

# Determination of potential allergenic fungal flora and its clinical reflection in suburban elementary schools in Izmir

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**Abstract** In this research, in order to determine mean fungus counts, indoor and outdoor air samples were taken in five elementary school buildings located in the city center of Seferihisar, Izmir (45 km from Izmir) within a 1-year period between March and April 2004, and between January and February 2005 representing similar climatic characteristics. Five samples, three from three classes where 5–8, 10–12, and 12–15 age group students attended, one from the corridors, and one from outside the buildings in all the schools, were taken for each period. Within the period of the study, in indoor and outdoor air samples, 7,122 microfungus colonies were counted. Sixty-four species were identified that belong to 17 genera as a result of the identification of isolates

obtained. Skin prick tests were applied to 55 out of a total of 117 teachers by the clinicians. According to these test results, 24 teachers were sensitive to at least one agent. Results revealed that, in terms of mold counts, the difference between the schools and the difference between the times (periods) were statistically significant ( $p < 0.05$ ).

**Keywords** Airborne fungal spores · Fungus species · Mold · Skin prick test

## Introduction

Distribution of airborne fungal spores is associated with serious health problems and is of importance in terms of its reflections both on quality of life and on economy (Asan et al. 2002, 2004; Aydoğdu and Asan 2005; Burge 2002; Gravesen 1985, 1999; Sarıca-Okten et al. 2005; Shelton et al. 2002). Health problems caused by indoor microfungi in buildings we live in are allergenic agents causing immune sensitivity and response, infections resulting from the growth of fungi in or on a living creature, and toxic effects (Kuhn and Ghannoum 2003; Nielsen 2003; Robins et al. 2000). Advanced respiratory tract sensitivities and infections are diseases caused by airborne contaminants which affect both children and adults. Schools are of great importance in

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terms of the development of diseases affecting both adults and especially children such as atopic dermatitis, allergic rhinitis, allergic asthma, and sick building syndrome resulting from such unfavorable factors as indoor moisture and growth of indoor fungi affecting indoor air quality (Burge 1985, 1989). Allergic reactions develop when an individual is exposed to several antigens present in mold spores and/or hyphae. Mold spores are hydrophobic spores taken in the body through respiration. They are resistant to drought and can spread to distant areas through air movements as particles sustaining in the air. Among the potential fungal allergen sources are ventilation systems, specifically made ceilings, fire-resistant indoor decoration materials, wallpapers, use of damp wooden materials and firewood, gaps around doors or windows, and the presence of indoor plants (Ergin 2006). The distance between the area in which fungi exist and the forests, pastures, and agricultural land leads to differences in fungus flora. Determination of the airborne fungal distribution in an area, urban sensitivity to fungi, and clinical diagnosis play an important role in combating seasonal allergic diseases. Fungi growing in moist buildings or in buildings damaged by water due to several reasons can be classified into three groups: primary, secondary, and tertiary. The most common fungi types are *Penicillium* 90%, *Cladosporium* 89%, and *Ulocladium* 62% (Robins et al. 2000). The most common fungi species causing allergic reactions are *Alternaria*, *Aspergillus*, *Cladosporium*, *Candida*, *Penicillium*, *Curvularia*, *Dreschlera*, *Epicoccum*, *Fusarium*, *Helminthosporium*, *Phoma*, *Scopulariopsis*, *Stachybotrys*, *Trichoderma*, *Trichophyton*, and *Ulocladium* (Crameri 2002; Frew 2004; Singh and Kumar 2003). These fungi which are also found in school buildings pose a risk to students, faculty, and staff. Moisture and high fungus counts due to moisture in school buildings can negatively affect the respiratory systems (respiration, breathing) of students and personnel who spend their days in those buildings. It is also known that indoor fungal flora represents outdoor atmospheric flora (Burge 2002; Burge and Rogers 2000). Therefore, in order to assess airborne fungi and the negative effects they lead to, the rate of indoor and outdoor airborne fungi popula-

tion should be known and detected, and sampling time, meteorological conditions, seasonal climatic changes, and the effects of vegetation on fungal spores should be determined (Pepeljnjak and Segvic 2003).

Our study, which was carried out in schools where students and teachers spent more or less 40 h a week, was aimed to detect indoor and outdoor fungi and their distribution rates in elementary schools in Seferihisar, a tourist town of Izmir famous for its citrus gardens, and thus to be used as a reference for future studies.

## Materials and methods

### Sampling sites and vegetation

Seferihisar, a tourist town 45 km away from Izmir, was chosen as a sampling site. The town, whose winter population is around 18,000, is very crowded and busy between April and October due to tourism and those who move to their summerhouses. During this season, the town's population ranges from 80,000 to 130,000. The town is famous with its citrus gardens. The area also has a rich flora.

### Distribution of herbs in Seferihisar district

*Amaranthus albus* L., *Pancratium maritimum* L. (AMARANTHACEAE); *Vinca major* L. (APOCYNACEAE); *Dracunculus vulgaris* Schott. (ARACEAE); *Aristolochia hirta* L. (ARISTOLOCHIACEAE); *Myosotis litoralis* Steven ex Bieb., *Heliotropium hirsutissimum* Graver, *Anchusa undulata* L. (BORAGINACEAE); *Campanula lyrata* Lam. (CAMPANULACEAE); *Silene behen* L., *S. cretica* L., *S. colorata* Poir., *S. italica* (L.) Pers., *S. vulgaris* (Moench.) *Spergularia marina* (L.) Gris., *Petrorhagia velutina* (Guss.) Ball. et Heywood, *Dianthus tripunctatus* Sibth. et Sm., Garche, *Cerastium brachypetalum* Pers., *Spergularia pentandra* (L.) J. et C. Presl., *S. rubra* L., (CARYOPHYLLACEAE); *Beta maritima* L., *Chenopodium murale* L.,

*Salicornia fruticosa* L. (CHENOPODIACEAE); *Scorzonera laciniata* L., *Helichrysum stoechas* (L.) Moench., *Bellis annua* L., *Centaurea maritima* L., *Notobasis syriaca* (L.) Gass., *Scorzonera lanata* M.Bieb., *Ptilostemon chamaepeuce* (L.) Less., *Anthemis tomentosa* L., *Chrysanthemum segetum* L., *Aster tripolium* L., *Centaurea solstitialis* L., *Inula viscosa* (L.) Aiton, *Pallenis spinosa* (L.) Cass. (COMPOSITAE); *Convolvulus arvensis* L. *Calystegia soldanella* (L.) (CONVOLVULACEAE); *Sedum caespitosum* (Cav.) DC. (CRASSULACEAE); *Cakile maritima* Scop, *Malcomia flexiosa* (Sibth&Sm) Sibth&Sm, *Arabis verna* (L.) DC., *Matthiola tricuspidata* (L.) R.Br. (CRUCIFERAE); *Tamus communis* L. (DIOSCORACEAE); *Scabiosa columbaria* L. (DIPSACACEAE); *Euphorbia helioscopia* L. (EUPHORBIACEAE); *Centaureum erythraea* Rafn. (GENTIANACEAE); *Erodium gruinum* (L.) L'Herit. (GERANIACEAE); *Cynodon dactylon* L., *Polypogon monspeliensis* (L.) Desf., *Dactylis glomerata* L., *Briza maxima* L., *Lagurus ovatus* L., *Aegilops umbellulata* Zhuk. (GRAMINEAE); *Iris orientalis* Miller. (IRIDACEAE); *Teucrium polium* L. (LABIATAE); *Lotus cytisoides* L., *Trifolium purpureum* Lois. (LEGUMINOSAE); *Malva sylvestris* L. (MALVACEAE); *Ophrys vernixia* Brot, *Orchis anatolica* Boiss., *Ophrys lutea* Cav. (ORCHIDACEAE); *Orobanche cernua* Loeffl. (OROBANCHACEAE); *Fumaria parviflora* Lam., *Glacium flavum* Crantz., *Hypecoum procumbens* L., *Papaver dubium* L., *P. rhoeas* L. (PAPAVERACEAE); *Plantago lagopus* L. (PLANTAGINACEAE); *P. lanceolata* L., *P. major* L., *Rumex bucephalophorus* L. (POLYGONACEAE), *R. conglomeratus* Murray.; *Anagallis arvensis* L. (PRIMULACEAE); *Anemone coronaria* L., *Ranunculus sprunerianus* Boiss., *Delphinium staphisagria* L. (RANUNCULACEAE); *Rosa canina* L. (ROSACEAE); *Galium aparine* L. (RUBIACEAE); *Veronica anagallis-auiatica* L., *Parentucellia latifolia* (L.) Carvel., *Bellardia trixago* (L.) All. (SCROPHULARIACEAE); *Datura stramonium* L. (SOLANACEAE); *Daucus carota* L., *Eryngium campestre* L., *E. creticum* Lam., *E. maritimum* L.,

*Oenanthe silaifolia* Brit., *Scandix australis* L. (UMBELLIFERAE) (Davis 1965–1988).

#### Distribution of shrubs in Seferihisar district

*Hedera helix* L. (ARALIACEAE); *Pistacia lentiscus* L. (ANACARDIACEAE), *Nerium oleander* L. (APOCYNACEAE); *Lonicera implexa* Ait. (CAPRIFOLIACEAE); *Cistus parviflorus* L., *C. salviifolius* L. (CISTACEAE); *Erica arborea* L., *E. verticillata* Forsk. (ERICACEAE); *Arbutus andrachne* L., *A. unedo* L. (ERICACEAE); *Hypericum empetrifolium* Willd., *H. perforatum* L., *H. triquetrifolium* Turra. (GUTTIFERAE); *Juncus heldreichianus* Marsson ex Pasl., *J. littoralis* C.A. Meyer (JUNCACEAE); *Origanum onites* L. (LABIATAE); *Laurus nobilis* L. (LAURACEAE); *Centaurea spinosa* L. (COMPOSITAE); *Genista acanthoclada* L., *Anthyllis hermanniae* L., *Calycotome villosa* Link., *Anthyllis vulneraria* L. (LEGUMINOSAE); *Myrtus communis* L. (MYRTACEAE); *Jasminum fruticans* L., *Phillyrea latifolia* L. (OLEACEAE); *Styrax officinalis* L. (STYRACACEAE); *Thymelaea tartonraria* (L.) All. (THYMELAEACEAE); *Quercus coccifera* L. (FAGACEAE); *Vitex agnus castus* L. (Verbenaceae) (Davis 1965–1988).

#### Distribution of tree shrubs in Seferihisar district

*Pinus brutia* Ten., *P. halepensis*, *Cedrus* spp. (PINACEAE); *Ligustrum vulgare*, *Olea europaea* L. (OLEACEAE); *Cupressus sempervirens*, *Juniperus oxycedrus*, *Thuja orientalis*, *T. occidentalis* (CUPRESSACEAE); *Eleagnus angustifolia* L. (ELEAGNACEAE); *Tamarix hampanana* Boiss. et Heldr., *T. parviflora* DC., *T. smyrnensis* Bunge. (TAMARICACEAE); *Ailanthus altissima* (Miller) Swingle (SIMAROUBACEAE); *Acer campestre* L., *A. negundo* L. (ACERACEAE); *Casuarina equisetifolia* L. (CASUARIANACEAE), *Ceratonia siliqua*, *Cercis siliquastrum* (FABACEAE), *Eriobotrya japonica*, *Sophora japonica* (ROSACEAE); *Eucalyptus camadulensis* (MYRTACEAE); *Ficus carica*, *Morus alba* (MORACEAE);

*Juglans regia* (JUGLANDACEAE); *Nerium oleander* (APOCYNACEAE); *Platanus orientalis* (PLATANACEAE); *Populus alba*, *Salix* spp. (SALICACEAE); *Tilia argentea* (TILIACEAE); *Ulmus* spp. (ULMACEAE); *Washingtonia filifera* (PALMAE) (Davis 1965–1988).

### Description of the study units in primary schools

Several features of five elementary school buildings located in Seferihisar are shown in Table 1. The study was carried out between March–April 2004 and January–February 2005 (Table 2). Although schools were constructed in different years, ventilation is assured between March and April 2004 and between January and February 2005 (open windows and no air conditioning or air filtration system). All the schools are heated with central heating systems. Although the number of cleaning personnel in the schools is limited, hygiene conditions in all schools are above average.

### Sampling and identification

In this research, in order to determine mean fungus counts, indoor and outdoor air samples were taken in five elementary school buildings located in the city center of Seferihisar, Izmir (45 km from Izmir) within a 1-year period between March and April 2004, and between January and February 2005 representing similar climatic characteristics. Five samples, three from three classes where 5–8, 10–12, and 12–15 age group students attended, one from the corridors, and one from outside the buildings in all the schools, were taken for each period.

As an active sampling method, volumetric impactor sampler MERCK Mas 100 air sampler was used. Air samples 1.40–1.50 m above ground

level were obtained between 11:00 and 13:00 during the third week of each month. As isolation medium, Rose Bengal Chloramphenicol Agar (Oxoid CM549) and Chloramphenicol Selective Supplement (Oxoid SR78) were used. Each petri dish was filled with 100 l of air and left for incubation for 5–7 days at 25–27°C, and the colonies reproduced were assessed and the results were assessed as colony forming units per cubic meter (Boyacioglu et al. 2007).

Based on their micro- and macromorphologies, fungal species grown on Czapek' solution agar, malt extract agar, and potato dextrose agar media were first determined at genus levels by using Barnett and Hunter (1999). Then, *Aspergillus* Micheli species were determined with Raper and Fennell (1965) and Klich (2002); *Penicillium* Link species with Pitt (2000); *Fusarium* species with Booth (1971); *Alternaria* Nees, *Chaetomium* Kunze ex Fr., *Cladosporium* Link, *Dreschlera* Ito, *Doratomyces* Corda, *Helminthosporium* Link ex Fr., *Neurospora* Shear&Dodge, *Mucor* Mic. ex St-Am., *Phoma* Desm., *Rhizopus* Ehrenberger, *Scopulariopsis* Bain, *Sordaria* Ces&de Not, *Trichoderma* Pers. ex Fr. genera with Barnett and Hunter (1999), and Hasenekoğlu (1991) monographs. Fungal author names as cited are standardized and in accordance with said names as listed in the website [www.indexfungorum.org](http://www.indexfungorum.org).

### Skin testing

Questionnaires were given to 117 teachers working in the five elementary schools included in the study. Questionnaires were evaluated by the Allergy-Immunology Department of Internal Diseases, Medical Faculty, Ege University; those teachers suspected to have allergic rhinitis and/or asthma were examined and then 55 teachers

**Table 1** Sample schools in Seferihisar, Izmir, and their characteristics

A, B, C, D, E primary schools

| Schools | Building year | Heating type    | Student count | Teacher count | Director count | Personnel count | Classroom count |
|---------|---------------|-----------------|---------------|---------------|----------------|-----------------|-----------------|
| A       | 1937          | Central heating | 370           | 16            | 2              | 3               | 13              |
| B       | 1970–1988     | Central heating | 384           | 20            | 2              | 1               | 12              |
| C       | 1995          | Central heating | 454           | 25            | 1              | 2               | 18              |
| D       | 1923          | Central heating | 206           | 12            | 2              | 1               | 9               |
| E       | 1987          | Central heating | 171           | 10            | 2              | 1               | 8               |

**Table 2** Identified fungus species and sampling time

| Species                            | Schools                                      |                                     |                                     |                                       |                                       |
|------------------------------------|----------------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|---------------------------------------|
|                                    | A                                            | B                                   | C                                   | D                                     | E                                     |
| <i>Aspergillus alliceus</i>        | P1(I,O), P5(I,O)                             | P2(I,O)                             | P2(I)                               | –                                     | P2(I,O)                               |
| <i>A. auricomus</i>                | –                                            | –                                   | –                                   | P2(I)                                 | –                                     |
| <i>A. japonicus</i>                | P3(I,O), P4(I,O)                             | P3(I,O), P4(I,O)                    | P3(I,O), P4(I)                      | P3(I,O), P4(I,O)                      | P1(I,O), P3(I,O),<br>P4(I,O)          |
| <i>A. flavus</i>                   | P3(I,O)                                      | P3(I,O)                             | P3(I), P6(I)                        | P3(I,O), P4(I,O),<br>P6(O)            | P3(I,O)                               |
| <i>A. fumigatus</i>                | –                                            | P1(I,O), P3(I)                      | P3(I), P5(O)                        | –                                     | –                                     |
| <i>A. melleus</i>                  | P4(I,O)                                      | P4(I,O)                             | P2(I), P4(I,O)                      | P4(I,O)                               | P2(O), P4(I,O)                        |
| <i>A. niger</i>                    | P1(I,O), P2(I), P3(I,O),<br>P5(I,O), P6(I,O) | P3(I), P4(I,O),<br>P5(I,O), P6(I,O) | P1(I,O), P3(I),<br>P4(I,O), P5(I,O) | P3(I), P4(I,O)                        | P1(I,O), P3(I,O),<br>P4(I,O), P5(I,O) |
| <i>A. parasiticus</i>              | P6(I)                                        | P4(O)                               | P1(O), P6(I,O)                      | P1(I,O)                               | P3(I,O), P6(I)                        |
| <i>A. puniceus</i>                 | –                                            | P2(I)                               | –                                   | –                                     | P2(I)                                 |
| <i>A. sydowi</i>                   | –                                            | P1(I), P2(I)                        | –                                   | P2(I,O)                               | –                                     |
| <i>A. terreus</i>                  | P1(I,O), P5(I)                               | P1(I,O), P5(I)                      | P5(I,O), P6(I)                      | P1(O), P3(O)                          | P2(I), P4(O),<br>P5(O)                |
| <i>A. ustus</i>                    | P1(I)                                        | P1(I,O), P3(I)                      | P1(I,O), P3(I)                      | P1(I,O)                               | P1(I,O), P2(I),<br>P3(I,O)            |
| <i>A. wentii</i>                   | P4(I,O)                                      | P2(I,O), P4(O)                      | P3(I), P4(I,O)                      | P2(I), P3(I,O)                        | P3(I)                                 |
| <i>Emmericella quadriliata</i>     | P3(I)                                        | P6(I,O)                             | P3(I), P6(I)                        | P3(I)                                 | P3(I)                                 |
| <i>E. nidulans</i>                 | –                                            | –                                   | –                                   | P1(I), P2(I),<br>P3(I)                | –                                     |
| <i>Penicillium aurantiogriseum</i> | –                                            | –                                   | –                                   | P1(I)                                 | –                                     |
| <i>P. brevicompactum</i>           | –                                            | P3(I), P4(I,O)                      | P2(I,O), P3(I),<br>P6(I), P4(O)     | P1(I), P4(I,O)                        | P4(I)                                 |
| <i>P. camemberti</i>               | P1(I,O)                                      | –                                   | P1(I,O)                             | P1(I)                                 | P1(I), P3(I,O)                        |
| <i>P. chrysogenum</i>              | P1(I,O), P2(I,O),<br>P3(O), P(O)             | P1(I,O), P3(I),<br>P5(I)            | P1(I,O), P5(I,O),<br>P6(I)          | P1(I), P5(I)                          | P2(I), P5(I), P6(I)                   |
| <i>P. citreonigrum</i>             | P1(I,O), P2(I), P6(O)                        | P1(I)                               | P1(I), P4(O)                        | P1(I)                                 | P1(I), P4(O)                          |
| <i>P. citrinum</i>                 | P2(I), P3(I,O)                               | P3(I,O)                             | P2(I,O), P3(I)                      | P2(I,O), P3(I,O)                      | P3(I,O)                               |
| <i>P. commune</i>                  | P1(I,O)                                      | P5(I), P(O)                         | –                                   | P1(I,O)                               | –                                     |
| <i>P. crustosum</i>                | P1(I), P2(I)                                 | P1(I,O)                             | P1(I), P(O)                         | P1(I,O)                               | P1(I,O), P4(O)                        |
| <i>P. decumbens</i>                | P3(I)                                        | –                                   | P1(I,O)                             | P1(I,O), P5(I,O)                      | P1(I,O), P2(I)                        |
| <i>P. digitatum</i>                | P5(I)                                        | –                                   | –                                   | P1(I)                                 | P1(I,O), P5(I)                        |
| <i>P. duclauxii</i>                | –                                            | P4(I,O)                             | P4(I)                               | –                                     | –                                     |
| <i>P. expansum</i>                 | P1(I,O), P2(I,O),<br>P5(I), P6(I,O)          | P2(I,O), P3(I),<br>P5(I,O), P6(I)   | P2(I,O), P3(I,O)                    | P2(I,O), P6(I,O)                      | P2(I,O), P5(I,O),<br>P6(I)            |
| <i>P. glabrum</i>                  | P2(I)                                        | –                                   | –                                   | P2(I)                                 | P2(I), P4(O)                          |
| <i>P. griseofulvum</i>             | P6(I,O)                                      | P6(I)                               | P6(I)                               | P6(I,O)                               | P6(I)                                 |
| <i>P. hirsutum</i>                 | –                                            | –                                   | –                                   | –                                     | P5(I)                                 |
| <i>P. italicum</i>                 | P1(I,O), P5(I)                               | P1(I,O), P5(I,O)                    | P4(I), P5(O)                        | P5(I,O)                               | P5(I)                                 |
| <i>P. marnefeii</i>                | –                                            | –                                   | –                                   | –                                     | P6(I,O)                               |
| <i>P. melinii</i>                  | –                                            | P6(I)                               | P6(I)                               | –                                     | P6(I)                                 |
| <i>P. paxilli</i>                  | P4(I,O)                                      | P4(I,O)                             | P4(I), P6(I)                        | P4(I,O)                               | P4(I,O)                               |
| <i>P. purpurogenum</i>             | P3(I)                                        | P3(I)                               | P2(I), P3(I)                        | P3(I,O)                               | P5(I,O)                               |
| <i>P. simplicissimum</i>           | P2(I,O)                                      | P2(I,O)                             | –                                   | P2(I,O)                               | P2(I,O)                               |
| <i>P. solitum</i>                  | P4(I,O)                                      | P1(I), P4(I,O),<br>P5(I)            | –                                   | P4(I)                                 | –                                     |
| <i>P. wakswanii</i>                | P6(I)                                        | P6(I)                               | P6(I)                               | P6(I)                                 | P6(I)                                 |
| <i>Alternaria alternate</i>        | P1(I,O), P4(I),<br>P5(I,O)                   | P4(I,O), P5(I,O),<br>P6(I)          | P4(I,O), P5(I,O)                    | P2(I,O), P4(I,O),<br>P5(I,O), P6(I,O) | P1(I,O), P2(O),<br>P4(I,O), P5(I,O)   |

**Table 2** (continued)

| Species                                     | Schools                             |                              |                                   |                                          |                                            |
|---------------------------------------------|-------------------------------------|------------------------------|-----------------------------------|------------------------------------------|--------------------------------------------|
|                                             | A                                   | B                            | C                                 | D                                        | E                                          |
| <i>A. citri</i>                             | P2(I,O), P1(O),<br>P5(O)            | P2(I,O), P5(I,O),<br>P6(I,O) | P2(I), P5(I),<br>P6(I)            | P1(O), P2(I,O),<br>P5(I,O), P6(I), P3(O) | P2(I,O), P3(I), P4(O),<br>P5(I,O), P6(I,O) |
| <i>A. dianthi</i>                           | –                                   | –                            | P3(I)                             | P3(I)                                    | P3(I)                                      |
| <i>A. longipes</i>                          | P6(I)                               | P6(I)                        | –                                 | P6(O)                                    | –                                          |
| <i>A. radicina</i>                          | –                                   | –                            | P3(I), P5(I)                      | –                                        | P3(O)                                      |
| <i>A. tenuissima</i>                        | P1(I,O), P4(I,O)                    | P1(I,O), P4(I,O)             | P1(I,O), P4(I,O)                  | P1(I), P4(I,O)                           | P1(I)                                      |
| <i>A. pluriseptata</i>                      | P4(I)                               | P4(I)                        | –                                 | –                                        | P4(I)                                      |
| <i>Cladosporium herbarum</i>                | –                                   | –                            | P6(O)                             | –                                        | P1(I)                                      |
| <i>C. oxysporum</i>                         | P1(I,O), P4(I),<br>P5(I), P6(I,O)   | P1(I,O), P4(I,O),<br>P5(I,O) | P1(I), P4(I,O),<br>P5(I,O), P6(I) | P4(I,O), P5(I,O),<br>P6(I,O)             | P1(I,O), P4(I,O),<br>P5(I,O)               |
| <i>C. spermum</i>                           | –                                   | P1(I,O)                      | P1(I), P4(O)                      | –                                        | P1(I)                                      |
| <i>Chaetomium</i> sp.                       | –                                   | –                            | P1(I,O)                           | P3(I,O)                                  | –                                          |
| <i>Dreschlera hawaiiensis</i>               | P5(I)                               | –                            | –                                 | –                                        | –                                          |
| <i>Doratomyces</i> sp.                      | –                                   | –                            | –                                 | –                                        | P6(I)                                      |
| <i>Fusarium flocciferum</i>                 | P6(O)                               | –                            | –                                 | P5(I)                                    | P6(O)                                      |
| <i>Fusarium oxysporum</i>                   | –                                   | P4(I)                        | –                                 | P5(I,O)                                  | –                                          |
| <i>Helminthosporium acaciae</i>             | P1(O)                               | P6(I)                        | P6(I)                             | P4(I)                                    | P6(I)                                      |
| <i>H. solani</i>                            | –                                   | –                            | –                                 | P2(I)                                    | –                                          |
| <i>Mucor plumbeus</i>                       | –                                   | –                            | –                                 | –                                        | P6(I)                                      |
| <i>Mucor rasemosus</i>                      | –                                   | –                            | P5(I)                             | –                                        | –                                          |
| <i>Neurospora crassa</i>                    | –                                   | –                            | –                                 | –                                        | P2(I)                                      |
| <i>Phoma</i> sp.                            | P6(I,O)                             | P6(I)                        | P6(O)                             | –                                        | –                                          |
| <i>Rhizopus oryzae</i>                      | P2(I,O), P3(I,O),<br>P4(I), P5(I,O) | P4(I,O), P5(I)               | P4(I,O), P5(I,O)                  | PI(O), P4(I,O), P5(I)                    | P2(I), P3(O),<br>P4(I), P5(I)              |
| <i>R. stolonifer</i> var. <i>stolonifer</i> | –                                   | –                            | –                                 | P3(I)                                    | –                                          |
| <i>Scopulariopsis acremonium</i>            | –                                   | –                            | –                                 | P3(I,O)                                  | –                                          |
| <i>Sordaria</i> sp.                         | –                                   | P6(I,O)                      | P6(I,O)                           | P6(I,O)                                  | –                                          |
| <i>Trichoderma hamatum</i>                  | P1(O), P5(I,O)                      | P6(I)                        | P3(I), P4(I,O)                    | P3(I,O)                                  | –                                          |
| <i>Miselyum</i>                             | P1(I,O), P6(I,O)                    | P1(I,O), P5(I,O),<br>P6(I,O) | P1(I,O), P6(I)                    | P1(I,O), P2(I,O),<br>P4(I,O), P6(I,O)    | P1(O), P4(O), P5(O)                        |

O outdoor, I indoor, P1 March–April 2004, P2 May–June 2004, P3 July–August 2004, P4 September–October 2004, P5 November–December 2004, P6 January–February 2005

were requested to undergo skin testing. Skin testing was performed with 18 commercially available mold allergens.

Skin prick test (SPT) was performed by two nurses according to European guidelines (Dreborg), with commercially available mold allergens (*Aspergillus*, *Alternaria*, *Botrytis*, *Candida*, *Chaetomium*, *Cladosporium*,

*Curvularia*, *Fusarium*, *Helminthosporium*, *Microsporium*, *Mucor*, *Neurospora*, *Penicillium*, *Phoma*, *Pullularia*, *Rhizopus*, *Serpula*, *Trichophyton*) (Allergopharma, Germany). Histamine phosphate (10 mg/ml) and glycerinated saline were the positive and the negative controls, respectively. The mean wheal size (longest plus shortest diameter divided by two) was recorded

after 15 min. The SPT was regarded as positive with a wheal size of minimum 3 mm larger than the negative control (Dreborg and Frew 1993).

### Statistical analyses

Firstly, descriptive statistics on mold counts were performed and graphics were prepared. Then, two-way analysis of variance (ANOVA) was used to investigate whether there was a difference between schools and times. For binary comparisons, Tukey test analysis was used. With regression analyses, the impacts of meteorological variables such as time, moisture, and temperature on the amount of mold were investigated. Then, between the schools, the similarity coefficients for microfungus flora determined at genus level were calculated for each month. After that, the similarity coefficients for the changes each school experienced over time were assessed. The relationship between meteorological factors and indoor and outdoor mold counts was compared with the Pearson correlation analysis. SPSS 10.0 was used for all the analysis (SPSS-10 2000). Value of  $\alpha = 0.05$  was used for the evaluation of the analysis (Daniel 1983; Dawson and Trapp 2001; Dunn and Everitt 1995; Motulsky 1995; Steel et al. 1997).

### Results and discussion

In our research, in 150 Petri dishes were counted 7,122 microfungus colonies. Of them, 1,079 microfungus colonies were from indoor and 6,043 were from outdoor. The number of colonies either indoor (1,613 colonies) or outdoor (469 colonies) was highest between October and November (fourth term). The lowest number of the colonies indoor (155) was between June and July and the lowest number of the colonies outdoor (90) was between February and March. Sixty-four species belonging to 17 genera were identified. These are *Alternaria* (seven species), *Aspergillus* (13 species), *Chaetomium* (one species), *Cladosporium* (three species), *Dreschleria* (one species), *Doratomyces* (one species), *Emericella* (two species), *Fusarium*

(two species), *Helminthosporium* (two species), *Neurospora* (one species), *Mucor* (two species), *Penicillium* (23 species), *Phoma* (one species), *Rhizopus* (two species), *Scopulariopsis* (one species), *Sordaria* (one species), and *Trichoderma* (one species). In addition, several sterile micelles and yeast colonies were isolated as well. *Penicillium* genus had a maximum species number of 23 (35.93%), followed by *Aspergillus* by 13 species (20.31%), *Alternaria* by seven species (10.93%), and *Cladosporium* by three species (4.68%). We were able to identify three microfungus genera—*Chaetomium*, *Doratomyces*, *Phoma*, and *Sordaria*—at the genus level (Table 2).

The distribution of the species in indoor air and outdoor air during the research periods in the schools is shown in Table 2. As seen in the table, species of *Alternaria* *dantii*, *A. pluriseptata*, *Aspergillus* *auricomus*, *A. puniceus*, *Dreschleria* *hawaiiensis*, *Doratomyces* sp., *Emericella* *nidulans*, *Helminthosporium* *solanii*, *Mucor* *plumbeus*, *M. rasemosus*, *Penicillium* *aurantiogriseum*, *P. hirsutum*, *P. melinii*, and *P. waksmani* were isolated from indoor air in different schools at different periods, whereas *Penicillium* *marneffeii* was isolated both from indoor and outdoor air but only in one school and during a single period.

*Alternaria* *citrii*, *A. tenuissima*, *Aspergillus* *japonicus*, *A. flavus*, *A. melleus*, *A. niger*, *A. terreus*, *A. ustus*, *A. wentii*, *Cladosporium* *oxysporum*, *Penicillium* *chrysogenum*, *P. citreonigrum*, *P. citrinum*, *P. crustosum*, *P. expansum*, *P. italicum*, *P. paxillii*, *P. purpurogenum*, and *Rhizopus* *oryzae* were isolated both from indoor and outdoor air in all the schools and during all sampling periods (Table 2).

Asan et al. (2002) compared airborne fungal concentration, its contents, and different meteorological data. Aydoğdu and Asan (2005) compared the relationship between indoor fungal flora and relative humidity in elementary schools and age of the schools. The source of indoor airborne fungi is outdoor air. Thus, indoor microfungus species and their counts are affected by meteorological conditions (Burge 2002). Indoor fungal counts are higher in summer months when the outdoor fungal counts increase. Therefore, since air ventilation in the schools researched is ensured

**Table 3** School–time and school–age descriptive statistics

|          |          | Mean   | Std. deviation |
|----------|----------|--------|----------------|
| School A | Time     |        |                |
|          | 4/2004   | 620    | 155.78         |
|          | 6/2004   | 260    | 184.03         |
|          | 8/2004   | 170    | 52.92          |
|          | 10/2004  | 1,050  | 469.47         |
|          | 12/2004  | 415    | 146.40         |
|          | 2/2005   | 350    | 73.94          |
|          | Total    | 477.5  | 359.05         |
| B        | 4/2004   | 225    | 50.00          |
|          | 6/2004   | 165    | 52.60          |
|          | 8/2004   | 220    | 150.55         |
|          | 10/2004  | 1,420  | 123.29         |
|          | 12/2004  | 845    | 196.89         |
|          | 2/2005   | 455    | 125.83         |
|          | Total    | 555    | 473.44         |
| C        | 4/2004   | 965    | 506.06         |
|          | 6/2004   | 60     | 43.20          |
|          | 8/2004   | 280    | 58.89          |
|          | 10/2004  | 1,225  | 411.95         |
|          | 12/2004  | 1,040  | 718.89         |
|          | 2/2005   | 595    | 137.9          |
|          | Total    | 694.2  | 556.93         |
| D        | 4/2004   | 270    | 150.11         |
|          | 6/2004   | 150    | 52.92          |
|          | 8/2004   | 400    | 176.64         |
|          | 10/2004  | 2,215  | 150.00         |
|          | 12/2004  | 750    | 116.05         |
|          | 2/2005   | 545    | 204.86         |
|          | Total    | 721.7  | 721.98         |
| E        | 4/2004   | 935    | 634.01         |
|          | 6/2004   | 140    | 104.56         |
|          | 8/2004   | 305    | 71.88          |
|          | 10/2004  | 2,155  | 603.41         |
|          | 12/2004  | 1,925  | 1,425.52       |
|          | 2/2005   | 470    | 171.66         |
|          | Total    | 988.3  | 1,006.88       |
| School A | Age      |        |                |
|          | (5–8)    | 366.67 | 222.23         |
|          | (9–11)   | 463.33 | 342.33         |
|          | (12–15)  | 556.67 | 555.47         |
|          | Corridor | 523.33 | 304.74         |
|          | Total    | 477.50 | 359.05         |
| B        | (5–8)    | 476.67 | 502.38         |
|          | (9–11)   | 606.67 | 548.99         |
|          | (12–15)  | 536.67 | 493.87         |
|          | Corridor | 600.00 | 469.04         |
|          | Total    | 555.00 | 473.44         |
| C        | (5–8)    | 603.33 | 414.81         |
|          | (9–11)   | 613.33 | 440.12         |
|          | (12–15)  | 830.00 | 857.69         |
|          | Corridor | 730.00 | 532.88         |
|          | Total    | 694.17 | 556.93         |
| D        | (5–8)    | 726.67 | 823.62         |

**Table 3** (continued)

|   | Mean     | Std. deviation |
|---|----------|----------------|
|   | (9–11)   | 676.67         |
|   | (12–15)  | 730.00         |
|   | Corridor | 753.33         |
|   | Total    | 721.67         |
| E | (5–8)    | 763.33         |
|   | (9–11)   | 1,526.67       |
|   | (12–15)  | 686.67         |
|   | Corridor | 976.67         |
|   | Total    | 988.33         |

by opening the windows, indoor air rich in fungal colony counts can partly be due to outdoor air containing high numbers of fungal colonies. In the area the research was conducted, since the temperature reaches quite high degrees between May and October, this increased temperature together with the increase in humidity can affect the increase in the number of fungal colonies.

Of the 117 teachers working in five elementary schools in the research area, 55 (47%) underwent skin prick tests. Twenty-four (20.5%) of these 55 teachers were sensitive to at least one agent. Of these 24 teachers, one was sensitive to *Aspergillus*, two to *Alternaria*, two to *Botrytis*, one to *Candida*, one to *Cladosporium*, one to *Curvularia*, two to *Fusarium*, one to *Helminthosporium*, one to *Mucor*, one to *Neurospora*, one to *Penicillium*, one to *Pullularia*, three to *Rhizopus*, one to *Serpula*, five to *Trichophyton*. Four of them were sensitive to both *Alternaria* and *Trichophyton*, one to both *Botrytis* and *Candida*, two to both *Curvularia* and *Rhizopus*, and two to *Botrytis*, *Fusarium*, *Penicillium*, *Rhizopus*, *Serpula*, and *Trichophyton*. The rate of sensitivity to microfungi among teachers living in non metropolitan area was found to be 28.2%.

There is an increasing concern about exposure to fungi and the effects of metabolites on health. Of these fungi, especially *Aspergillus*, *Cladosporium*, and *Penicillium* cause allergic symptoms mostly in the respiratory system since they exist in indoor air (Aydoğdu and Asan 2008; Fisher and Dott 2003). Exposure to *Alternaria* spores is known to be the leading cause of asthma and allergic rhinoconjunctivitis (Dreborg and Frew 1993).

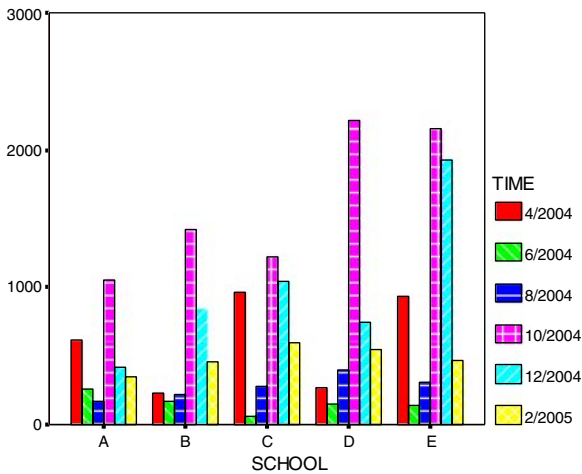


**Fig. 1** Histogram graphics showing mold counts in Seferihisar (for period)

It is also reported that especially *Alternaria*, *Cladosporium*, and *Aspergillus* cause sensitivity and thus allergic symptoms in children (Aydoğdu and Asan 2008; Pastuszka et al. 2000). Since *Alternaria* spores are bigger than 10  $\mu\text{m}$ , they are usually retained in the nose and/or pharynx. However, since *Aspergillus* and *Penicillium* spores are smaller than 5  $\mu\text{m}$ , they cause asthma and allergies by colonizing the lungs (Fang et al. 2005). In our research, *Alternaria*, *Aspergillus*, *Cladosporium*,

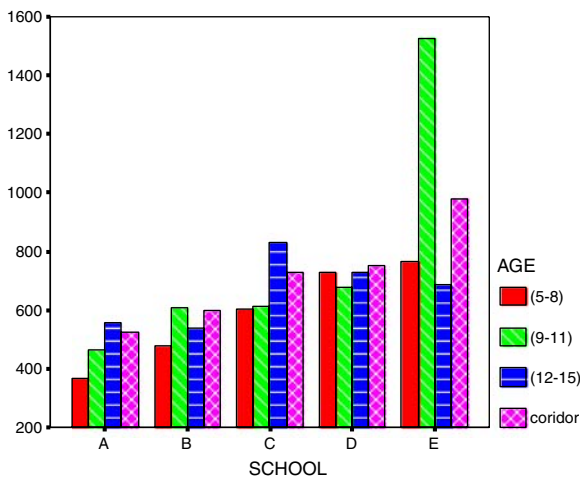


**Fig. 2** Histogram graphics showing mold counts in Seferihisar (for age)

and *Penicillium* spores were profoundly present and can be said to affect both the students and the teachers.

In Table 3, descriptive statistics of school–time and school–age are assessed. When school–time relationship is considered, it was found that mold counts reached the highest points during October 2004 in school D ( $2,215 \pm 150$ ) and the lowest points during June 2004 in school C ( $60 \pm 43.20$ ) (Table 3; Fig. 1). When school–age relationship is considered, it was found that mold counts were at their highest in the 9–11 age group in school E ( $1,526 \pm 1,562.90$ ), and lowest in the 5–8 age group in school A ( $366.67 \pm 222.23$ ) (Table 3; Fig. 2).

ANOVA revealed that, in terms of mold counts, the difference between the schools and the difference between the times (periods) were statistically significant ( $F_{\text{school}} = 6.345$ ,  $p = 0.00 < 0.05$ ;  $F_{\text{time}} = 40.094$ ,  $p = 0.00 < 0.05$ ). When the

**Table 4** Tukey test results comparing the mold counts in the school and time

|            |            | p value |
|------------|------------|---------|
| (I) School | (J) School |         |
| A          | B          | 0.955   |
|            | C          | 0.289   |
|            | D          | 0.181   |
|            | E          | 0.000   |
| B          | C          | 0.713   |
|            | D          | 0.555   |
|            | E          | 0.001   |
| C          | D          | 0.999   |
|            | E          | 0.066   |
| D          | E          | 0.118   |
| (I) Time   | (J) Time   |         |
| 4/2004     | 6/2004     | 0.005   |
|            | 8/2004     | 0.081   |
|            | 10/2004    | 0.000   |
|            | 12/2004    | 0.019   |
|            | 2/2005     | 0.918   |
| 6/2004     | 8/2004     | 0.918   |
|            | 10/2004    | 0.000   |
|            | 12/2004    | 0.000   |
|            | 2/2005     | 0.081   |
| 8/2004     | 10/2004    | 0.000   |
|            | 12/2004    | 0.000   |
|            | 2/2005     | 0.518   |
| 10/2004    | 12/2004    | 0.000   |
|            | 2/2005     | 0.000   |
| 12/2004    | 2/2005     | 0.001   |

**Table 5** Similarity coefficients regarding microfungus flora detected at genus level between the schools on a monthly basis and similarity coefficients for each school for a period

| School          | 4/2004 | 6/2004 | 8/2004 | 10/2004 | 12/2004 | 02/2005 |
|-----------------|--------|--------|--------|---------|---------|---------|
| A–B             | 1      | 0.80   | 1      | 0.86    | 0.67    | 0.67    |
| A–C             | 1      | 0.80   | 0.80   | 0.86    | 0.67    | 1       |
| A–D             | 0.86   | 0.80   | 0.80   | 0.86    | 0.40    | 1       |
| A–E             | 1      | 0.80   | 1      | 0.86    | 0.67    | 0.86    |
| B–C             | 1      | 1      | 0.80   | 1       | 1       | 0.67    |
| B–D             | 0.86   | 1      | 0.80   | 1       | 0.86    | 0.67    |
| B–E             | 1      | 1      | 1      | 1       | 1       | 0.40    |
| C–D             | 0.86   | 1      | 1      | 1       | 0.86    | 1       |
| C–E             | 1      | 1      | 0.80   | 1       | 1       | 0.86    |
| D–E             | 0.86   | 1      | 0.80   | 1       | 0.86    | 0.86    |
| Time            | A      | B      | C      | D       | E       |         |
| 4/2004–6/2004   | 0.67   | 0.86   | 0.86   | 1       | 0.86    |         |
| 4/2004–8/2004   | 0.67   | 0.67   | 0.86   | 1       | 0.67    |         |
| 4/2004–10/2004  | 0.86   | 1      | 1      | 0.86    | 1       |         |
| 4/2004–12/2004  | 0.67   | 1      | 1      | 0.67    | 1       |         |
| 4/2004–2/2005   | 1      | 0.67   | 1      | 0.86    | 0.86    |         |
| 6/2004–8/2004   | 0.50   | 0.67   | 1      | 1       | 0.80    |         |
| 6/2004–10/2004  | 0.80   | 0.86   | 0.86   | 0.86    | 0.86    |         |
| 6/2004–12/2004  | 0.50   | 0.86   | 0.86   | 0.67    | 0.86    |         |
| 6/2004–2/2005   | 0.67   | 0.80   | 0.86   | 0.86    | 0.67    |         |
| 8/2004–10/2004  | 0.80   | 0.67   | 0.86   | 0.86    | 0.67    |         |
| 8/2004–12/2004  | 0.50   | 0.67   | 0.86   | 0.67    | 0.67    |         |
| 8/2004–2/2005   | 0.67   | 0.50   | 0.86   | 0.86    | 0.40    |         |
| 10/2004–12/2004 | 0.80   | 1      | 1      | 0.86    | 1       |         |
| 10/2004–2/2005  | 0.86   | 0.67   | 1      | 1       | 0.86    |         |
| 12/2004–2/2005  | 0.67   | 0.67   | 1      | 0.86    | 0.86    |         |

schools were compared with the Tukey test, there was a statistically significant difference in mold counts between A and E, and between B and E schools ( $p < 0.05$ ) (Table 4). When the periods were compared with the same method, there was a statistically significant difference between the following periods: 4/2004–6/2004; 4/2004–

10/2004; 4/2004–12/2004; 6/2004–10/2004; 6/2004–12/2004; 8/2004–10/2004; 8/2004–12/2004; 10/2004–12/2004; 10/2004–2/2005; 12/2004–2/2005 ( $p < 0.05$ ) (Table 4).

Similarity coefficients regarding microfungus flora were calculated and the results were found as shown in Table 5. The effects of meteorological

**Table 6** Regression analysis of fungus

| Variable     | <i>B</i>        | SE <i>B</i>  | Beta       | <i>T</i> | <i>p</i> value |
|--------------|-----------------|--------------|------------|----------|----------------|
| Indoor       |                 |              |            |          |                |
| $R^2 = 0.96$ |                 |              |            |          |                |
| WET          | 5,816.484711    | 757.752506   | 55.124781  | 7.676    | 0.0046         |
| WET**2       | −44.401122      | 5.823560     | −54.754403 | −7.624   | 0.0047         |
| (Constant)   | −188,861.223000 | 24,533.64112 |            | −7.698   | 0.0046         |
| Outdoor      |                 |              |            |          |                |
| $R^2 = 0.94$ |                 |              |            |          |                |
| WET          | 5,954.991542    | 865.769954   | 58.139976  | 6.878    | 0.0063         |
| WET**2       | −45.641341      | 6.653707     | −57.981696 | −6.860   | 0.0063         |
| (Constant)   | −192,673.714691 | 28,030.90608 |            | −6.874   | 0.0063         |

factors (temperature, humidity) on indoor and outdoor mold counts were investigated with the regression analysis and it was determined that the effect of humidity on the amounts of mold was significant, which can be explained with a quadratic model either indoor or outdoor ( $p < 0.05$ ) (Table 6). The relationship between meteorological factors and indoor and outdoor mold counts was compared with the Pearson correlation analysis and it was found that the association between humidity and temperature ( $p = 0.028$ ) and the indoor and outdoor fungus ( $p = 0.001$ ) counts was statistically significant ( $p < 0.05$ ).

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