



## Mycobiota of outdoor air that can cause asthma: a case study from Lake Manzala, Egypt

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### Abstract

Continuous sampling of airspora in Lake Manzala was carried out monthly over a period of 1 year at five sites with an automated air sampler on Czapek's yeast extract, DG-18 and potato dextrose agar media plates. A total of 71780 mould- and 560 yeast colony-forming units were recovered from 600 exposures and the isolated taxa were assigned to 28 genera and 43 species. A greater presence of fungal spores occurred in the summer. *Aspergillus niger*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Aureobasidium pullulans*, *Alternaria cheiranthi*, *Penicillium chrysogenum*, *Aspergillus fumigatus* and *Alternaria alternata* were the predominant species. Many of the identified species have an aerodynamic diameter ( $d_{ae}$ ) of 1.5-10  $\mu\text{m}$  that can deeply penetrate into the lungs. *Aspergillus*, *Cladosporium*, *Penicillium*, and *Alternaria* that had the greatest frequencies in air of Lake Manzala are strongly associated with allergic respiratory disease, especially asthma, in Port Said and Ismailia governorates. A comprehensive model of factors that contribute to asthma in the region is needed and this can be a useful tool for planning efforts and disease prevention. The obtained results indicated that the fungi of air should be considered when the quality of Egyptian air is assessed.

**Key words** – Aerodynamic diameter – aeromycobiota- allergy – diversity – Protectorate

### Introduction

Manzala's lagoon is the largest body of brackish water (~1071km<sup>2</sup>) of the Nile Delta coastal lakes. The lagoon has a rhombohedral-shape formed in the actively subsiding delta plain (Stanley 1988), and is shallow, with a depth rarely exceeding one meter (Tahoun 2007). It lies between 31° 00" and 31° 30" latitude and 31° 45" and 32° 20" longitude, in the northern quadrant of the delta between the Mediterranean Sea to the north, the Suez Canal, Port Said and Ismailia governorates to the east, the Damietta Branch of the River Nile and the governorates of Sharkiya and Dakahliya to the west (Zahran 2010). The total population of the governorates and their surrounds overlooking the Manzala lagoon is 14 514 000 (<http://www.citypopulation.de/Egypt.html>). It is connected with the Mediterranean Sea by five straits, permitting the exchange of water and biota between the lake and the sea (Rashad and Abdel-Azeem 2010). The Lake is considered an important and valuable natural resource ecosystem for fish catch, wildlife, hydrological and biological regime in Egypt.

Lake Manzala attracts attention of many scientists because of its important economical aspects. Rashad and Abdel-Azeem (2010) listed a set of 447 references that could be considered an updated bibliography on Lake Manzala published during the last two hundred years, out of which 8 references were about fungi.

Research on mycobiota of the protected areas in Egypt is accumulating in the last decade but with little or no concern is given to air fungi as a group playing an important role in the environment except the sole study carried by Abdel-Azeem (2009) on mycobiota of different habitats in Saint Katherine Protectorate.

In other parts of Egypt, little attention has been paid to outdoor airborne mycobiota (Saad 1958, Zaky 1960, Moustafa 1971, Moubasher and Moustafa 1974, Abdel-Hafez et al. 1986). Airborne fungal concentrations have been monitored in some Egyptian cities including Ismailia (Abdul Wahid et al. 1996, Abdel-Azeem 2003), Cairo (Abdel Hameed et al. 1999), Menofia (Abdel Hameed and Khoder 2001), Western Desert (Ismail et al. 2002), Giza (Abdel Hameed et al. 2007), and New Damietta (El-Morsy 2006). Diurnal distribution of airborne fungi was only studied in Upper Egypt, at Assiut (Abu-El-Souod 1974), Qena (Abdel-Fattah et al. 1981) and Helwan (Abdel Hameed et al. 2009).

Airborne fungi have been found to be associated with certain respiratory illnesses and allergies (Kowalski and Bahnfleth 1998), while exposure to fungi and other microbes, their fragments and metabolites may constitute a health risk: for example, increases in asthma attacks and bronchial hyperactivity and other respiratory symptoms such as lung cancer have been correlated to increased microbial and particulate levels in the aerosphere (Ross et al. 2000). More than 80 genera of fungi have been linked with symptoms of respiratory tract allergies (Horner et al. 1995), with the most common allergenic genera being *Cladosporium*, *Alternaria*, *Aspergillus* and *Fusarium*. Exposure to large concentrations of the spores of these four genera causes aspergillosis (Anderson et al. 1996), asthma and pneumonitis (Cuijpers et al. 1995, Hu et al. 1997), allergic alveolitis and toxicosis (Flannigan et al. 1991).

Asthma is one of the most common chronic diseases in the world; with reported estimates of 300 million people worldwide currently having asthma, and suggesting that such figure increases by 50% every decade (Masoli et al. 2004). Many environmental risk factors are thought to play an important role in asthma e.g. dust mites, pet dander, cockroaches and fungi among others (Bush 2001, King et al. 2004). Asthma burden in some countries has warranted government intervention and consideration in their health strategies. Some estimates have stated that asthma may account for 1 in every 250 deaths worldwide (Masoli et al. 2004).

The prevalence of asthma among Egyptians ranged between 4.8% to 9.4 (Khallaf et al. 1993, Georgy et al. 2006, Halim et al. 2013). Despite a large volume of clinical and epidemiological researches within affected populations, the etiology and risk factors of these conditions remains poorly understood.

According to Levetin (1995) fungal spores are between 2  $\mu\text{m}$  and 100  $\mu\text{m}$ . Eduard et al. (2001) have studied spores from aerosols by microscopy and they have discriminated fungal spores by size always greater than 1.5  $\mu\text{m}$ . Table 1 presents aerodynamic diameters ( $d_{ae}$ ) of fungi in aerosols and shows that fungi are often present as particles with  $d_{ae}$  between 2 and 6  $\mu\text{m}$ . The small fungal particles in aerosols from pure fungal cultures have  $d_{ae}$  of different sizes and are ~0.4 to 1.1  $\mu\text{m}$  (Table 1).

Following the recent publications on the assessment of Egyptian fungi (Abdel-Azeem 2010) and published work on Lake Manzala (Rashad and Abdel-Azeem 2010) the objectives of this study were to: 1- close gaps in biodiversity knowledge on the diversity of Egyptian fungi, 2- determine the structure and diversity of aeromycobiota in Ashtoum El-Gamil protectorate, Lake Manzala, Egypt which has not never been the target of any study before and 3- raise the awareness about the adverse health effects due to exposure of air fungi.

**Table 1** Diameters (d) and aerodynamic diameters ( $d_{ae}$ ) of some common fungi.

| Species                             | Spore dimension ( $\mu\text{m}$ ) | References           | $d_{ae}$ of spores | References                  |
|-------------------------------------|-----------------------------------|----------------------|--------------------|-----------------------------|
| <i>Aspergillus flavus</i>           | 3.5–4.5                           | Domsch et al. (2007) | 3.3–3.8a           | Madelin and Johnson (1992)  |
| <i>A. fumigatus</i>                 | 2.5–3.0                           | Domsch et al. (2007) | 3.1b               | Lacey and Dutkiewicz (1976) |
| <i>Cladosporium cladosporioides</i> | 2–11                              | Domsch et al. (2007) | 2.3–2.5 a          | Madelin and Johnson (1992)  |
| <i>C. sphaerospermum</i>            | 3–7                               | Domsch et al. (2007) | 1.1 a              | Madsen et al. (2009)        |
| <i>Penicillium chrysogenum</i>      | 2.8–4.0                           | Domsch et al. (2007) | 1.0 a              | Madsen et al. (2006)        |
| <i>Stachybotrys chartarum</i>       | 7–12                              | Domsch et al. (2007) | 4.5 a              | Sørensen et al. (1987)      |
| <i>Scopulariopsis brevicaulis</i>   | 5–9                               | Domsch et al. (2007) | 5.1–5.5 a          | Madelin and Johnson (1992)  |
| Fungi                               | >1.5                              | Eduard et al. (2001) | 2.1–3.3 b          | Lin and Li (1996)           |
|                                     |                                   |                      | <2.1 b             | Yeo and Kim (2002)          |
| Fungal hyphal fragments             | Length 5–100                      | Green et al. (2005)  |                    |                             |

<sup>a</sup> $d_{ae}$  measured by an aerodynamic particle sizer.

<sup>b</sup>Peak number of cultivable organisms measured by an Andersen sampler.

## Materials & Methods

### Study area

Ashtoum El-Gamil and Tanis island protectorate is located in western Port Said, about 13 km of the town, and covers an area of about 180 km<sup>2</sup> lying completely inside Lake Manzala. Global Positioning system (GPS) of the protectorate's coordinates are: point A (N: 31° 17' 21", E: 32° 13' 07"), point B (N: 31° 12' 55", E: 32° 13' 45"), point C (N: 31° 12' 20", E: 32° 15' 19"), point D (N: 31° 11' 17", E: 32° 14' 37"), point E (N: 31° 12' 20", E: 32° 12' 56"), point F (N: 31° 12' 20", E: 32° 04' 00"), point G (N: 31° 19' 07", E: 32° 04' 00"), point H (N: 31° 18' 26", E: 32° 08' 45").

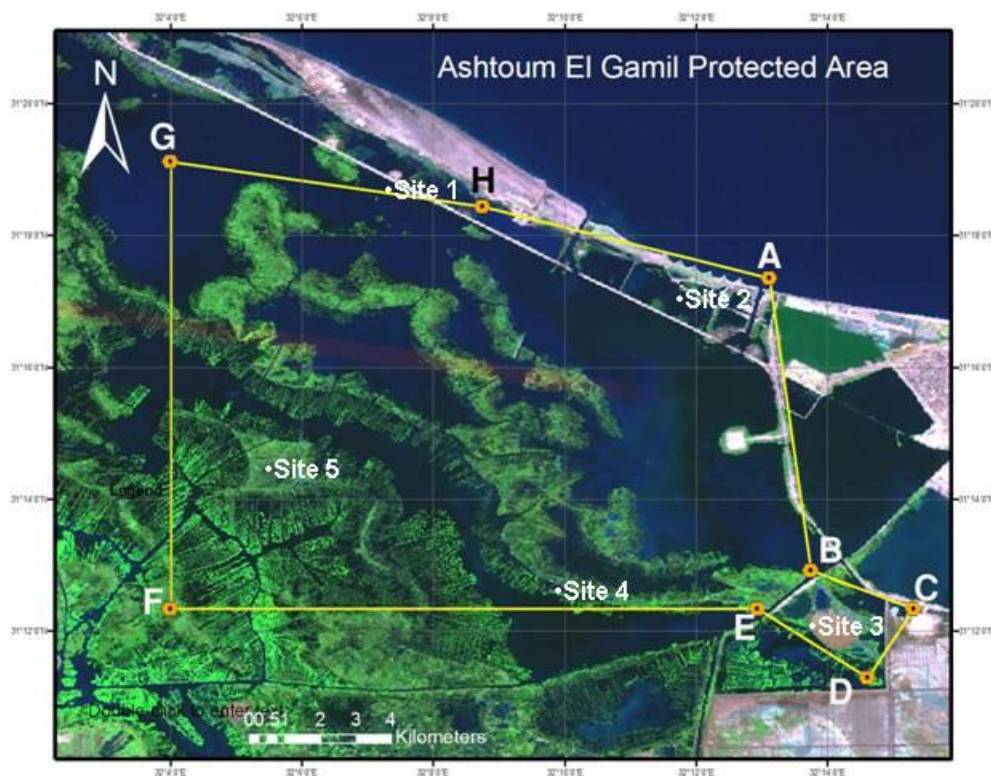
The climate affecting Lake Manzala is generally arid. The absolute minimum temperature ranges between 10°C in January and 23.7°C in August whereas the absolute maximum temperature ranges between 19°C in January and 33.3°C in August. Relative humidity is very similar throughout the year, the air being humid all the year round; relative humidity is rarely less than 70% or more than 80%. The rainfall of Lake Manzala ranges between 47 mm/year and 88 mm/year; the rainy months are usually November, December, January and February. The total rainfall in any of these months varies between 5 mm and 20 mm and rarely amounts to 30 mm. Wind velocity is almost uniform throughout the year; usually there is a gentle breeze. The wind velocity normally ranges between 10 and 20 km/h (Zahran and Willis 2008).

Data such as monthly mean of temperature, relative humidity, wind speed and direction were kindly provided by Port-Said Metrological Station.

### Sampling, isolation and identification of fungi

Five sites for spore trapping were selected in Ashtoum El-Gamil protectorate and designated as site 1 up to site 5 (Fig. 1 and Table 2). Fungi were sampled using a Microbio single-stage air sampler Model MB2 (Parrett LTD, UK). The sampler was operated for 10 min at a flow rate of 100 l/min. The sampler was loaded with Czapek's agar supplemented with 0.5% yeast extract (CYA), Potato Dextrose Agar (PDA) and Dichloran Glycerol Agar (DG-18) amended with Rose bengal (1/1500) and chloramphenicol (50 ppm) as isolation media. Between measurements the samplers were swabbed with 70% ethanol. At each stands, ten isolation plates were used for catching air fungi and were incubated at 28 °C for 7 days and the developing fungi were identified and counted. Samples were collected monthly over a period of 12 months between January 2012 and December 2012.

The fungi were identified morphologically and the aerosol concentrations (CFU/m<sup>3</sup>) were calculated. The number of CFU per cubic meter was calculated as: number of colonies x1000/sampling time x velocity of air flow (Fanga et al. 2005). Taxonomic identification using morphology characteristics of fungal isolates down to the species level on standard media was mainly based on the following identification keys: Pitt (1980) for *Penicillium* (on Czapek Yeast



**Fig. 1** – Map of Ashtoum El-Gamil Protectorate within Lake Mazala.

**Table 2** GPS data of air sampling sites.

| No. | Site name          | GPS            |                |
|-----|--------------------|----------------|----------------|
|     |                    | N              | E              |
| 1   | Ashtoum-El-Gamil   | 31° 18' 12.34" | 32° 09' 27.49" |
| 2   | El-Gamil           | 31° 17' 43.74" | 32° 11' 00.44" |
| 3   | Kawm-Tanis         | 31° 12' 0.53"  | 32° 14' 4.57"  |
| 4   | Barr-El-Samariyyat | 31° 12' 22.82" | 32° 11' 22.49" |
| 5   | Ligan              | 31° 14' 26.49" | 32° 05' 35.60" |

Extract Agar (CYA) ad Malt-Extract Agar (MEA)); Raper and Fennell (1965), Klich (2002) for *Aspergillus* (on Czapek Agar (CZ)); Ellis (1971, 1976) for dematiaceous hyphomycetes (Potato Carrot Agar (PCA)); Booth (1971) for *Fusarium* (Potato Dextrose Agar (PDA)), Domsch et al. (2007) for miscellaneous fungi (on MEA, PDA, CYA) and Guarro et al. (2012) for ascomycetes (on MEA, PDA, Oat Meal Agar (OA)).

The names of authors of fungal taxa are abbreviated according to Kirk and Ansell (1992). The systematic arrangement in the present list follows the latest system of classification appearing in the 10<sup>th</sup> edition of Anisworth and Bisby's Dictionary of the Fungi (Kirk et al. 2008). Name corrections, authorities, and taxonomic assignments of all taxa reported in this work were checked against the Index Fungorum database ([www.indexfungorum.org](http://www.indexfungorum.org)).

### Data analyses

The frequency of isolated taxa is expressed as number of cases of isolation of each species out of the total number of isolation plates. To estimate the similarity of species composition among different sites, the similarity coefficient suggested by Sørensen (1948) have been applied, while species diversity is calculated as Simpson's diversity index (Lande, 1996).

## Results and Discussion

### Meteorological parameters

During this study, the temperature ranged between 13.9 and 28 °C, relative humidity ranged between 68.4 and 75.6 % and wind speed records varied between 15.2 and 17.3 km/h. Wind direction through this study was mainly northern (30 %), western (17 %), northern east and northern west (15 % each), eastern (9 %), southern west (7%), southern (5%) and southern east (2 %).

### General features of isolated mycobiota

From the 600 exposures that have been conducted, a total of 71780 fungi and 560 yeast colony-forming units (CFU) were recovered during the entire study. Mould colonies were assigned to 28 genera and 43 species from the different sites under investigation (Table 3).

The results show that Zygomycota were represented by three species (6.9 % of the total species number), teleomorphic Ascomycota (3 species, 6.9 %), anamorphic Ascomycota (36 species, 83.7 %) and Basidiomycota (1 species, 2.3 %). The prevailing genera were *Aspergillus* (8 species 18.60 % of the total taxa), *Cladosporium*, *Penicillium* and *Ulocladium* (three species each, 11.86%), *Alternaria*, *Fusarium* (2 species each; 4.65 %). The remaining taxa were represented only by one species each.

Taxonomically, isolated species were assigned to 3 phyla with five classes, 11 orders, and 13 families (Table 4). Taxa with uncertain position were distributed among classes, orders and families. While order Eurotiales accommodates the greatest range of species (12 species) followed by Pleosporales (10 species), the remaining orders accommodate the lowest range between one to three species each. Family Trichocomaceae had the highest contribution to the isolated fungi (12 species out of 43) followed by Pleosporaceae (10 species), Davidiellaceae (3 species) and the remaining families were represented each only by one or two species.

The species genus ratio (S/G) per family however shows that family Trichocomaceae was the most diverse taxonomical rank by recording a ratio of 4 followed by Davidiellaceae (3), Nectriaceae (2) and Pleosporaceae (1.42). In view of species richness, site 5 showed the highest richness index of fungi species (species richness= 36) among all studied sites and site 2 showed the poor species richness (25).

### The distribution of airborne fungi

Site number 5 came first among all studied sites by recording 16750 CFU, while site number 2 showed the lowest amount (11960), as shown in figure 2. The distribution pattern of mycobiota based on the presence/absence in sites under investigation showed that recorded taxa could be tentatively classified into three groups. Group 1, comprises taxa of occurrence restricted to a single site (5 species) e.g. *Phoma herbarum*, *Pithomyces chartarum* and *Stagonospora caricinella*. Group 2, consists of species occurring in two or more sites (18) e.g. *Mucor circinelloides*, *Paecilomyces variotii*, *Stemphylium piriforme* and *Curvularia lunata*. Group 3, contains species of common occurrence to almost all sites (20 species) e.g. *Aspergillus niger*, *A. flavus*, *Cladosporium cladosporioides* and *Ulocladium atrum*.

Similarity coefficient values indicated that site 2 is a less similar to the other sites by showing the lowest similarity values. Other sites showed high similarity index values ranging from 0.84 to 0.94.

The diversity of fungi was measured for each site by calculating Simpson's diversity index (Lande 1996). Based on the results, site 5 showed the highest diversity index of 0.937 while site 2 showed the lowest value (0.918).

**Table 3** Fungi recorded, total counts (TC, calculated per 600 plates), percentage counts (% C, calculated per total fungal catch), number of cases of isolation (NCI, out of 12 exposures) and occurrence remarks (OR) of all fungi recovered from the air of Ashtoum El-Gamil protectorate.

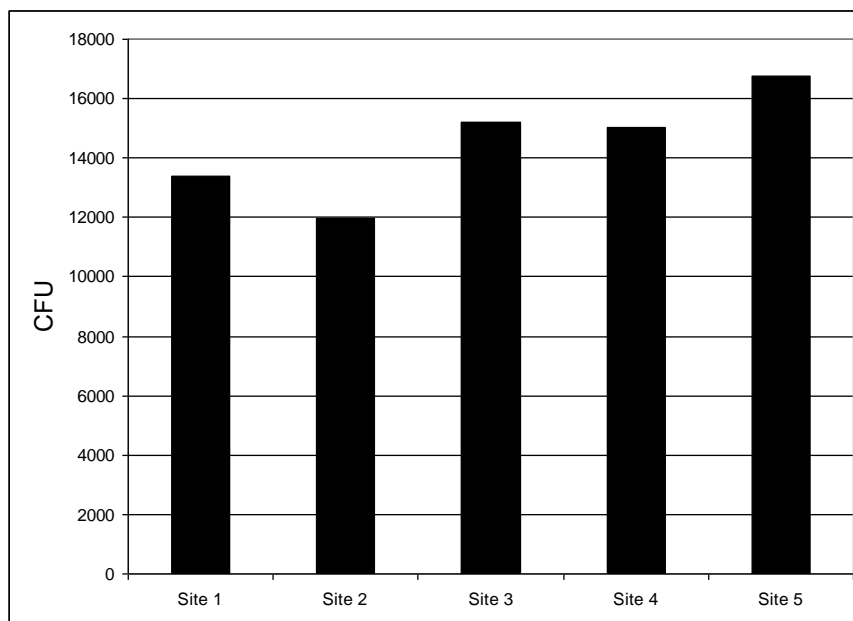
| Fungal species  | TC    | C %    | NCI  | OR |
|---|-------|--------|------|----|
| <i>Alternaria alternata</i> (Fr.) Keissl.                     | 3300  | 4.56   | 9.0  | H  |
| <i>A. cheiranthi</i> (Lib.) P.C. Bolle                        | 5700  | 7.88   | 12.0 | H  |
| <i>Aspergillus flavus</i> Link                                | 2900  | 4.01   | 8.0  | H  |
| <i>A. fumigatus</i> Fresen.                                   | 4620  | 6.39   | 8.0  | H  |
| <i>A. nidulans</i> (Eidam) G. Winter                          | 1300  | 1.80   | 6.0  | H  |
| <i>A. niger</i> Tiegh   | 9200  | 12.72  | 12.0 | H  |
| <i>A. ochraceus</i> G. Wilh.                                  | 460   | 0.64   | 4.0  | M  |
| <i>A. sydowii</i> (Bainier & Sartory) Thom & Church           | 680   | 0.94   | 3.0  | M  |
| <i>A. terreus</i> Thom  | 4320  | 5.97   | 8.0  | H  |
| <i>A. versicolor</i> (Vuill.) Tirab.                          | 360   | 0.50   | 3.0  | M  |
| <i>Aurobasidium pullulans</i> (de Bary) Arnaud                | 5960  | 8.24   | 11.0 | H  |
| <i>Candida tropicalis</i> (Castell.) Berkhout                 | 340   | 0.47   | 9.0  | H  |
| <i>Chaetomium globosum</i> Kunze                              | 300   | 0.41   | 3.0  | M  |
| <i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries   | 7740  | 10.70  | 10.0 | H  |
| <i>C. herbarum</i> (Pers.) Link                               | 3320  | 4.59   | 8.0  | H  |
| <i>C. sphaerospermum</i> Penz.                                | 900   | 1.24   | 4.0  | M  |
| <i>Curvularia lunata</i> (Wakker) Boedijn                     | 140   | 0.19   | 4.0  | M  |
| <i>Drechslera hawaiiensis</i> Bugnic. ex Subram. & B.L. Jain  | 160   | 0.22   | 5.0  | M  |
| <i>Epicoccum nigrum</i> Link                                  | 6040  | 8.35   | 10.0 | H  |
| <i>Fusarium oxysporum</i> Schldtl.                            | 280   | 0.39   | 6.0  | H  |
| <i>F. solani</i> (Mart.) Sacc.                                | 100   | 0.14   | 4.0  | M  |
| <i>Gymnascella dankaliensis</i> (Castell.) Currah             | 40    | 0.06   | 1.0  | L  |
| <i>Lichtheimia corymbifera</i> (Cohn) Vuill.                  | 120   | 0.17   | 4.0  | M  |
| <i>Microascus brevicaulis</i> S.P. Abbott                     | 160   | 0.22   | 5.0  | M  |
| <i>Mucor circinelloides</i> Tiegh.                            | 220   | 0.30   | 3.0  | M  |
| <i>Paecilomyces variotii</i> Bainier                          | 260   | 0.36   | 2.0  | L  |
| <i>Penicillium chrysogenum</i> Thom                           | 5460  | 7.55   | 12.0 | H  |
| <i>P. funiculosum</i> Thom                                    | 1220  | 1.69   | 7.0  | H  |
| <i>P. roquefortii</i> Thom                                    | 480   | 0.66   | 6.0  | H  |
| <i>Phoma herbarum</i> Westend.                                | 120   | 0.17   | 2.0  | L  |
| <i>Pithomyces chartarum</i> (Berk. & M.A. Curtis) M.B. Ellis  | 100   | 0.14   | 2.0  | L  |
| <i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.                   | 1180  | 1.63   | 12.0 | H  |
| <i>Rhodotorula glutinis</i> (Fresen.) F.C. Harrison           | 560   | 0.77   | 6.0  | H  |
| <i>Sarocladium strictum</i> (W. Gams) Summerb.                | 420   | 0.58   | 3.0  | M  |
| <i>Scopulariopsis chartarum</i> (G. Sm.) F.J. Morton & G. Sm. | 220   | 0.30   | 3.0  | M  |
| <i>Stachybotrys chartarum</i> (Ehrenb.) S. Hughes             | 280   | 0.39   | 3.0  | M  |
| <i>Stagonospora caricinella</i> Brunaud                       | 20    | 0.03   | 1.0  | L  |
| <i>Stemphylium piriforme</i> Bonorden                         | 180   | 0.25   | 3.0  | M  |
| <i>Trichoderma koningii</i> Oudem.                            | 1500  | 2.07   | 5.0  | M  |
| <i>Trichothecium roseum</i> (Pers.) Link                      | 60    | 0.08   | 1.0  | L  |
| <i>Ulocladium atrum</i> Preuss.                               | 900   | 1.24   | 9.0  | H  |
| <i>U. chartarum</i> (Preuss) E.G. Simmons                     | 220   | 0.30   | 6.0  | H  |
| <i>U. oudemansii</i> E.G. Simmons                             | 500   | 0.69   | 5.0  | M  |
| Total   | 72340 | 100.00 | 12   |    |

Occurrence remarks (OR): H: high, 6-12 of the exposures; M: moderate, 3-5 of the exposures; L: low, 1-2 of the exposures.

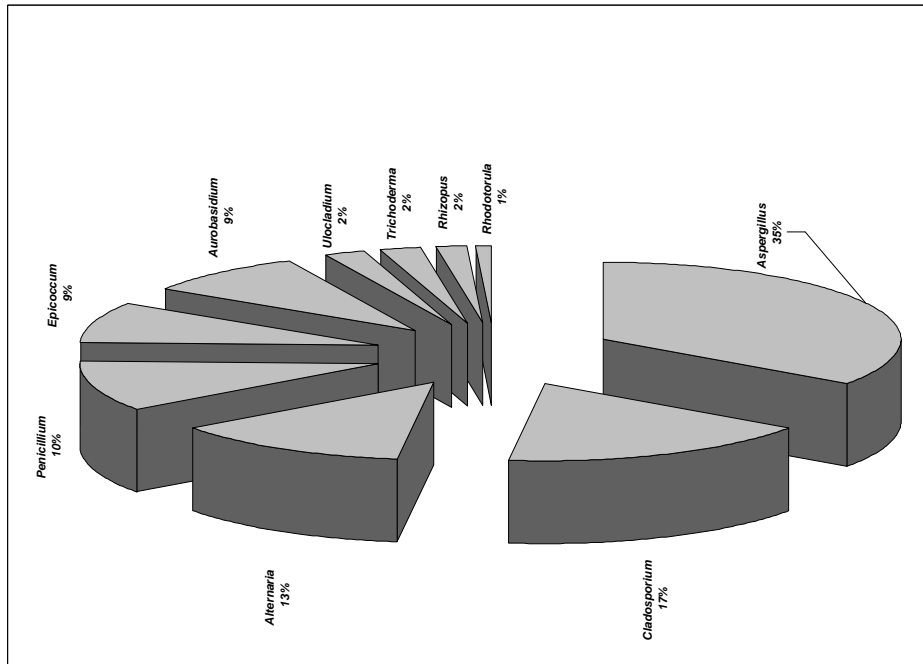
**Table 4** Taxonomic assignment of the isolated taxa according to Kirk et al. (2008)

| Phylum     | Class                 | Order              | Family                | Genus        | Species               |   |    |
|------------|-----------------------|--------------------|-----------------------|--------------|-----------------------|---|----|
| Zygomycota | <i>Incertae sedis</i> | Mucorales          | Lichtheimiaceae       | 1            | 1                     |   |    |
|            |                       |                    | Mucoraceae            | 1            | 1                     |   |    |
|            |                       |                    | Rhizopodaceae         | 1            | 1                     |   |    |
|            |                       |                    | Capnodiales           | 1            | 3                     |   |    |
|            |                       |                    | Dothidiales           | 1            | 1                     |   |    |
|            |                       |                    | Dothideomycetes       | Pleosporales | Phaeosphaeriaceae     | 1 | 1  |
|            |                       |                    |                       |              | Pleosporaceae         | 7 | 10 |
|            |                       |                    |                       |              | <i>Incertae sedis</i> | 1 | 1  |
|            |                       |                    |                       |              | Eurotiales            | 3 | 12 |
|            |                       |                    |                       |              | Onygenales            | 1 | 1  |
| Ascomycota | Eurotiomycetes        | Saccharomycetales  | <i>Incertae sedis</i> | 1            | 1                     |   |    |
|            |                       |                    | Hypocreaceae          | 1            | 1                     |   |    |
|            | Sordariomycetes       | Hypocreales        | <i>Incertae sedis</i> | 3            | 3                     |   |    |
|            |                       |                    | Nectriaceae           | 1            | 2                     |   |    |
|            |                       |                    | Microascales          | 2            | 2                     |   |    |
|            | Basidiomycota         | Microbotryomycetes | Sordariales           | 1            | 1                     |   |    |
|            |                       |                    | Sporidiobolales       | 1            | 1                     |   |    |
| Total      | 5                     | 11                 | 13                    | 28           | 43                    |   |    |

The greatest colony count was attributed to *Aspergillus niger* (12.7 % of the total isolate number), *Cladosporium cladosporioides* (10.69%), *Epicoccum nigrum* (8.34%), *Alternaria cheiranthi* (7.87 %), *Penicillium chrysogenum* (7.54%), *Aspergillus fumigatus* (6.38%), *Aspergillus terreus* (5.97%), and *Alternaria alternata* (4.56%) respectively. Figure 3 represents the ten major genera observed in the study as they represent more than 95% of total colony counts obtained (Table 2). The greatest total colony count was attributed to *Aspergillus* with 35% followed by *Cladosporium*, *Alternaria* and *Penicillium* with 17, 13 and 10% respectively (Table 3, Fig. 3).

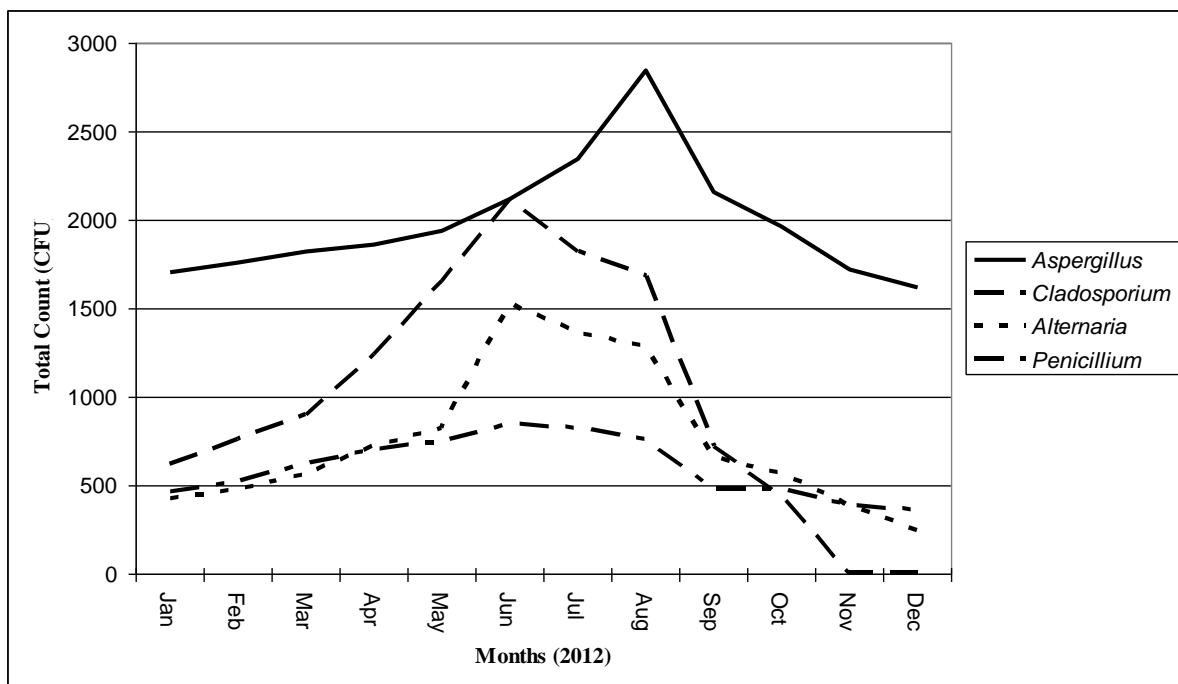


**Fig. 2** – Total fungal counts in the study sites.



**Fig. 3** – Relative contributions (% of total colony count) of the major airborne fungi in Manzala lagoon, Egypt.

*Aspergillus*, *Alternaria* and *Penicillium* occurred every month of the entire study (12 months), followed by *Cladosporium* (10 months), *Ulocladium* (9 months) and *Fusarium* (6 months). During the entire year of 2012, the number of monthly recorded species showed that August recorded 24 species while September recorded 10 species only. *Aspergillus*, showed one peak in August while *Cladosporium* and *Alternaria* peaked in June (Fig. 4). Lake Manzala airborne fungal counts increased with temperature and decreased with rainfall and relative humidity and this in agreement with findings of Awad (2005).



**Fig. 4** – Dominant genera recorded every month throughout the study course.



Vegetation of Lake Manzala is typically Mediterranean (Maswada and Elzaawely, 2013) which includes halophytic and helophytic species growing mainly on shores and islands of the lake. Water habitat of Lake Manzala is characterized by five dominant hydrophytes and four associates (Khedr 1989). Grasses e.g. *Phragmites australis* is widespread and its community occurs in all islands where it forms dense thickets along shore-lines with surface deposits of sand and silt (Zahran et al. 1989).

Vegetation plays a major role in addition of different fungal types into the air and their numbers vary according to vegetation type and weather conditions (Awad 2005). Some of the most the abundant spores found in the atmosphere have the capability to interact with plants, where they may become endophytes first and saprobes later. These fungi are extremely abundant as endophytes in grasses and other plant taxa, and are likely to have important roles as saprobes, in plant decomposition and nutrient cycling. Furthermore, the spores of these fungi also interact with human immune systems, being respiratory allergens (Vázquez de Aldana et al. 2013).

Many studies have been performed to identify the concentrations and types of aeromycobiota in outdoors in Egypt. Outdoor concentrations averaged 540 CFU/m<sup>3</sup> across the US (Shelton et al. 2002), and 1,042 CFU/m<sup>3</sup> in Latrobe Valley, Australia (Garrett et al. 1997). In the present study, fungal concentrations, 120.6 CFU/m<sup>3</sup>, were found to be higher than those reported in some studies in different localities in Egypt. Fungal concentrations were found in the range of 25–222 CFU/m<sup>3</sup> outdoors at the coastal buildings in Damietta (El-Morsy 2006).

There are no numeric guidelines for outdoor airborne fungi, however a number of numeric guidelines have been proposed throughout the years (Li and Kuo 1993, WHO 1998, Wu et al. 2000), but none of them are currently widely accepted by the scientific community (Codina et al. 2008). In comparison with previous studies in Egypt, our findings indicate that the Lake Manzala air (120.6 CFU/m<sup>3</sup>) had much higher than acceptable levels of fungi. In the present study, concentrations of fungal spores in the size range of  $\leq 5 \mu\text{m}$  were the predominant fraction. In most parts of the world the main core of fungal aerosols is likely to be similar, but the dominance of genera may differ from one area to another depending on geographical location, local sources, and climatic conditions (Abdel Wahid *et al.* 1996). Qualitative determination of fungi may be more useful than determining concentrations, as many species may have health effects. The frequent detection of *Aspergillus* spp., *Penicillium*, and *Cladosporium* is attributed to their ready dissemination into the air. These findings in our study are similar to those observed in other geographical locations in Italy (Dacarro et al. 2003), Egypt (El-Morsy 2006, Abdel Hameed et al. 2009) and Florida, USA (Codina et al. 2008).

Horner et al. (2004) grouped fungi into three categories with different ecological relevance: (1) phylloplane fungi (*Cladosporium*, *Curvularia* and *Alternaria*); (2) soil fungi (*Penicillium*, *Paecilomyces* and *Aspergillus*), and (3), water indicator fungi (*Chaetomium*, *Stachybotrys* and *Ulocladium*). In the present study water indicator fungi were found in low counts, and their presence associated with rain time or the presence of damped materials, however phylloplane and soil fungi were found in higher counts during the collection of air samples (January 2012 to December 2012). Taxa such as *Cladosporium*, *Penicillium* and *Alternaria* could increase the risk of asthma and allergic rhinitis and allergic alveolitis (Lacey and Dutkiewicz 1994). While *Sarocladium* (*Acremonium*), *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Stachybotrys* and *Trichoderma* are well known mycotoxin producers (Davis 2013). Both of *Aspergillus flavus* and *A. fumigatus* can lead to aspergillosis (Abarca 2000). As the potential implications of the fungal contamination on health have not been studied in Egypt, it should be reported that exposure to such concentrations of this study (120.6 CFU/m<sup>3</sup>) and types is a risk factor for resident's respiratory symptoms. Fungi impact human health in four different ways as they can infect humans or act as allergens or can be toxic or can cause inflammatory reactions (Fischer and Dott 2003).

Some fungal spores such as *Cladosporium* and *Alternaria* are considered as integral parts of the outdoor air and they comprise more than 29% of the total fungal load in Lake Manzala. These spores are subject to the individual geoclimatic characteristics of their surrounding

environment. Aerobiology studies suggest that the release of spores is subject to factors such as species, texture and air velocity above the contaminated surfaces or vibrations that cause their release (Górny et al. 2002). Our results may clarify why the prevalence of asthma in Ismailia and Port Said governorates increased dramatically between 9.6% and 11.4% respectively (El-Baz, 1994; Halim et al. 2013).

## Conclusions

This work has shown that fungi which may cause diseases of the respiratory system and have the power to produce mycotoxins were encountered frequently in the research areas. Lake Manzala with its climatic conditions and vegetation presents a suitable habitat for the increase of fungi. The presence of fungal spores in air, in spite of their counts, may raise arguments about their role in health complaints in a particular region, i.e., the fungal concentration may be low but the predominant aeroallergen may be dangerous. Asthma is a multi-factorial condition and environmental factors such as particulate matter and other contaminants coupled with possible genetic predisposition, behavioral and social factors interacted to produce the high asthma prevalence. A comprehensive model of factors that contribute to asthma in the region is needed and this can be a useful tool for planning efforts and disease prevention. The obtained results indicated that the fungi of air should be considered when the quality of Egyptian air is assessed.

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