bengal agar media and incubated as previously mentioned to count and identify bacteria and fungi associated dust, respectively. The concentrations of microorganisms associated dust were calculated and expressed as the colony forming unit per gram of dust (CFU/gm).

Particulate matter (PM)

Particulate matter (PM) samples were collected, indoors and outdoors at both libraries, using sterilized pre-weighted cellulose nitrate membrane filters (0.45µm pore size and 25 mm diameter) equipped in open face filter holders and vacuum pumps calibrated to draw 16 l/ min. for 3 hrs. The filters were weighted (Sartorius TE2145, Germany), and along with sampling time and flow rate, the concentration was calculated and expressed as microgram per cubic meter of the air ($\mu g/m^3$).

Identification of microorganisms

Bacterial and fungal isolates that were isolated from the air and dust were identified. Bacterial isolates were identified by using BIOLOG 21124 (Cabot Blvd, Hayward, CA 94545, Water Pollution Dept., NRC, Egypt). Biolog's microbial identification system software (OmniLog ® Data Collection) was used to identify the bacterium from its phenotypic pattern in the GEN III MicroPlate. On the other hand, fungal isolates were identified by the direct observation on the basis of micro-and macro- morphological features on Sabouraud dextrose agar, Czapek's dox agar and malt extract agar, reverse and surface coloration of colonies using various literature (Ellis, 1971; Barnett, 1972 and Raper & Fennell, 1977; Singh *et al.,* 1991 and Barnett & Hunter, 1999; Hussein, 2002 and Pitt & Hocking, 2009).

Fungal enzymatic activity

Cellulose, lipid and protein decomposing fungi were qualitatively screened using selective media. Cellulolytic activity was determined using cellulose-containing agar medium (0.03% urea, 0.02% KH_2PO_4, 0.14% $(NH_4)_2SO_4$, 0.03% $MgSO_4.7H_2O$, 0.5% cellulose, 0.1% peptone, 0.00001% $FeSO_4.7H_2O$, 0.00016% $MnSO_4.7H_2O$, 0.00017% $ZnSO_4.7H_2O$, 0.0002% $COCl_2$, 2% agar, pH 5), (Bland and Douglas, 1977). Mycelium discs, 5 mm diameter, from 7 days old culture fungal taxa were cut and placed onto the center of cellulose agar plates. The plates were incubated at 28 ± 2°C for 72 hrs, and further incubated at 50°C for 8 hrs (Osman *et al.,* 2008). The plates were flooded with rose-bengal (0.005%). The appearance of clear zone around the colony discs indicates cellulolytic activity (Bland and Douglas, 1977).

Lipase activity was screened using phenol red agar plates (0.01% phenol red, 1% olive oil, 0.1% $CaCl_2$ and 2% agar, pH 7.4), (Singh *et al.,* 2006). Mycelium discs, 5 mm diameter, from 7 days old culture fungal taxa were cut and placed

onto the center of agar plates. The plates were incubated at 37 °C for 4 days. The appearance of yellow color, due to release of fatty acid, indicates lipase enzymatic activity.

Protease activity was determined using Gelatin-agar medium plates (1% gelatin, 2% agar and 0.02% M phosphate buffer, pH. 7.5), (Ammar et al., 1991). Mycelium discs, 5 mm diameter, from 7 days old culture fungal taxa were cut and placed onto the center of agar plates and incubated at 25°C for 4 days. The plates were flooded with 10 ml acidic mercuric chloride solution (Cowan, 1974), and the appearance of clear zone around the colonies (discs) indicates protease enzymatic activity.

Medical effects

An interview was performed among all employees working at both libraries. The questionnaire contained general questions on the frequency of the symptoms for 3 days per week in the previous 4 weeks before survey, or which appear every working day and got better when the employee went away from the work (Crandell *et al.,* 1996). The questionnaire contained the following general symptoms:

- Shortness of breath, cough and wheezing;
- Dry eye, watering eyes, irritation of the eyes, and allergic conjunctivitis;
- Sneezing, stuffy nose, coryza and throat irritation;
- Headache, fatigue, drowsiness, dizziness and nausea and
- Dermatitis

Statistical analysis

The Mann Whitney U test was used to ascertain the significance of differences between microclimatic conditions at both libraries as well as airborne microorganisms inside and outside the libraries. Spearman's rank correlation test was used to determine the relationships between airborne microbial concentrations with both microclimatic conditions and particulate matter (PM). A probability of less or equal to $P \leq 0.05$ was considered significant.

Results

Microbiological air quality

Table 1 and Fig.2. show the mean total airborne microbial concentrations at the two libraries. Environmental bacteria ranged from 0-1060.4 CFU/m^3 indoors and 11.77-2514.71 CFU/m^3 outdoors, higher concentrations shifted toward the old library. The maximum mean concentrations of environmental bacteria were 296.2 CFU/m^3 indoor and 1455.5 CFU/m^3 outdoor. Mesophilic bacteria ranged within 0 - 405 CFU/m^3 indoors and 11.77 - 2157.82 CFU/m^3 outdoors, with higher concentrations outdoors.

Fungal concentrations ranged between 11.77 - 315.66 CFU/m^3 indoors and 0-713.77 CFU/m^3 outdoors. Generally, indoor and outdoor airborne microbial

concentrations were higher at the old library than the new one. No significant differences were found between airborne microbial concentrations indoors and outdoors at both libraries, except mesophilic bacteria that showed a significant difference in the outdoor and indoor concentrations (P= 0.0286) at the old library, higher concentrations shifted toward outdoor.

TABLE 1. The range and mean concentrations (CFU/m^3) of total airborne microorganisms at two libraries at the main campus of the National Research Centre (NRC), Dokki, Giza.

Microbial type	CFU/m^3			
	Old Library		New Library	
	Indoor	Outdoor	Indoor	Outdoor
Environmental bacteria	0.0-1060.4 (167.61±296.2)	35.32-1107.18 (961.41±1455.5)	0.0-353.34 (101.41±106.7)	11.77-2514.71 (738.72±644.9)
Mesophilic bacteria	0.0-107.17 (32.45±28.9)	23.55-2157.82 (392.362±554.7)	0-405.17 (67.57±99.3)	11.77-1571.25 (312.34±243)
Fungi	11.77-315.66 (88.03±83.3)	0.0-713.77 (185.35±185.4)	11.77-252.05 (88.47±67.7)	47.11-374.55 (188.89±136.6)

Range, (Mean ±SD)

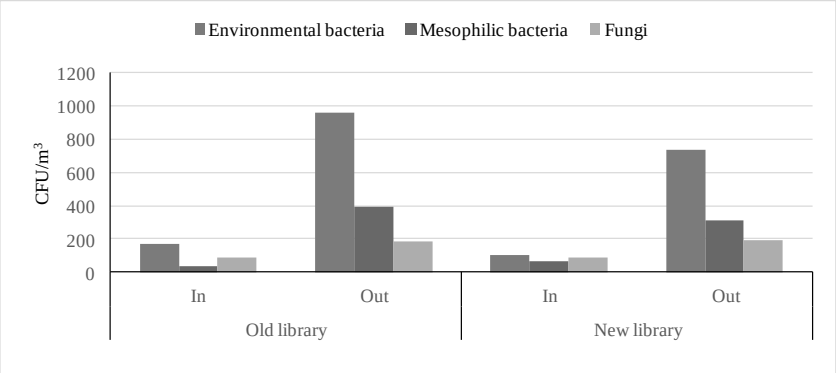

Fig. 2. Mean concentrations of total airborne microorganisms registered at the both libraries.

Microbial size fraction

The diameter of a microbial particle will impact how deep the particle may penetrate into the lungs. The mean concentrations of fine (≤8µm) and coarse (≥ 8µm) culturable airborne microbial concentrations are shown in Table 2. The fine size fraction of environmental bacteria ranged from 0-1036.51 CFU/m^3 indoors and 0-4030.62 CFU/m^3 outdoors, while mesophilic bacterial concentrations ranged within 0 - 35.33 CFU/m^3 indoors and 0-254.41 CFU/m^3 outdoors. In contrast, fine size fraction of fungal concentrations ranged within 0 - 315.6 CFU/m^{3}indoors and 0- 508.83 CFU/m^{3}outdoors.

TABLE 2. The range and mean concentrations of coarse (≥8 µm) and fine (8≤µm) culturable airborne microorganisms at two libraries at the main campus of the National Research Centre (NRC), Dokki, Giza.

Location	CFU/m^3					
	Bacteria				Fungi	
	Environmental		Mesophilic			
	≥ 8µm	≤ 8µm	≥ 8µm	≤ 8µm	≥ 8µm	≤ 8µm
Old Indoor	0.0-1036.51 (149.2±294.9)	0.0-58.89 (18.4±17.6)	0.0-83.62 (24.4±23.2)	0.0-35.33 (8.09±9.9)	0.0-23.55 (5.15±7.2)	11.77-315.66 (82.9±81.7)
Outdoor	23.6-4030.62 (858.2±1241.9)	0.0-849.23 (103.2±225.3)	23.55-1903.4 (356.4±496.18)	0.0-254.41 (35.9±68.6)	0.0-204.94 (53.4±48.3)	0.0-508.83 (131.1±144.5)
New Indoor	0.0-329.79 (89.6±101.9)	0.0-58.33 (11.8±15.6)	0.0-393.4 (60.2±96.6)	0.0-23.55 (7.4±7.0)	0.0-35.33 (15. 5±14.3)	0.0-216.72 (73 ±64.2)
Outdoor	0.0-1765.6 (719.5±623.8)	0.0-107.2 (19.2±28.7)	0.0-1418.1 (298.4±377.9)	0.0-153.12 (13.9±36.4)	35.3-130.74 (59.8±24.8)	0.0-405.18 (129.1±128.4)

Range, (Mean ±SD).

Fine fraction of environmental bacteria represented small percentages in indoors (11-11.6%) and outdoors (2.6- 10.74%), while mesophilic bacteria represented relatively higher percentages indoors (10.87 - 24.94 %) and outdoors (4.48 - 9.16 %). Fine fraction of fungi constituted the majority of size ranges ~ 82.54-94.15% indoors and 68.35- 71.16% outdoors. Significant differences were found between the concentrations of coarse fractions of mesophilic bacteria (*P*= 0.0286) and fungi (P= 0.0143) registered in the outdoor and indoor at the old library and the new library, respectively, higher concentrations shifted toward outdoors.

Indoor / Outdoor (I/O) ratio

The I/O ratios of airborne microbial concentrations are illustrated in Fig. 3. I/O ratios were always less than 1 at both libraries. The I/O ratios for environmental bacteria, mesophilic bacteria and fungi reached 0.24, 0.08 and 0.47, respectively at the old library, and they were 0.13, 0.21 and 0.46 for the corresponding microbial parameters at the new library. I/O ratios of environmental bacteria were relatively higher at the old library while mesophilic bacteria at the new library (Fig. 3).

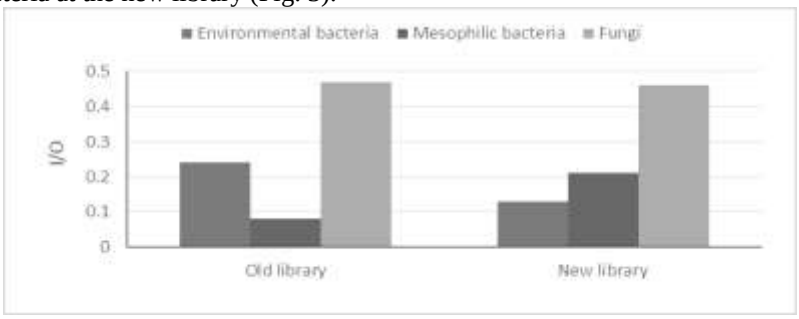

Fig. 3. I/O ratios of total airborne microorganisms at the both libraries

Microorganisms associated surface dust

Table 3 shows range and mean concentrations of bacteria and fungi associated surface settled dust at the two libraries. Concentrations of bacteria associated dust exceeded fungi; however the environmental bacteria were higher than mesophilic ones. Environmental bacteria associated settled dust ranged from $1.2x10^5$ – 7.9×10^6 CFU/gm indoors and $6 x10^4$ -10×10^6 CFU/gm outdoors, while mesophilic bacteria ranged within $4x10^4$ -5.68×10^6 CFU/gm indoors and $6x10^4$ - 3.3×10^6 CFU/gm outdoors. Fungal concentrations were found in the range of 0- 6.3×10^5 CFU/gm indoors and $1x10^4$- 7.5×10^5 CFU/gm outdoors. At the old library, fungi associated dust concentrations significantly differed (P=0.0143) indoor and outdoor. Moreover indoor fungal concentrations (P = 0.0143) significantly differed at both libraries, higher concentrations shifted towards the new library.

TABLE 3. The range and mean concentrations of microorganisms associated settled dust (≤ 45µm) at two libraries at the main campus of the National Research Centre (NRC), Dokki, Giza.

Location	CFU/gm×10^5				CFU/gm×10^4	
	bacteria				Fungi	
	Environmental		Mesophilic			
	Range	Mean± SD	Range	Mean± SD	Range	Mean±SD
Old Indoor	1.2-79.0	12.08±18.55	0.4-48.8	10.72±13.91	0.0-7.0	2.4±1.5
Outdoor	0.6-100	19.93±26.11	0.6-30.0	6.17±8.16	1.0-75.0	12.1±14.7
NewIndoor	3.2-47.2	19.55±13.69	1.8-56.8	9.51±11.16	2.0-63.0	11.0±12.1
Outdoor	10.0-60.6	31.52±19.08	3.2-33.0	14.45±8.27	1.0-50.0	10.0±11.1

Particulate matter

The mean concentrations of PM at both libraries are graphically illustrated in Fig. 4. PM concentrations ranged between 203.52 - 395.16 µg/m^3, with the greatest mean values of 395.16 µg/m^3 indoors and 306 µg/m^3 outdoors. PM concentrations were higher at the old library with I/O ratio exceeded 1. Significant difference was found between the outdoor PM concentrations at the new and old libraries (P =0.0143), higher concentrations shifted toward the old library.

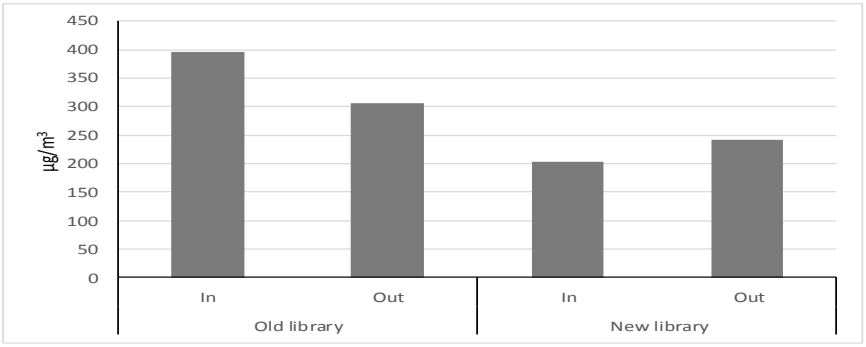

Fig. 4. The mean concentrations of PM indoors and outdoors of the both libraries.

Influence of environmental factors on microbial survivability

Spearman's rank correlations between airborne microorganisms with temperature, relative humidity and PM are shown in Table 4. Complex correlations were found between airborne microorganisms and environmental factors. Generally, temperature negatively affected bacterial survivability at both libraries. Significant negative correlations ($P \leq 0.05$) were found between temperature and airborne bacteria, both environmental and mesophilic bacteria, at the old library. Significant negative correlations were found between temperature and fungi in outdoor the old library ($r = -0.67$) and indoor the new library ($r = -0.61$), (Table 4). Relative humidity (RH%) showed significant positive correlations with indoor and outdoor environmental bacteria at the old library. However, significant positive correlations were found between relative humidity and mesophilic bacteria outdoor the old library ($r = 0.77$) and indoor the new library ($r = 0.51$). In contrast, negative and positive correlations were found between RH% with indoor and outdoor fungi at the old and new libraries, respectively.

TABLE 4. Spearman's rank correlation coefficients between airborne microorganisms with microclimatic parameters and particulate matter (PM).

Location	T°C			RH%			PM		
	Bacteria		Fungi	Bacteria		Fungi	Bacteria		Fungi
	Env.	Meso.		Env.	Meso.		Env.	Meso.	
Old Indoor	–0.61*	–0.67*	0.22	0.76*	0.12	–0.32	0.33	0.15	–0.62*
Outdoor	–0.82*	–0.75*	–0.67*	0.66*	0.77*	–0.58*	0.2	0.59*	0.052
New Indoor	–0.42	–0.29	–0.61*	0.42	0.51*	0.61*	–0.8*	–0.77*	–0.44*
Outdoor	–0.48*	–0.13	0.1	0.40	–0.06	0.16	–0.26	0.26	0.20

Env: Environmental bacteria; Meso: Mesophilic bacteria;* significant ($p \leq 0.05$).

PM showed positive correlations with airborne bacteria at the old library and negative correlations with indoor and outdoor airborne bacteria at the new library, except with outdoor airborne mesophilic bacteria (positively correlated with PM). Positive and negative correlations were found between PM with fungi outdoors and indoors, respectively (Table 4).

Microbial biodiversity

Table 5 shows percentages of bacterial taxa isolated from the air state and surface settled dust. No Gram negative bacteria were isolated, however Gram positive cocci and bacilli constituted 14.28 - 47.06% and 52.94-85.72%, respectively of the total bacterial isolates. *Bacillus pseudomycoides* and *B. subtilis* were frequently found indoors while *B. subtilis* and *Staphylococcus* outdoors. *Alloiococcus otitis* and *Macrococcus caseolyticus* were only found in the air state at the new library. *Bacillus* was found to be the only genus associated surface settled dust, and represented by: *B. atrophaeus*, *B. pumilus*, *B. subtilis* and *Paenibacillus glucanolyticus*.

TABLE 5. Bacterial taxa isolated from the air state and settled dust at two libraries at the main campus of the National Research Centre (NRC), Dokki, Giza.

Species	Medium	Bacteria %							
		Environmental				Mesophilic			
		Old Library		New Library		Old Library		New Library	
		In	Out	In	Out	In	Out	In	Out
Alloiococcus otitis	Air Dust	0 0	0 0	9.52 0	0 0	0 0	0 0	0 0	5 0
Bacillus atrophaeus	Air Dust	0 50	0 12.5	4.76 27.28	5 50	15 44.45	15.38 22.22	14.28 27.28	20 44.44
Bacillus licheniformis	Air Dust	50 0	0 0	9.52 0	5 0	5 0	0 0	0 0	0 0
Bacillus pseudomycoides	Air Dust	10 0	0 0	4.76 0	15 0	15 0	7.69 0	14.28 0	10 0
Bacillus pumilus	Air Dust	0 37.5	11.76 25	0 36.36	5 25	0 55.55	0 44.45	14.28 54.54	5 11.12
Bacillus subtilis	Air Dust	20 12.5	41.18 50	19.04 36.36	15 25	30 0	26.92 33.33	28.6 18.18	15 44.44
Macrococcus caseolyticus	Air Dust	0 0	0 0	4.76 0	0 0	0 0	0 0	0 0	0 0
Paenibacillus amylolyticus	Air Dust	0 0	0 0	14.28 0	25 0	0 0	3.84 0	7.14 0	30 0
Paenibacillus glucanolyticus	Air Dust	0 0	0 12.5	9.52 0	0 0	5 0	0 0	7.14 0	0 0
Rothia nasimurium	Air Dust	0 0	11.76 0	4.76 0	0 0	10 0	23.1 0	14.28 0	10 0
Staphylococcus aureus	Air Dust	10 0	17.65 0	19.08 0	15 0	5 0	15.38 0	0 0	0 0
Staphylococcus species	Air Dust	10 0	17.65 0	0 0	15 0	15 0	7.69 0	0 0	5 0

The biodiversity of fungal taxa isolated from air state and surface settled dust is illustrated graphically in Fig. 5 a &b. A total of 15 fungal taxa were identified as *Aspergillus*, *Penicillium* and *Cladosporium* were the predominant types at both libraries. A relatively different fungal content was found between the air state and surface settled dust. *Monilia* and *Mucor* species were only isolated from the air state at the old library. *Epicoccum* and *Nigrospora* species were only isolated from the surface settled dust at the old library while *Trichoderma viride* at the new library.

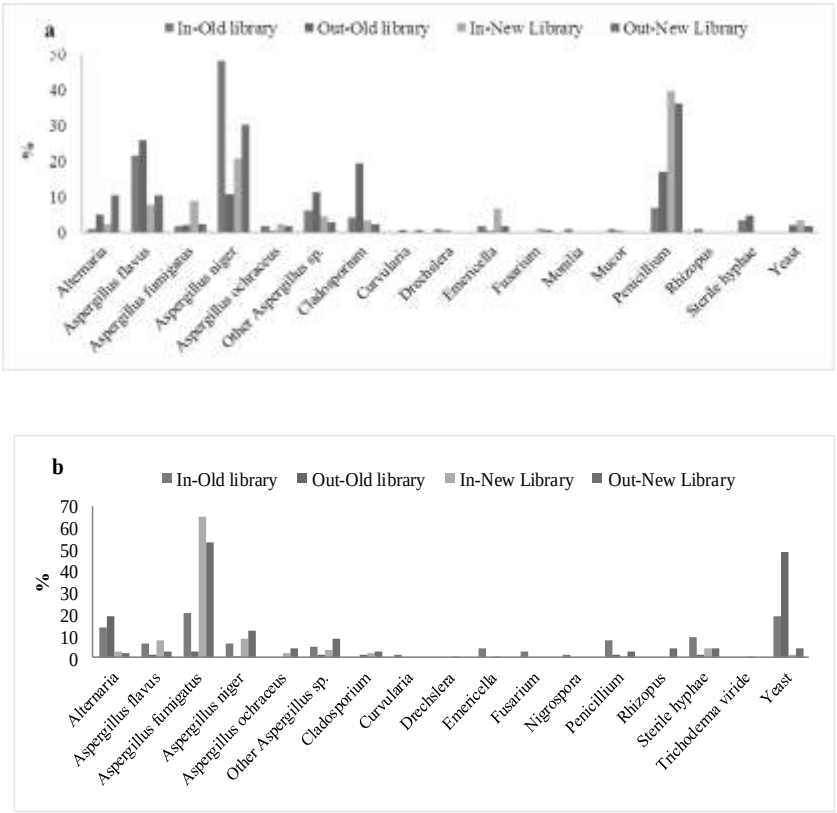

Fig. 5. Percentages of the identified fungal taxa isolated from air state (a) and settled dust (b) at two libraries at the main campus of the National Research Centre (NRC), Dokki, Giza.

Enzymatic activities

A total of 22 fungal isolates were screened for proteolytic, lipolytic and cellulolytic enzyme activities (Table 6). Many of the isolated fungal taxa had more than one of the enzymatic properties/activities. The majority of fungal taxa had cellulolytic (16 taxa) and lipolytic (15 taxa) properties and only 10 fungal

taxa had proteolytic properties. Five fungal taxa namely: *Aspergillus flavus*, *Curvularia pallescens*, *Fusarium oxysporium*, *Penicillium notatum* and *Trichoderma viride* had proteolytic, lipolytic and cellulolytic properties.

Medical effects

Table 7 shows the health complaints among employees at both libraries. The employees at the new library had lower complaints for most of symptoms than those at the old library. Symptoms of the central nervous system including headache and fatigue were the common complaints among employees at both libraries. Higher portions of employees at the old library reported wheezing, shortness of breath, irritation of the eyes, cough, drowsiness, dizziness, dermatitis and throat irritation compared to those reported among employees at the new library.

TABLE 6. Qualitative detection of cellulases, lipases and proteases in the isolated fungal taxa.

Species	Enzyme		
	Cellulase	**Lipase**	**Protease**
Alternaria alternata	√	√	*ND*
Aspergillus flavus[1]	√	√	*ND*
A. flavus[2]	√	√	√
A. fumigatus	*ND*	√	*ND*
A. nidulans	√	*ND*	√
A. niger	*ND*	√	*ND*
A. ochraceus	√	*ND*	√
Cladosporium cladosporioides	√	*ND*	*ND*
Curvularia lunata	√	*ND*	√
Curvularia pallescens	√	√	√
Dreschlera species	√	*ND*	√
Epicoccum species	√	*ND*	*ND*
Fusarium oxysporium	√	√	√
Mucor species	√	√	*ND*
Penicillium chrysogenum	*ND*	√	*ND*
P. digitatum	√	√	*ND*
P. expansum	√	√	*ND*
P. italicum	*ND*	√	*ND*
P. notatum	√	√	√
Rhizopus stolonifer	*ND*	√	√
Trichoderma viride	√	√	√
Yeast species	*ND*	*ND*	*ND*

ND: not detected.

TABLE 7. Symptoms indicated by questionnaire for employees at two libraries at the main campus of the National Research Centre (NRC), Dokki, Giza during the period of study (January to December 2013).

Symptom %	library	
	Old	New
Shortness of breath	51.61	0.00
Cough	45.16	12.50
Wheezing	38.71	0.00
Dry Eye	35.48	8.33
Watering eyes	29.03	4.17
Irritation of the eyes	54.84	16.67
Allergic Conjunctivitis	58.06	12.50
Sneezing	61.29	12.50
Stuffy nose	64.52	4.17
Coryza	67.74	16.67
Throat irritation	58.06	0.00
Headache	61.29	41.67
Fatigue	77.42	45.83
Drowsiness	64.52	4.17
Dizziness	54.84	4.17
Nausea	35.48	4.17
Dermatitis	64.52	4.17
Employees interviewed (n)	**31**	**24**

Discussion

Airborne bacteria in the present study showed variations ranged between 10^2-10^3 CFU/m^3 and fungi between 10^1-10^2 CFU/m^3 for indoors and outdoors concentrations. Environmental bacteria achieved the greatest microbial concentrations and mesophilic bacteria significantly differed indoors and outdoors at the old library. Generally microorganisms of indoor air depend on many factors including for example, number of people present and their hygienic standards, ventilation type and geographical location (Abdel Hameed *et al.*, 2012). These conditions vary greatly between regions and buildings. Bacteria are mainly related to human activities, bacteria mainly shed from human skin and respiratory tracts (Fraser *et al.*, 1977 and Chen & Hildemann, 2009).

Higher airborne microbial concentrations indoors and outdoors were found at the old library compared to the new one. These variations are attributed to building location and ventilation type (natural ventilation). Mechanical ventilation reduces infiltration of microorganisms derived from outdoor at the new library. Moreover the old library is more impacted by traffic activities; it is faced to a busy traffic street. The age of library may also be a factor in microbial

generation. Older library is more likely to have issues such as dust buildup, or collected debris that could contribute to microbial generation, accumulated dust may create microenvironment for microbial growth. Moreover air movement within the libraries and outdoors help aerosolize microbial particles or keep them aloft.

Concentrations of airborne fungi inside the tested libraries were low. Indoor fungal concentrations were found in the range of 19-86 CFU/m^3in Poland (Karbowska-Berent *et al.,* 2011), 524-1992 CFU/m^3 in Ethiopia (Hayleeyesus and Manaye, 2014), 29-2000 CFU/m^3 in Italy (Micheluz *et al.,* 2015) and 59-327 CFU/m^3 in Cuba (Anaya et al., 2016), these results are compatible with the results in the present study. On the other hand the results in the present study were lower than those previously found in libraries Egypt. Awad and Farag (1999) found airborne bacterial and fungal concentrations at mean values of 8.9×10^3 CFU/m^3 and 1.337×10^3 CFU/m^3, respectively, in the National Research Center library (old library). In addition Sahab *et al.* (2014) found airborne bacteria and fungi concentrations in the range of 94.35-660.48 CFU $x10^3$ /m^3 and 15.72-369.45 CFU $x10^3$ /m^3 in the National Library and Archives of Egypt. These differences may be attributed to different sampling methods and culture media used for microbial growth in addition to variations in human activities and number of persons visit libraries, nowadays less number of people visit book libraries depending on website and electronic books.

There are no acceptable threshold limit values (TLVs) for bioaerosols, however some recommendations are available (Cappitelli *et al.,* 2010). Concentrations of airborne microorganisms during the present study did not exceed the acceptable limit of 5000 CFU/m^3 for public facilities (Górny, 2004; Augustynska *et al.,* 2010). Moreover fungal concentrations were lower than the TLVs of the occupational exposure specified by the Polish Committee for the Highest Permissible Concentrations and Intensities of Noxious Agents at the Workplace (5×10^4 CFU/m^3 for total fungi), (Skowron and Górny, 2012). Indoor fungal concentrations were < 150 CFU/m^3 value recommended for Italian libraries (MIBAC, 2001) and the maximum value of 1000 CFU/m^3 for archives (Nunes *et al.,* 2013).

The variation of microbial concentrations may be attributed to differences in their size distribution. The size of microbial particles depends on many factors for example, ventilation type, location, anthropogenic activities and nature of the major source. Size of microorganism determines its behavior in the air (Nicholson, 1988). The majority of airborne bacterial concentrations were found in the coarse size fraction (≥8µm). This is due to bacteria are mainly attached to PM, depending on the degree of urbanization, or present as aggregates, although coarse particles are settled down faster than fine ones. Bacteria are usually found in the range of 4-20 µm equivalent diameters (Noble *et al.,* 1963). Re-suspension of dust particles increases bacterial loads in the air (Fröhlich-Nowoisky, 2011).

However, fine fractions of fungi were found in high percentages ≥ 60%, as fungal spores are found individually in the environment and/or not hydrophilic. Species of *Aspergillus, Penicillium* and *Cladosporium* with aerodynamic diameters less than 8 µm were found in high percentage in air of museum store room, indicated that fungi found as single cells, spores or small fragments of mycelium (Skóra *et al.*, 2015) It is important to sample microbial particles as size fractions, since particles with smaller diameters are more likely to enter the lungs and thus may negatively impact human health. Fine size microbial fractions are able to penetrate the respiratory system of human, causing irritation to mucous membrane of nose and eyes, inflammatory responses and allergic reactions (Kulkarni *et al.*, 2011).

I/O ratio documents the presence/ or absence of indoor biocontaminant sources (ACGIH, 1999). In this work I/O ratios were always ≤1, suggesting no indoor bio-contaminant sources inside both libraries. I/O ratios of the environmental bacteria were relatively higher at the old library while mesophilic bacteria at the new library. This reflects role of ventilation type and number of visitors at each library respectively. A relatively high I/O ratios of fungi (averaged ~ 0.46) confirm derived of fungi from outdoors /or attached clothes and foot wear and settled dust.

In this work microorganism associated settled dust averaged 10^5 CFU/g for bacteria and 10^4 CFU/g for fungi. Microbial contents of settled dust varied depending on the amount and type of outdoor dust particles infiltrated indoors and hygienic rules. Dust can absorb moisture and provide nutrients and protection to microorganisms (Simoes *et al.*, 2011) and it may form a microenvironment for microbial growth (Florian, 1997). Microorganisms associated dust may have potential damage of surfaces (Gallo *et al.*, 1996) and settled dust can be dispersed elsewhere in the building under the influence of air current and human activities, constituting a source of indoor air microbial contamination (CEC, 1993). Outdoor dust particles are mainly emitted from traffic activities nearby the old library while garden activities and sweeping NRC's streets near to the new library.

Indoor and outdoor PM concentrations during the present study exceeded the limit value of 120 µg/m^3 recommended by the World Health Organization (WHO, 2000) and 230 µg/m^3 suggested by the Egyptian Environmental law (EEAA, 1994). However no obvious correlations were found between PM and airborne microorganisms, i.e. PM may not affect microbial concentrations. PM may serve as a carrier/ and source of nutrients for microorganisms, and at the same time may affect microbial viability and behavior (Alghamdi *et al.*, 2014), depending on particle composition and microbial type and its tenacity to PM. PM showed positive and negative correlations with bacteria and fungi at the old and the new library. It is suggested that indoor fungi actively released indoors and dust played an important role in releasing of fungi outdoors. The negative correlations between bacteria and PM at the new library confirmed the role of PM as an essential agent for nutrient and protection of bacteria (the new library is cleaner

than the old one). Wan *et al.* (2011) found a significant correlation between bacteria and PM.

Microclimatic factors had complex influences on survival of airborne microorganisms. Relative humidity was the crucial factor supporting microbial survivability and temperature was the most detrimental factor, the influence of microclimatic factors varied depending on microbial type. Microclimatic conditions mainly cause desiccation/ or hygroscopicity effects on microorganisms (Cole and cook, 1998). Tang (2009) and Abdel Hameed *et al.* (2013) found different responds between bacteria and fungi to microclimatic conditions and environmental factors. Karbowska-Berent *et al.* (2011) found that RH% $\leq$ 65% detrimentally affected bacterial growth, and RH% had significant influence on fungal concentrations, while in other cases T°C and RH% had no significant influence on microbial populations.

Gram positive cocci and bacilli were quite numerous in the air state and settled dust. These organisms normally exist on human skin and related to human activities. Gram positive bacteria are more resistant to sever environmental conditions (Beggs, 2003). *Staphylococcus* bacteria are related to human skin and *Bacillus* and spore forming bacteria are numerous in dust particles and their concentrations differed from place to place depending on microenvironment and human activities (Burkowska *et al.*, 2012). It has been reported that high concentration of *Bacillus* species in indoor air is usually indicative of water damage/ or lack of building maintenance (Baxter *et al.*, 1981). *Bacillus* bacteria have ability to form spores, allow them to resist the unfavorable conditions long period of time (Karbowska-Berent *et al.*, 2011). *Bacillus* and *Streptomyces* species constitute potential risk for paper, because they can produce cellulose degrading enzyme (Ramırez and Coha, 2003).

The results in the present study agree with the findings obtained by other investigators. Borrego and Perdomo (2012) found that Gram positive cocci and bacilli bacteria are abundant in libraries in Cuba. Moreover, species of *Micrococcus* (Harkawy *et al.*, 2011), *Staphylococcus* (Hayleeyesus and Manaye, 2014) and *Bacillus* (Skóra *et al.*, 2015) were found to be the common bacterial genera in indoors environment of libraries and archives.

The majority of fungal taxa isolated from libraries are commonly existed in the ambient air such as *Aspergillus*, *Penicillium*, and *Cladosporium*. *Aspergillus* and *Penicillium* are able to colonize different substrates and resist unfavorable conditions (Araujo and Cabral, 2010). *Cladosporium*, *Penicillium* and *Aspergillus* were the common indoor fungi in libraries and archives (Micheluz *et al.*, 2015; Skóra *et al.*, 2015 and Anaya *et al.*, 2016). *A. versicolor*, *Paecilomyces* and *A. fumigatus* were numerous in the library in Poland (Cieplik, 1997). *Penicillium*, *A. flavus*, *Trichoderma viride*, *Alternaria tenuis* and *A. niger* constituted 54.42% of the total air spores in the National Library of Egypt (Sahab *et al.*, 2014).

The identification of fungi associated settled dust (Fig. 5b) showed that composition of fungal taxa reflected composition of airborne fungi (Fig. 5a), confirming that settled dust is an important source of airborne fungi. *Nigrospora* species and *Trichoderma viride* were only found in the settled dust whereas *Monilia* and *Mucor* in the air state. *Cladosporium*, *Penicillium* dominated in the air state, and yeast in the settled dust. This may be attributed to periodicity of source, location and, and microclimatic conditions. Moreover aerodynamic diameter determines fungal transport in the air, and different fungal size distribution would be expected to cause different behavior in the air (Baron and Willeke, 1993). Pasanen *et al.* (1991) reported that the minimum air velocity at which *Cladosporium* released spores was 1 m/s and 0.5 m/s for release of *Aspergillus* and *Penicillium*, respectively.

The qualitative composition of air microorganisms is important indoor issue due to the fact that they may pose threats to book collections and human health. Some fungi can cause allergy–mediated diseases (Karbowska-Bernet *et al.*, 2007) and mycotoxicosis in human (Flanningan, 2001). *Aspergillus*, *Mucor* and *Rhizopus* pose threat to vulnerable individuals (Soleimani *et al.*, 2013). *Aspergillus fumigatus*, *A. flavus* and *Stachybotrys chartarum* should not be present in the air of library and their concentrations should be zero CFU/m^3 (Górny *et al.*, 2004 and Augustynska *et al.*, 2010). *Staphylococcus* and *Bacillus* bacteria were numerous in the environment and may have been transferred to book as a result of contact with readers causing respiratory diseases.

In this work, many of fungal isolates had enzymatic properties; *A. flavus*, *Curvulari apallescens*, *Fusarium oxysporium*, *Penicillium notatum* and *Trichoderma viride* had proteolytic, lipolytic and cellulolytic activities, which may damage library materials. May *et al.* (1993) listed fungi among the major agents of microbial deteriorations of surfaces and Leznicka *et al.* (1988) pointed to the significance of dematiaceous fungi in the staining and deterioration of works of arts. Urzi *et al.* (2001) reported that once fungi settle on the colonize surface they attribute a great variety of alternation like black paints, intergranular growth and biopitting. Fungal species exhibit strong cellulolytic (*e.g. Trichoderma, Penicillium, Botrytis, Chaetomium, Stemphylium* and *Alternaria*), proteolytic (*e.g. Mucor, Aureobasidium, Chaetomium, Trichoderma, Verticillium* and *Epicoccum*) and lipolytic (as above plus *Paecilomyces*) properties (Karbowska-Berent *et al.*, 2011).

Although the obtained viable microbial concentrations were low in the air or settled dust, they may pose health threats. Indoor microorganisms may cause health symptoms (Mishra *et al.*, 1992). In the present work the severity of health symptoms was higher among employees at the older library, indoor air quality was worse at the older library. The development of specific symptoms (*e.g.*, multiple respiratory symptoms and atopic group) may not obviously clear among employees because several symptoms may be related to indoor environmental factors including PM, bio-contamination, ventilation, Job stress, space and heat comfort (Mendell *et al.*, 1996). Generally, there is a lack of information of how

the different physical, psychological stress, chemical and biological contaminants interacts to produce discomfort and complaints in non-industrial settings in Egypt. The limitation in the interview is the employee cannot accurately determine that the health symptoms may be building related.

Conclusion

Little information is available on microbiological air quality in non–industrial settings in Egypt**.** This work aims to evaluate microbial indoor air quality at libraries and factors affecting their prevalence. The concentrations and types of microorganisms were library dependent, the older library had higher airborne microbial concentrations than the new one. Dust buildup and ventilation were considered to be the main factors influence indoor microbial concentrations. Environmental bacteria achieved the greatest airborne microbial concentrations while mesophilic bacteria significantly differed between indoors and outdoors at the older library. I/O ratios of airborne microorganisms were always ≤ 1 confirming no permanent indoor bio-contaminant sources at both libraries. Microclimatic factors had complex influences on airborne microbial survivability; however relative humidity was the crucial factor supporting their survival, depending on microbial type. Gram positive cocci and bacilli were commonly found in the air state and settled the dust. *Aspergillus*, *Penicillium* and *Cladosporium* were the common fungal species. A composition of fungi taxa associated settled dust reflected the composition of airborne fungi. Many of the isolated fungal taxa had enzymatic properties which may damage library materials. Although the microbial concentrations were low but the obtained microorganisms might pose health threats.

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تلوث الهواء الميكروبي و العوامل التى تؤثر في حدوثه في بعض مكتبات الكتب، مصر.

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تهدف الدراسة الى تقييم جودة الهواء الميكروبية داخل المكتبات المصرية. تم تجميع العينات من مكتبتى المركز القومي للبحوث (المكتبة القديمة و المكتبة الجديدة) والتى تختلفان من حيث عمر المبنى ، المساحة ، عدد الزائرين و طرق التهويه .تم تجميع الدلائل الميكروبية " بكتيريا و فطريات الهواء" باستخدام جهاز اندرسون ذو المرحلتين (الاكبر من و الاقل من ٨ ميكروميتر). اوضحت النتائج ان تركيزات البكتيريا في الهواء تراوحت ما بين ٠-١٠٦٠,٤ مستعمرة/ م^{٣} والفطريات ما بين ١١,٨- ٣١٥,٦مستعمرة / م^{٣}. اظهرت الدراسة تواجد البكتيريا و الفطريات المرتبطة بالاتربة المترسبة على الاسطح بتركيزات تراوحت بين ٠,٤-١٠٠×١٠^{5}و ٠-٧٣×١٠^{٤} على التوالي.

مثلت البكتيريا الموجبة للجرام الكروية و العصوية نسب تراوحت بين (١٤,٣-٤٧%) و (٥٢,٩-٨٥,٧%) من اجمالي العزلات البكتيريه المعزولة من الهواء على التوالي، بينما شكلت البكتيريا العصوية الموجبة لجرام نسبة ١٠٠% من اجمالي العزلات البكتيريه المعزولة من الاتربة . كان فطري الاسبرجلس و البنسليوم من الفطريات الاكثر شيوعا بالهواء و الاتربة داخل المكتبات ، واظهرت العديد من الفطريات قدرتها على افراز انزيمات السليوليز، الليبيزو والبروتيزمما يشير الى مدى خطورتها على الصحه العامة للعاملين اضافه الى التاثير البيولوجي الضار على الكتب و المواد المحفوظه بالمكتبات. اوضحت النتائج ان الرطوبه النسبية من العوامل الاساسية التى تؤثر على حيوية الكائنات الحية بالبيئة الداخلية للمكتبات.