The International

Journal of Ventilation

Volume 12 Number 1
June 2013

ISSN 1473 - 3315

Contents

Editorial Board

Published by VEETECH Ltd
7A Barclay's Venture Centre,
The University of Warwick Science Park,
Sir William Lyons Road,
COVENTRY
United Kingdom
CV4 7EZ
Tel +44(0) 1189477231
Email mliddament@coventry.org.uk Web www.veetech.org.uk

Cover Design: Zoe Lawson, *Wyatt International Ltd.*
Paper: Meets Nordic and European environmental standards and is sourced from sustainable forests, totally chlorine free.
Binding of printed edition: Kew Bookbinding
E-Edition: *This Journal is available on-line via Allen Press and major providers*

Correlation of Particulate Matter with Airborne Fungi in Schools in Greece

P.V. Dorizas[1], E. Kapsanaki-Gotsi[2], M.N. Assimakopoulos[1] and M. Santamouris[1]

[1] Faculty of Physics, Department of Environmental Physics and Meteorology, University of Athens, University Campus, Building of Physics -5, GR-157 84, Athens, Greece
[2] Faculty of Biology, Department of Ecology and Systematics, University of Athens, Panepistimiopolis, GR-157 84, Athens, Greece

Abstract

The concentration levels of particulate matter (PM), airborne fungi, carbon dioxide as well as temperature and relative humidity were investigated in the indoor and outdoor environment of two schools in Athens, Greece during the period January to May 2011. The overall concentration ranges of the indoor measured pollutants were: PM_{10}: 14.92-166.18 $\mu g/m^3$, $PM_{2.5}$: 3.16-31.27 $\mu g/m^3$, PM_1: 0.72-9.01 $\mu g/m^3$, UFP: 4188-63093 pt/cm^3, total airborne fungi: 28-2098 CFU/m^3 and CO_2: 389-1717 ppm. The relationships between PM and airborne fungi were mainly examined, and bivariate correlations of all the measured environmental parameters are also reported. The results indicate that PM of certain aerodynamic diameters significantly correlate to the total airborne fungi and their prevalent genera, *Penicillium*, *Cladosporium* and *Aspergillus*. Principal Component Analysis (PCA) was conducted so as to cluster variables of common characteristics. Furthermore, simple and multiple linear regression models were developed to investigate several cases of dependent variables to be used for prediction purposes in health risk assessments.

Key words: indoor air quality, schools, particulate matter, airborne fungi, PCA, multiple linear regression models.

1. Introduction

The study of Indoor Air Quality (IAQ) has attracted the interest of the scientific community in recent years, especially since people spend approximately 85-90 % of their time in indoor environments (EPA 1995a; Jenkins et al, 1992; Long et al, 2001). Epidemiological studies have associated exposure to indoor air pollutants, such as particulate matter (PM) and airborne fungi, to human health effects (Chapman 1999; Pope III et al, 1995). The effects of PM include headaches, asthma symptoms, allergies, respiratory and cardiovascular diseases and even mortality (Brunekreef and Holgate, 2002; Dockery et al, 1993; Donaldson and Stone, 2003). The small size and relatively large surface are to volume ratio of particles in the ultrafine size range (UFP <100 nm in diameter) result in their ability to penetrate deeper into the lungs and alveoli and may have higher reactivity and toxicity compared to larger particles (Oberdörster, 2000). Effects of airborne fungi include irritations, infections, respiratory allergies and asthma (Adhikari et al, 2004; Burge and Rogers, 2000; Bush and Portnoy, 2001; Pongracic et al, 2010). Children are more vulnerable to health problems compared to adults, due to the greater volume of air inhaled in proportion to their body weight. Owing to their developing lungs, they may be especially susceptible to particle inhalation (EPA, 1995b). Students, in particular, spend a substantial amount of their day time within school premises (Silvers et al, 1994). Internationally, several epidemiological publications report exposure to air pollutants in school environments in association to health impacts (Guo et al, 1999). Human activities and classrooms overcrowded for several hours, poor ventilation and infrequent cleaning constitute some of the most common sources of air pollutants in school environments. In addition, the indoor air is greatly affected by outdoor pollutant concentrations (Fromme et al, 2008). Degraded IAQ in school classrooms impacts on students' performance, attendance and comfort and may also lead to increased energy consumption, leading to massive societal and economic costs (EPA 1995b; Mendell and Heath, 2005; Wargocki and Wyon, 2013). Therefore, the quality of air inside school classrooms is of primary concern.

Even though both particulate matter and airborne fungi constitute important indoor air pollutants that cause a great number of adverse health effects, there are only a small number of studies in the international literature reporting their simultaneous measurement, interrelationships and interaction (Adhikari et al, 2006; Degobbi et al, 2011; Grinn-Gofroń et al, 2011; Hargreaves, 2003; Sousa et al, 2008). However, these studies have not been extensively evaluated due to the complex interrelation of the pollutants. In Greece, a quantitative evaluation of bioaerosol in comparison to particulate matter has been made in the ambient air and in residential, office and school buildings (Kalogerakis et al, 2005; Raisi et al, 2010, Dorizas et al, 2012). Nevertheless in school buildings, several studies have been carried out investigating concentration levels of only non-biological air pollutants such as PM, carbon dioxide (CO_2), carbon monoxide (CO), ozone (O_3) and Total Volatile Organic Compounds (TVOCs) (Diapouli et al, 2007; Lazaridis and Aleksandropoulou, 2009; Santamouris et al, 2008; Synnefa et al, 2003; Triantafyllou et al, 2008).

In this study, an experimental campaign was conducted in two high schools in Athens, Greece. Particulate matter of several size ranges (PM_{10}, $PM_{2.5}$, PM_1, UFP), CO_2, temperature (T) and relative humidity (RH) were measured in school classrooms and the outdoor environment. Air sampling was simultaneously conducted for airborne fungi. The fungi were identified to genus level and the prevalent genera were *Penicillium*, *Cladosporium* and *Aspergillus*. The objectives of this work were to:

(i) report the indoor and outdoor concentration levels of the aforementioned air pollutants;
(ii) characterize the indoor environments of classrooms against proposed limit values by international certification bodies;
(iii) examine the correlations between the measured parameters focusing on relationships between PM and airborne fungi;
(iv) deliver simple and multivariate linear regression models.

2. Materials and Methods

2.1 Sampling Site Description

Measurements were carried out in two vocational high schools from nearby areas outside the city centre of Athens. The first school is in the Kaisariani urban area, hereafter denoted by K. This school is located away from major highways and is next to a park. It was constructed in 2001 and has double glazed windows installed. The second school is in the Ymittos urban area, hereafter denoted by Y, where the traffic in the adjoining streets is moderate. This school was constructed in 1930 and all of the building's windows are single glazed. Central heating systems are used in both of the two schools and are oil-fired boilers that heat water to provide central heating via radiators. Both schools are naturally ventilated.

2.2 Sampling Strategy

The sampling period was from January until May 2011 and measurements were conducted once every two weeks in each school. Air samples were collected from eight sampling sites denoted by K1 to K8 and Y1 to Y8 for schools K and Y respectively. One of the measurement sites was the outdoor environment where the instruments were placed on the schools' roof-yards (positions K8 and Y8). The other seven sampling positions were classrooms and laboratories. Certain classrooms were occupied by students during sampling throughout the measurement period. The number of students before and during the measurements, the prevailing conditions such as open windows before the measurement or any detected odours was logged. The windows were kept closed while the measurements were carried out. The sampling apparatus were synchronized and were placed each time at the same location of 0.8 m above the floor.

2.3 Parameters Measured and Instrumentation

PM_{10}, $PM_{2.5}$, PM_1 (where 10, 2.5 and 1 refer to the aerodynamic diameter of the particles in μm) were measured in units of mass per unit volume ($\mu g/m^3$) using Osiris, an airborne particulate monitor (Turnkey Instruments Ltd). UFP concentrations were measured in units of number of particles per unit volume ($particles/cm^3$) using P-Trak, a portable particle counter (TSI, Model 8525). The particle detection range of this instrument is from 20 nm to about 1 μm. Carbon dioxide (CO_2) was recorded using IAQ-CALC, a portable instrument meter from TSI (TSI model 8732). Temperature (T) and relative humidity (RH) were recorded using self-contained sensors incorporated in Tinytag dataloggers (Gemini data loggers TGP-4500). All these parameters were monitored with a sampling step of 1 second and the duration of each measurement, per position, was 10 minutes.

Airborne fungi were recovered using a Burkard (Burkard Manufacturing Co. Ltd. Hertfordshire, UK), a portable air sampler for agar plates. Three plates with Malt Agar were exposed consecutively in each sampling site for 3 min/plate and then incubated for 2 weeks at 28 °C. The colony count was corrected and expressed as colony forming units per cubic meter (CFU/m^3), a measure of viable spore concentrations. The fungal colonies were identified to genus level and *Penicillium*, *Cladosporium* and *Aspergillus* were the predominant genera.

2.4 Statistical Analysis

Statistical analysis was performed using the SPSS (SPSS Inc PASW Statistics 18) statistical software package as well as data analysis of Microsoft Excel 2007. The concentrations of the pollutants per measurement position and day were studied by temporal variation graphs, and the range of values which the PM concentrations laid are presented in box-plots. Furthermore, Principal Component Analysis (PCA) was performed in order to cluster variables of common characteristics. In addition Spearman's correlation coefficients were calculated among all possible pairs of measured variables so as to investigate bivariate relationships. Simple and multiple stepwise linear regression analyses were also performed to examine combined and individual influence between the environmental variables.

3. Measurements Results

3.1 Particulate Matter Concentrations

The variation in time of PM_{10} concentrations, in units of μg/m^3, in the course of the campaign is presented in Figure 1 (Figure 1, left is for school K and right is for school Y) (Dorizas et al, 2012). Each diagram contains 8 graphs (one for each sampling position). K8 and Y8 graphs refer to the measurements in the outdoor environment. The horizontal line denoted by LV corresponds to the 24-h limit value of PM_{10} (50 μg/m^3) recommended by the World Health Organization (WHO, 2005). The overall indoor PM_{10} concentrations in both schools ranged between 14.92 and 166.18 μg/m^3. In particular, the mean concentration of PM_{10} indoors was 64.53 μg/m^3 and ranged between 18.27-166.18 μg/m^3 in school K (Figure 1 left), and 38.65 μg/m^3 and 14.92-116.14 μg/m^3 in school Y respectively (Figure 1 right). Higher concentrations appear at school K exceeding the limit values in many cases (Figure 1 left e.g. K3 graph, st.dev. = 46), whereas in school Y the concentrations are significantly lower in almost all measurement positions, with a few exceptions (Figure 1 right e.g. Y3 graph, st.dev. = 32). The intense indoor peaks for certain dates are possibly attributed to:

(i) the increased presence of students being in the classrooms before and during the

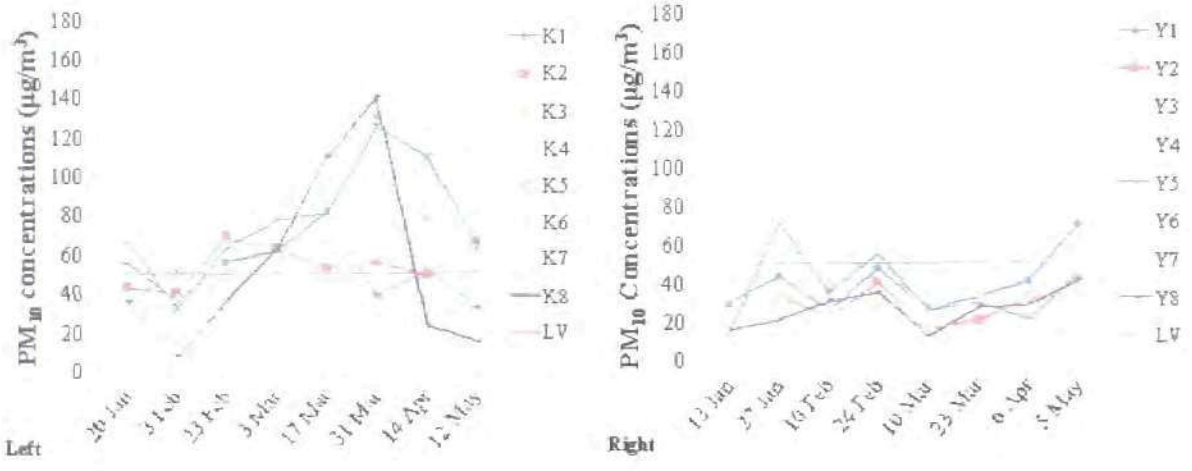

Figure 1. Temporal variation of PM_{10} concentrations for all of the measurement sites for schools K (left) and Y (right).

measurements; since students' presence and physical movement in the classroom may cause re-suspension of coarse particles and therefore affect their concentrations (Guo et al, 2010);

(ii) inadequate ventilation rates;

(iii) infiltration of particles from construction works that were taking place outside school K in certain days. It should be noted that in most cases outdoor fresh air did not enter the classrooms so as to meet the ventilation requirements and to remove odours and contaminants.

The averaged PM_{10} concentrations at the 7 indoor sites (K1 to K7 and Y1 to Y7) are depicted versus the outdoor concentrations in Figure 2 (Figure 2, left is for school K and right is for school Y). The outdoor concentrations ranged from 7 to 139 $\mu g/m^3$ in school K and from 12 to 41 $\mu g/m^3$ in school Y. Most indoor measurements were greater than the corresponding outdoor ones for both schools and they seem to follow the same trend, especially at school Y. The majority of the indoor measurements of school K, exceeded the limit value. The exteme outdoor PM_{10} concentrations on the 17th and 31st of March in school K are possibly linked to the increased levels of relative humidity, as in both of these days it was drizzling during the measurements. In addition, over these days a strong smell of smoke was detected outside school K, which could have also affected the concentrations. Average indoor and outdoor PM_{10} concentrations in school Y were below the recommended limit value, excluding the indoor measurements on the 5th of May.

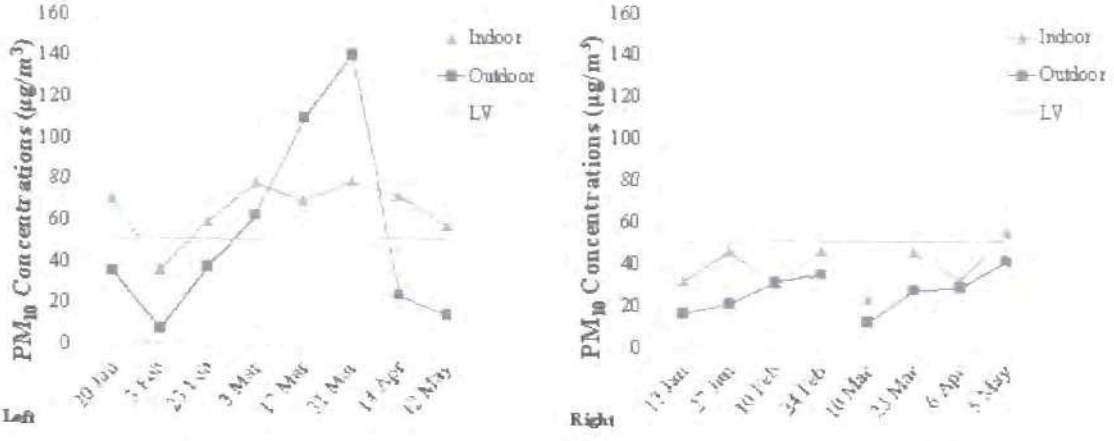

Figure 2. Averaged indoor versus outdoor PM_{10} concentration fluctuations for schools K (left) and Y (right).

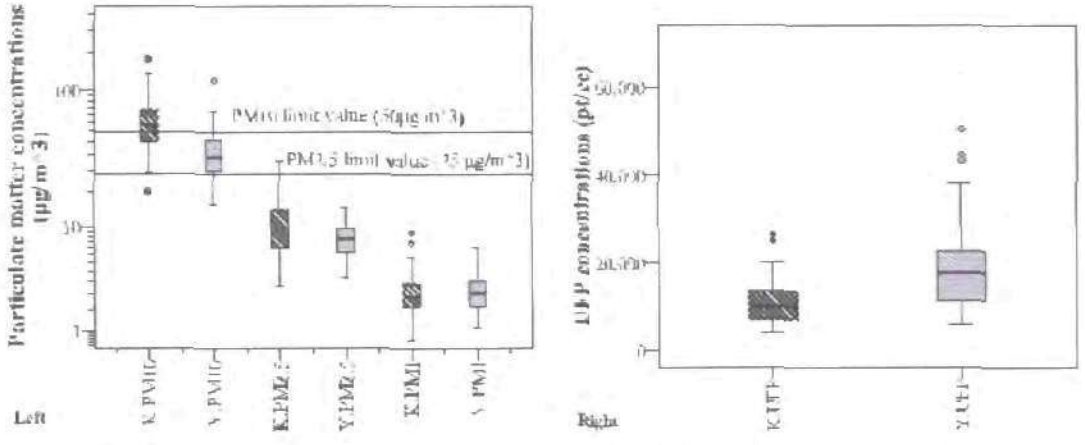

Figure 3. Concentration distributions of PM_{10}, $PM_{2.5}$, and PM_1 (left) and of UFP (right) in schools K (striped) and Y (grey), presented in box plots.

The indoor concentration distributions of PM_{10}, $PM_{2.5}$, PM_1 (Figure 3, left) and of UFP concentrations (Figure 3, right) for both schools are illustrated as box plots. A percentage of 60% of the total average PM_{10} concentrations in school K and of 17% in school Y, exceeded the limit value of 50 $\mu g/m^3$. Although school K is more recently constructed compared to school Y, the increased PM_{10} concentrations indoors may have been due to the increased concentrations outdoors (Figure 2 left). It is possible that PM_{10} concentrations were influenced by construction works that were carried out on certain days outside school K, where dust clouds were frequently visible. Furthermore, in certain cases, the surface area per pupil in school K was smaller than in school Y and the intense movement and activity from the increased presence of students caused resuspension of particles of greater size, increasing their concentrations. $PM_{2.5}$ concentrations indoors ranged from 3.16 to 31.27 $\mu g/m^3$ in school K and from 3.75 to 18.05 $\mu g/m^3$ in school Y. The limit value of 25 $\mu g/m^3$ for $PM_{2.5}$ was only exceeded in school K by 5% (Figure 3 left). The UFP concentrations ranged from 4188 to 26518 pt/cm^3 in school K and from 5789 to 63093 pt/cm^3 in school Y (Figure 3 right). The UFP concentrations in school Y were greater than the corresponding ones of school K and also present a stronger dispersion around the mean value. The increased UFP concentrations in school Y were possibly linked to vehicle emissions from the adjoining streets. Furthermore, due to the single and unsealed glazed windows of the construction of school Y, outdoor air pollutants may infiltrate more easily into the indoor environment.

3.2 Airborne Fungi Concentrations

The temporal variation of the total airborne fungi concentrations for both schools, expressed in CFU/m^3, is presented in a logarithmic scale in Figure 4 (Dorizas et al, 2012). Each diagram contains 8 graphs (one for each sampling site). K8 and Y8 graphs refer to the outdoor measurements. The overall indoor airborne fungi concentrations in both schools ranged between 28 and 2098 CFU/m^3. In particular, the mean concentration of the total airborne fungi concentrations indoors was 191 CFU/m^3 and ranged between 28-2098 CFU/m^3 excluding 1 extreme peak (5685 CFU/m^3) in school K (Figure 4 left) and 146 CFU/m^3 and 28-866 CFU/m^3 in school Y respectively (Figure 4 right). School K presents higher concentrations of airborne fungi as is the case for PM. There does not exist a universally acceptable threshold limit value for fungal concentrations.

The averaged total airborne fungi concentrations at the 7 indoor sites (K1 to K7 and Y1 to Y7) are presented versus the outdoor concentrations in Figure 5. The outdoor concentrations of the total airborne fungi ranged from 51 to 410 CFU/m^3 in school K and from 69 to 236 CFU/m^3 in school Y. In most of the measurements, the indoor concentrations follow the trend of the corresponding outdoor ones. In the case of school Y (Figure 5 right), the indoor concentration levels seemed to be mostly affected by the outdoor concentrations, which probably indicates the absence of major indoor sources.

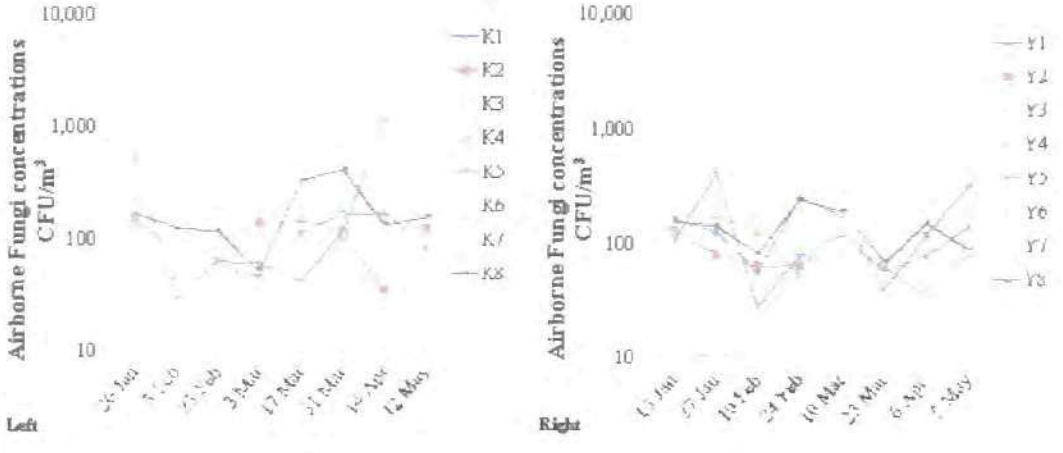

Figure 4. Temporal variation of airborne fungi concentrations in all of the measurement sites, for schools K (left) and Y (right).

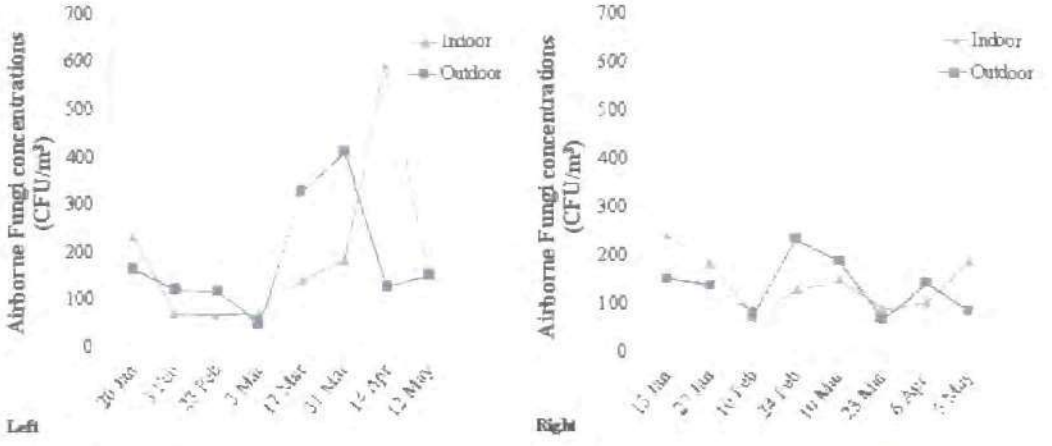

Figure 5. Averaged indoor versus outdoor airborne fungi concentration fluctuations for schools K (left) and Y (right).

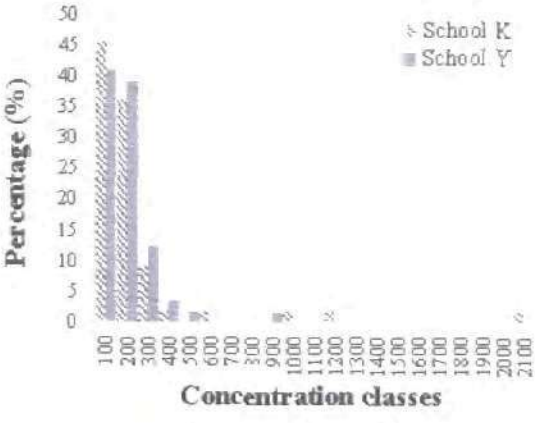

Figure 6. Frequency distribution of the total airborne fungi concentrations in classes of 100 CFU/m³ in schools K (striped) and Y (grey).

The frequency distribution of the total airborne fungi concentrations in the two schools is indicated in Figure 6, along 21 classes of 100 CFU/m^3, ranging from 100 to 2100. More than 90% of the total airborne fungi concentrations are between 100 and 300 CFU/m^3 for both schools. However, there was a small percentage, especially in school K, which distributed from 400 to 2098 CFU/m^3.

The predominant genera found in the indoor environment of the classrooms were *Penicillium*, *Cladosporium* and *Aspergillus*. *Penicillium* concentrations ranged from 0 to 872 CFU/m^3 in school K and from 6 to 476 CFU/m^3 in school Y. The concentration ranges for *Cladosporium*, were from 0 to 585 CFU/m^3 in school K and from 0 to 134 CFU/m^3 in school Y. *Aspergillus* concentrations extended from 0 to 17 and 0 to 176 CFU/m^3 in schools K and Y respectively.

3.3 CO_2 Concentration

CO_2 concentrations are often used as an indicator of ventilation efficiency and the assessment of indoor air quality and the excess of occupancy (Mumovic et al, 2009, Santamouris et al, 2008). In this case, CO_2 concentrations ranged from 389 to 1717 ppm in school K and from 460 to 1377 ppm in school Y throughout the measurement period. On every sampling day the number of students occupying the classrooms was marked down. The linear correlation between indoor CO_2 concentrations and

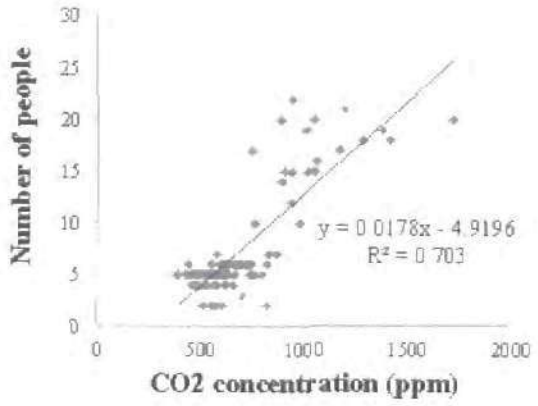

Figure 7. Scatter plot of CO_2 concentrations versus number of people.

the number of people being present during sampling for both schools in all indoor sites of measurement is presented in Figure 7. As expected CO_2 concentrations are highly correlated to the number of occupants ($r^2 = 0.703, N = 112$). In many cases CO_2 concentrations exceeded the more recent recommended standards of ASHRAE (62-2001 and 62-2004) of 700 ppm indicating inadequate ventilation rates and overcrowded classrooms. Insufficient ventilation contributes to the inhibition of the removal of larger particles from the indoor to the outdoor environment (Almeida et al, 2010).

4. Common Data Set of Measurements of the Two School

The areas to which the datasets of the measured parameters in the two schools extend, are visualized in a rose diagram in Figure 8. The maximum values of each variable have been used as input for the design of the diagram. The area where the variables of school K occur, is presented in black and for school Y is shown in grey. The common area of the data is where the data from the two schools overlap, and is presented in dark grey. The common area represents a percentage of approximately 66% of the total number of measurements from both schools.

Descriptive statistics of all the indoor measured environmental parameters of schools K (N=55), Y (N=56) and of the common area (denoted by C, N=73) of the dataset are summarized in Table 1. Both mean PM_{10} and $PM_{2.5}$ concentrations of the common area were below the recommended WHO limit values. The extreme values (outside the common area of Figure 8) were filtered out and hereafter any statistical analysis refers to the data set of the common area of size N=73.

5. Results and Discussion

This study primarily investigated correlations between particulate matter and airborne fungi concentrations, as well as further correlations such as PM_{10} versus temperature. To this end, a four-step analysis procedure was applied as follows:

Step 1. Principal Component Analysis (PCA) was applied to the entire set of variables of the common area so as to identify hidden pattern correlations in the data. These were categorized according to the amount of information stored in the data set they represent.

Step 2. Spearman's rank correlation coefficients were calculated amongst all the pairs of variables.

Step 3. Linear regression models were developed from all the statistically significant pairs of values.

Step 4. Several multiple linear regression models were further delivered aiming to be used for prediction purposes. According to the findings from PCA, certain variables were excluded from the regression models in each case.

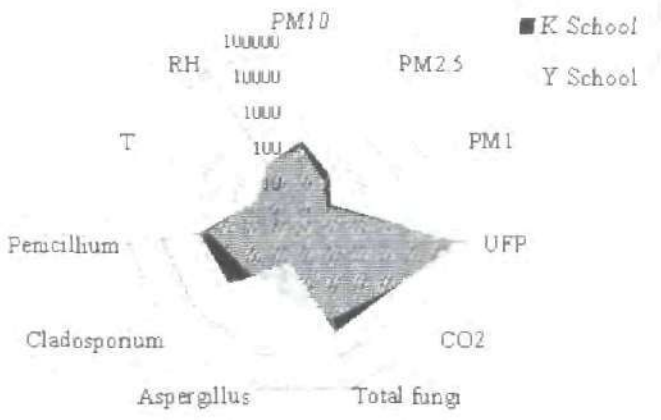

Figure 8. Rose diagram created from the maximum values of each variable in each of the two schools.

Table 1. Descriptive statistics of the indoor environmental parameters of Schools K, Y and of the common filtered area (denoted by C).

Variables	School	Minimum	Maximum	Mean	Median	Std. Deviation
PM_{10} (μg/m^3)	K (N=55)	18.27	166.18	64.53	55.94	32.07
	Y (N=56)	14.92	116.14	38.65	33.74	19.35
	C (N=73)	14.92	116.14	47.83	43.69	20.66
$PM_{2.5}$ (μg/m^3)	K (N=55)	3.16	31.27	10.90	9.76	5.52
	Y (N=56)	3.75	18.05	8.19	7.81	2.78
	C (N=73)	3.16	17.58	8.71	7.99	2.97
PM_1 (μg/m^3)	K (N=55)	.72	9.01	2.83	2.42	1.58
	Y (N=56)	1.09	6.81	2.94	2.75	1.29
	C (N=73)	0.72	6.81	2.59	2.39	1.08
UFP (pt/cm^3)	K (N=55)	4188	26518	10853	9774	4895
	Y (N=56)	5789	63093	19333	17530	11392
	C (N=73)	4188	26518	12365	10059	5865
CO_2 (ppm)	K (N=55)	389	1717	666	588	265
	Y (N=56)	460	1377	674	601	187
	C (N=73)	389	1377	613	570	166
Total Fungi (CFU/m^3)	K (N=55)	28	2098	191	104	329
	Y (N=56)	28	866	146	123	128
	C (N=73)	28	310	102	80	67
Aspergillus (CFU/m^3)	K (N=55)	0	17	3	0	4
	Y (N=56)	0	176	15	6	34
	C (N=73)	0	17	3	0	4
Cladosporium (CFU/m^3)	K (N=55)	0	585	43	23	85
	Y (N=56)	0	134	22	17	24
	C (N=73)	0	116	22	17	21
Penicillium (CFU/m^3)	K (N=55)	0	872	99	40	177
	Y (N=56)	6	476	76	66	75
	C (N=73)	0	227	51	34	45
T(^{0}C)	K (N=55)	15.83	22.19	19.67	20.18	1.51
	Y (N=56)	11.95	23.23	18.84	18.68	2.21
	C (N=73)	14.83	22.19	19.37	19.82	1.8
RH (%)	K (N=55)	47.14	66.90	53.88	53.20	4.67
	Y (N=56)	26.31	75.48	50.87	52.17	12.65
	C (N=73)	26.31	65.14	51.44	52.42	7.53

5.1 Principal Component Analysis

Principal Component Analysis (PCA) is a method of reducing the dimensionality of the dataset while keeping the information and variability of the data. It is a mathematical procedure which transforms a set of possibly related variables into a set on uncorrelated variables. The new variables are called principal components (PCs) and are linear correlations of the initial set of variables. The first PC has the largest possible variability and each next PC has the largest proportion of the variability that has not been explained by the first component (Gaitani et al, 2010; Jolliffe 2002). PCA also gives a hint concerning the correlations between parameters.

PCA was applied on the eleven parameters of the data set of the common area (N=73). These parameters were PM_{10}, $PM_{2.5}$, PM_1, UFP, CO_2, Total Fungi, *Penicillium*, *Cladosporium*, *Aspergillus*, temperature and relative humidity. In order to identify the number of significant PCs, the Varimax Rotation method was applied. Four components which had eigenvalues greater than one were examined (Table 2). The four component solution accumulates 71.848% of the total variance.

Table 2. Rotated component matrix.

	Component			
	1	2	3	4
PM_{10}	162	**.755**	169	138
$PM_{2.5}$	066	**.586**	086	**.737**
PM_1	-.041	-.082	059	**.952**
UFP	276	-.104	**.848**	100
CO_2	-.124	194	**.793**	018
Total fungi	**.960**	139	094	-.043
Aspergillus	098	493	202	-.066
Cladosporium	**.828**	183	-.183	000
Penicillium	**.876**	-.044	238	026
T	025	**.808**	-.151	-.022
RH	-.041	**.506**	-.302	302
Eigenvalue	2.950	2.195	1.595	1.164
% of variance	26.816	19.952	14.500	10.581
Cumulative %	26.816	46.768	61.268	71.848

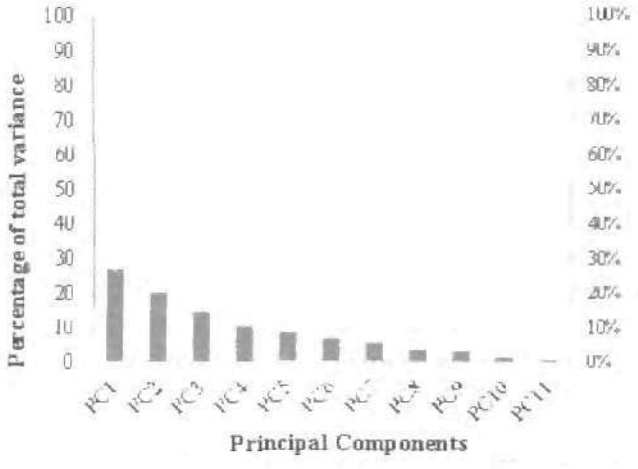

Figure 9. Percentage of the total variance of all the principal components.

The first component accounts for 26.816% of the total variance and presents strong positive contribution from total fungi, *Penicillium* and *Cladosporium*. This component represents the biological agent.

The second component is associated with loadings of coarse particulate matter (PM_{10} and $PM_{2.5}$) and environmental parameters (T and RH) explaining 19.952% of the total variance.

The third component representing 14.5% of the variance is highly correlated with UFP and CO_2

Finally the fourth component, which explains 10.581% of the variance, is associated with high

Table 3. Correlation matrix of Spearman's rank correlation coefficients between the measured environmental variables (N=73).

Variables	PM_{10}	$PM_{2.5}$	PM_1	UFP	CO_2	Total fungi	*Aspergillus*	*Cladosporium*	*Penicillium*	T	RH
PM_{10}	1	0.584**	0.012	-0.006	0.157	0.240*	0.301**	0.210	0.120	0.423**	0.354**
$PM_{2.5}$		1	0.636**	0.098	0.199	0.038	0.318**	0.149	-0.008	0.408**	0.434**
PM_1			1	0.097	0.156	-0.110	0.104	0.047	0.010	-0.047	0.182
UFP				1	0.558**	0.261*	0.068	0.061	0.364**	-0.116	-0.075
CO_2					1	0.076	-0.038	-0.151	0.190	-0.107	0.085
Total fungi						1	0.194	0.782**	0.832**	0.118	0.086
Aspergillus							1	0.162	0.136	0.308**	0.157
Cladosporium								1	0.547**	0.216	0.015
Penicillium									1	-0.075	0.002
T										1	0.389**
RH											1

*. Correlation is significant at the level 0.05, ** Correlation is significant at the level 0.01

loadings of fine particulate matter ($PM_{2.5}$ and PM_1). This component represents the agent of fine particulate matter (Table 2).

The percentage of the total variance of all the principal components (11 PCs) created is presented in Figure 9. The curved line corresponds to the cumulative proportion of the total variance when selecting a certain number of components to be kept.

5.2 Spearman's Rank Correlation Coefficients

Bivariate correlations of pair wise associations between the variables were calculated. In particular, Table 3 presents a correlation matrix of Spearman's rank correlation coefficients. Statistically significant positive correlations at the 0.01 level were found for the following cases:

1. PM_{10} versus $PM_{2.5}$, *Aspergillus*, temperature and relative humidity.

Statistically significant positive correlations between both $PM_{2.5}$ and PM_{10} with temperature and *Aspergillus*, at the 0.01 level of significance, are consistent with the findings of Adhikari et al, (2006). Additionally, Srimuruganandam and Nagendra (2011) and Raisi et al (2010) found associations between PM_{10} with $PM_{2.5}$ ($R_{Pearson}^2$=0.54-0.76 and R^2=0.77 respectively) similar to results obtained in this work. PM_{10} and $PM_{2.5}$ are also correlated with relative humidity, a fact that confirms the exteme outdoor PM_{10} concentrations on the 17th and the 31st of March in school K of Figure 2 (left) in which rainfall was observed.

2. $PM_{2.5}$ versus PM_1, *Aspergillus*, temperature and relative humidity

Deggobi et al (2011) also report significant positive correlation between $PM_{2.5}$ and *Aspergillus* (R^2=0.186, $p<0.05$). Srimuruganandam and Nagendra (2011) found stronger correlations between $PM_{2.5}$ and PM_1 ($R_{Pearson}^2$=0.92-0.98).

3. UFP versus CO_2 and *Penicillium*

UFP were found to be positively correlated with *Penicillium* at the 0.01 level. According to Hargreaves et al (2003) the similarities between airborne fungi and supermicrometre particles can be attributed to the fact that they are both measured in units of number. Particle concentrations are expressed in units of number per unit volume (pt/cc) and airborne fungi are expressed in number of colony forming units (CFU). UFP also correlated to CO_2. This certain relationship was also examined by Weichenthal (2008), however the correlation they found was not significant (R^2=0.04).

4. Total fungi versus *Cladosporium* and *Penicillium*

5. *Aspergillus* versus temperature

Adhikari et al. (2006) and also Hammed et al. (2012) report statistically significant correlations between *Aspergillus* and temperature.

6. *Cladosporium* versus *Penicillium*

Correspondingly, according to Table 3 statistically significant positive correlations at the 0.05 level were found for the following cases:

1. PM_{10} versus Total fungi.

Contrary to the findings of Lin and Li (2000) in which PM_{10} correlated with *Penicillium*, in this case PM_{10} significantly correlated with the total airborne fungi at the 0.05 level.

2. UFP versus total fungi

Relative humidity for the period of measurement did not have any linear correlation neither to the total airborne fungi nor the prevalent genera, a fact which is in agreement to the findings of Sousa et al, (2008).

Several of the above mentioned correlations are also confirmed with the findings of PCA.

5.3 Linear Regression Models

Linear regression models that were developed from the statistically significant pairs of variables of Table 3 at the 0.01 level of significance are presented in Table 4. Dependent and independent variables, β and α values (of linear regression equation: $y=\beta x + \alpha$), r^2 values, r_{adj}^2 (adjusted) values and F values of the linear models are shown in Table 4. The r^2 values ranged from 0.044 to 0.748 and p-values were all below 0.05, rejecting the null hypothesis H_0 which supports no actual correlation between the variables.

5.4 Multivariate Linear Regression Models

Multivariate linear regression models were further developed to investigate several cases of dependent variables. Backward stepwise multiple regression analysis was used so as to examine the predictive strength of each variable (Table 5).

Table 4. Linear regression models of the statistically significant pairs of measured environmental variables at the 0.01 level of significance (N=73).

Dependent variable	Independent variable	β	α	N	r^2	r_{adj}^2	F	p
PM_{10}	$PM_{2.5}$	4.311	10.30	73	0.385	0.376	44.398	0.000
PM_{10}	*Aspergillus*	1.261	44.62	73	0.057	0.044	4.303	0.042
PM_{10}	T	4.783	-44.81	73	0.174	0.162	14.941	0.000
PM_{10}	RH	0.669	13.42	73	0.059	0.046	4.488	0.038
$PM_{2.5}$	PM_1	1.669	4.38	73	0.371	0.362	41.829	0.000
$PM_{2.5}$	*Aspergillus*	0.190	8.22	73	0.062	0.049	4.725	0.033
$PM_{2.5}$	T	0.694	-4.74	73	0.177	0.165	15.245	0.000
$PM_{2.5}$	RH	0.143	1.33	73	0.132	0.120	10.798	0.002
UFP	CO_2	16.748	2095.99	73	0.225	0.214	20.612	0.000
UFP	*Penicillium*	50.917	9761.54	73	0.151	0.139	12.647	0.001
Total Fungi	*Cladosporium*	2.371	50.57	73	0.581	0.575	98.467	0.000
Total Fungi	*Penicillium*	1.289	35.89	73	0.751	0.748	214.545	0.000
Aspergillus	T	0.560	-8.29	73	0.066	0.053	5.041	0.028
Cladosporium	*Penicillium*	0.240	9.33	73	0.252	0.242	23.967	0.000

Table 5. Stepwise multivariate linear regression models of several combinations of the measured environmental variables.

Multivariate regression models	N	r^2	r_{adj}^2	F	p
1. UFP = 22840.979 +360.269 x $PM_{2.5}$ +16.584 x CO_2 -821.626xT -217.655 x RH +25.644 x Total fungi +282.739 x *Aspergillus*	73	0.476	0.429	10.009	0.000
2. PM_{10}= -51.87 +4.895 x T +0.095 x *Penicillium*	73	0.216	0.194	9.669	0.000
3. $PM_{2.5}$= -10.516 +0.00012 x UFP +0.592 x T +0.121 x RH	73	0.289	0.259	9.37	0.000
4. Total fungi = 57.9 +0.868 x PM_{10} +0.004 x UFP	73	0.181	0.146	5.089	0.003
5. *Aspergillus*= -11.784 +0.00016 x UFP +0.639 x T	73	0.121	0.095	4.8	0.011

According to the findings of PCA certain independent variables were excluded from certain models which were straightforwardly related to the dependent variable (see Table 2). For instance, $PM_{2.5}$ and PM_1 were excluded from the 2nd model in which PM_{10} was the dependent variable, and *Penicillium*, *Cladosporium* and *Aspergillus* were excluded from the 4th model that had the total airborne fungi as dependent variable (Table 5). The presence of these variables would account for most of the variance in the model, restricting by far the variability of the other parameters.

The p-values of every model were found to be less than 0.05, indicating that the variation of the models is not due to chance, and the models are useful. The r^2 values ranged between 0.121 and 0.476 (Table 5). Temperature was present in most of the linear

regression models. A rather moderate correlation stands for the prediction of UFP concentrations given $PM_{2.5}$, CO_2, temperature, relative humidity, total airborne fungi and *Aspergillus*. It is anticipated that this model can be used in the future for prediction purposes (Table 5, model 1).

6. Conclusions

The findings from an experimental campaign conducted in two schools in Athens measuring air pollutants were evaluated in this paper. The indoor concentrations of PM and airborne fungi were in many cases similar to the corresponding outdoor values indicating that, in the absence of major indoor sources, the indoor concentrations are greatly affected by the outdoor concentrations. However, in certain cases the indoor concentrations outreached the outdoor ones, possibly due to inadequate levels of ventilation and to overcrowded classrooms. The overall concentration range of the pollutants indicates a rather good indoor air quality with the exception of overload in certain sites. Increased levels of UFP may be linked to vehicle emissions from adjoining streets.

In order to analyse these findings, the total measured environmental parameters were presented in a rose diagram where the common area of the data represented approximately 66% of the total measurements. Statistical analyses were performed on the filtered from extremes common area of data. PCA was conducted in order to cluster variables of common characteristics. It was found that almost 72% of the total variance was explained by four principal components. Findings of PCA were further used for the removal of certain parameters from certain multivariate regression models. Spearman's rank correlation coefficients were also calculated for all pairs of the measured data. Significant positive correlations were found between PM_{10} and total fungi as well as PM_{10} and $PM_{2.5}$ with *Aspergillus*. UFP positively correlated to total fungi and *Penicillium*. From the statistically significant bivariate correlations, linear regression models were created where p-values for all relationships were below 0.05. Multivariate regression models with different cases of dependent variables were also developed using stepwise regression analysis. Weak correlations do exist between particulate matter and airborne fungi concentrations. Moderate correlation stands for the prediction of UFP given $PM_{2.5}$, CO_2, meteorological parameters and airborne fungi. It is anticipated that these multivariate regression models can be used in the future for prediction purposes. Further measurements are necessary as well as further evaluation of the developed models, in order to improve the understanding of the environmental health risks.

Acknowledgments

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Funding.

References

Adhikari A, Sen MM, Gupta-Bhattacharya S and Chanda S: (2004) "Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: A 2-year study at five outdoor sampling stations", *Science of the Total Environment*, **326**, (1-3), pp123-141.

Adhikari A, Reponen T, Grinshpun SA, Martuzevicius D and LeMasters G: (2006) "Correlation of ambient inhalable bioaerosols with particulate matter and ozone: A two-year study", *Environmental Pollution*, **140**, (1), pp16-28.

Almeida SM, Canha N, Silva A, Freitas MdC, Pegas P, Alves C, Evtyugina M and Pio CA: (2001) "Children exposure to atmospheric particles in indoor of Lisbon primary schools", *Atmospheric Environment*, **45**, (40), pp7594-7599.

Brunekreef B and Holgate ST: (2002) "Air pollution and health", *The Lancet*, **360**, (9341), pp1233-1242.

Chapman JA: (1999) "Update on airborne mold and mold allergy", *Allergy and Asthma Proceedings*, **20**, (5), pp289-292.

Burge HA and Rogers CA: (2000) "Outdoor allergens", *Environmental Health Perspectives*, **108**, (Suppl. 4), pp653-659.

Bush RK and Portnoy JM: (2001) "The role and abatement of fungal allergens in allergic diseases", *Journal of Allergy and Clinical Immunology*, **109**, ppS430-S440.

Degobbi C, Lopes FDTQS, Carvalho-Oliveira R, Muñoz JE and Saldiva PHN: (2011) "Correlation of fungi and endotoxin with PM2.5 and meteorological parameters in atmosphere of Sao Paulo, Brazil", *Atmospheric Environment*, **45**, (13), pp2277-2283.

Diapouli E, Chaloulakou A and Spyrellis N: (2007) "Indoor and Outdoor Particulate Matter Concentrations at Schools in the Athens Area", *Indoor and Built Environment*, **16**, (1), pp55-61.

Dockery DW, Pope Iii CA, Xu X, Spengler JD, Ware JH, Fay ME, Fay ME, Ferris BG and Speizer FE: (1993) "An association between air pollution and mortality in six U.S. cities", *New England Journal of Medicine*, **329**, (24), pp1753-1759.

Donaldson K, and Stone V: (2003) "Current hypotheses on the mechanisms of toxicity of ultrafine particles", *Annali dell'Istituto Superiore di Sanita*, **39**, (3), pp405-410.

Dorizas PV, Kapsanaki-Gotsi E, Assimakopoulos MN and Santamouris M: (2012) "Particulate matter and airborne fungi concentrations in schools in Athens", *Advances in Meteorology, Climatology and Atmospheric Physics* (Helmis CG and Nastos PT eds.), Springer-Verlag, Berlin Heidelberg, Springer Atmospheric Sciences, **2**, pp931-937.

EPA United States Environmental Protection Agency: (1995a) "The Inside Story: A Guide to Indoor Air Quality". Publications and Resources. http://www.epa.gov/iaq/pubs/insidestory.html. Accessed 13 March 2012.

EPA United States Environmental Protection Agency: (1995b) "Indoor Air Quality tools for schools", Indoor Air. http://www.epa.gov/iaq/schools/actionkit.html. Accessed 13 March 2012.

Fromme H, Diemer J, Dietrich S, Cyrys S, Heinrich J, Lang W, Kiranoglu M and Twardella D: (2008) "Chemical and morphological properties of particulate matter (PM10, PM2.5) in school classrooms and outdoor air", *Atmospheric Environment*, **42**, (27), pp6597-6605.

Gaitani N, Lehmann C, Santamouris M, Mihalakakou G and Patargias P: (2010) "Using principal component and cluster analysis in the heating evaluation of the school building sector", *Applied Energy*, **87**, (6), pp2079-2086.

Grinn-Gofroń A, Strzelczak A and Wolski T: (2011) "The relationships between air pollutants, meteorological parameters and concentration of airborne fungal spores", *Environmental Pollution*, **159**, (2), pp602-608.

Guo YL, Lin YC, Sung FC, Huang SL, Ko YC, Lai JS, Su HJ, Shaw CK, Lin RS and Dockery DW: (1999) "Climate, traffic-related air pollutants, and asthma prevalence in middle-school children in Taiwan", *Environmental Health Perspectives*, **107**, (12), pp1001-1006.

Guo H, Morawska L, He C, Zhang YL, Ayoko G and Cao M: (2010) "Characterization of particle number concentrations and PM2.5 in a school: influence of outdoor air pollution on indoor air", *Environmental science and pollution research international*, **17**, (6), pp1268-1278.

Hammed Abdel AA, Khoder MI, Ibrahim YH, Saeed Y, Osman ME and Ghanem S: (2012) "Study on some factors affecting survivability of airborne fungi". *Science of the total environment*, **414**, pp696-700.

Hargreaves M, Parappukkaran S, Morawska L, Hitchins J, He C and Gilbert D: (2003) "A pilot investigation into associations between indoor airborne fungal and non-biological particle concentrations in residential houses in Brisbane, Australia", *Science of the total environment*, **312**, (1-3), pp89-101.

Jenkins PL, Phillips TJ, Mulberg EJ and Hui SP: (1992) "Activity patterns of Californians: Use of and proximity to indoor pollutant sources", *Atmospheric Environment - Part A General Topics*, **26 A**, (12), pp2141-2148.

Jolliffe IT: (2002) "Principal Component Analysis-Second Edition", *Springer Series in Statistics*, Springer-Verlag New York Berlin Heidelberg.

Kalogerakis N, Paschali D, Lekaditis V, Pantidou A, Eleftheriadis K and Lazaridis M: (2005) "Indoor air quality—bioaerosol measurements in domestic and office premises", *Journal of Aerosol Science*, **36**, (5-6), pp751-761.

Lazaridis M and Aleksandropoulou V: (2009) "Sources and variability of indoor and outdoor gaseous aerosol precursors (O3, NOx and VOCs) ", *Water, Air and Soil Pollution: Focus* **9**, (1-2), pp3-13.

Lin WH and Li CS: (2000) "Associations of fungal aerosols, air pollutants, and meteorological factors", *Aerosol Science and technology*, **32**, (4), pp359-368.

Long CM, Suh HH, Catalano PJ and Koutrakis P: (2001) "Using time- and size-resolved particulate data to ouantify indoor penetration and deposition behavior", *Environmental Science and Technology*, **35**, (10), pp2089-2099.

Mendell MJ and Heath GA: (2005) "Do indoor pollutants and termal conditions in schools influence student performance? A critical review of the literature", *Indoor Air*, **15**, (1), pp27-52.

Mumovic D, Palmer J, Davies M, Orme M, Ridley I, Oreszczyn T, Judd C, Critchlow R, Medina HA, Pilmoor G, Pearson C and Way P: (2009) "Winter indoor air quality, thermal comfort and acoustic performance of newly built secondary schools in England", *Building and Environment*, **44**, (7), pp1466-1477.

Oberdörster G: (2000) "Toxicology of ultrafine particles: In vivo studies", *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, **358**, (1775), pp2719-2740.

Pongracic JA, O'Connor GT, Muilenberg ML, Vaughn B, Gold DR, Kattan M, Morgan WJ, Gruchalla RS, Smartt E and Mitchell HE: (2010) "Differential effects of outdoor versus indoor fungal spores on asthma morbidity in inner-city children", *Journal of Allergy and Clinical Immunology*, **125**, (3), pp593-599.

Pope Iii CA, Bates DV and Raizenne ME: (1995) "Health effects of particulate air pollution: Time for reassessment? ", *Environmental Health Perspectives*, **103**, (5), pp472-480.

Raisi L, Lazaridis M and Katsivela E: (2010) "Relationship between airborne microbial and particulate matter concentrations in the ambient air at a Mediterranean site", *Global Nest Journal*, **12**, (1), pp84-91.

Santamouris M, Synnefa A, Assimakopoulos M, Livada I, Pavlou K, Papaglastra M, Gaitani N, Kolokotsa D and Assimakopoulos V: (2008) "Experimental investigation of the air flow and indoor carbon dioxide concentration in classrooms with intermittent natural ventilation", *Energy and Buildings*, **40**, (10), pp1833-1843.

Silvers A, Florence BT, Rourke DL and Loromor RJ: (1994) "How Children Spend Their Time A Sample Survey for Use in Exposure and Risk Assessments", *Risk Analysis*, **14**, (6), pp931-944.

Sousa SIV, Martins FG, Pereira MC, Alvim-Ferraz MCM, Ribeiro H, Oliveira M and Abreu I: (2008) "Influence of atmospheric ozone, PM10 and meteorological factors on the concentration of airborne pollen and fungal spores", *Atmospheric Environment*, **42**, (32), pp7452-7464.

Srimuruganandam B and Shiva Nagendra SM: (2011) "Characteristics of particulate matter and heterogeneous traffic in the urban area of India", *Atmospheric Environment*, **45**, (18), pp3091-3102.

Synnefa A, Polichronaki E, Papagiannopoulou E, Santamouris M. Mihalakakou G, Doukas P, Siskos PA, Bakeas E, Dremetsika A, Geranios A and Delakou A: (2003) "An Experimental Investigation of the Indoor Air Quality in Fifteen School Buildings in Athens, Greece", *International Journal of Ventilation*, **2**, (3), pp185-201.

Triantafyllou AG, Zoras S, Evagelopoulos V and Garas S: (2008) "PM10, O3, CO concentrations and elemental analysis of airborne particles in a school building", *Water, Air, and Soil Pollution*: Focus, **8**, (1), pp77-87.

Wargocki P and Wyon DP: (2013) "Providing better thermal and air quality conditions in school classrooms would be cost-effective", *Building and Environment*, **59**, pp581-589.

Weichenthal S, Dufresne A, Infante-Rivard C and Joseph L: (2008) "Characterizing and predicting ultrafine particle pounts in Canadian classrooms during the winter months: Model development and evaluation", *Environmental Research*, **106**, (3), pp349-360.

WHO, World Health Organization. "Air Quality Guidelines- Global update 2005. Particulate matter, ozone, nitrogen dioxide and sulfur dioxide", World Health Organization Regional Office for Europe, Copenhagen, Denmark 2005.
http://www.euro.who.int/__data/assets/pdf_file/0005/78638/E90038.pdf. Accessed 13 March 2012