Atmos. Chem. Phys. Discuss., 10, 27725–27758, 2010
www.atmos-chem-phys-discuss.net/10/27725/2010/
doi:10.5194/acpd-10-27725-2010

Atmospheric Chemistry and Physics Discussions

Ergosterol, arabitol and mannitol as tracers for biogenic aerosols in the Eastern Mediterranean

N. Burshtein, N. Lang Yona, and Y. Rudich

Department of Environmental Sciences and Energy Research, The Weizmann Institute of Science, Rehovot, 76100, Israel

Received: 13 September 2010 – Accepted: 18 October 2010 – Published: 12 November 2010

Correspondence to: Y. Rudich (yinon.rudich@weizmann.ac.il)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Aerosols containing biological components can have a significant effect on human health by causing primarily irritation, infection and allergies. Specifically, airborne fungi can cause a wide array of adverse responses in humans depending on the type and quantity present. In this study we used chemical biomarkers for analyzing fungi-containing aerosols in the eastern Mediterranean region during the year 2009 in order to quantify annual fungal abundances. The prime marker for fungi used in this study was ergosterol, and its concentrations were compared with those of mannitol and arabitol, which were recently suggested to also correlate with fungal spores concentrations (Bauer et al., 2008a). Back trajectory analysis, inorganic ions, humidity and temperature were used in an attempt to identify sources as well as the dependence on seasonal and environmental conditions. We found that the ambient concentrations of ergosterol, arabitol and mannitol range between 0 and 2.73 ng m^{-3}, 1.85 and 58.27 ng m^{-3}, 5.57 and 138.03 ng m^{-3}, respectively. The highest levels for all biomarkers were during the autumn, probably from local terrestrial sources, as deduced from the inorganic ions and back trajectory analysis. Significant correlations were observed between arabitol and mannitol during the entire year except for the winter months. Both sugars correlated with ergosterol only during the spring and autumn. We conclude that mannitol and arabitol might not be specific biomarkers for fungi and that the observed correlations during spring and autumn may be attributed to high levels of vegetation during spring blossoms and autumn decomposition.

1 Introduction

Airborne particles that originate from living organisms, plants, cell parts, pollen, bacteria and fungal spores are termed bioaerosols. Uplifting of biological particles to the atmosphere is possible when mechanical disturbances such as dust storms, activated sludge systems and irrigation with reclaimed water occur (Crook et al., 1997;

ACPD
10, 27725–27758, 2010

Schlesinger et al., 2006; Taha et al., 2006). Bioaerosols have been implicated in many environmental processes such as modification of clouds by serving as cloud and ice condensation nuclei (Hoose et al., 2009; Kurup et al., 2000, 2003; Mohler et al., 2007; Vali et al., 1996). They are also known to affect human health by causing primarily irritation, infection and allergies (Pei-Chih et al., 2000; Shelton et al., 2002).

Measurements of aerosol phospholipids content suggest that pollen and fungal spores contribute 4 to 11% of the total mass in particulate matter less than 2.5 µm in aerodynamic diameter ($PM_{2.5}$) or 12 to 22% of the total organic carbon fraction of ambient aerosols (Womiloju et al., 2003). More recent estimations suggest that fungal spores account for about 60% of the coarse atmospheric organic carbon, and for 40 ± 5% of the coarse PM mass as was measured in Vienna during spring and summer (Bauer et al., 2008b).

Fungi are important microorganisms in nature, for they decompose most of the organic material. The vast majority of fungi have terrestrial habitats, in soil or dead plant matter (Brock, 1997). Most fungi emit spores that range between 1.5 and 12 µm (Cox et al., 1995; Ruzer et al., 2005). These spores constitute a significant portion of the natural bioaerosols. Due to their long retention time in the atmosphere and their ability to travel long distances.

Fungal spores can penetrate into the human airways and irritate the respiratory system, cause allergies and infectious acute diseases such as alvioletis and upper airway blockage as well as chronic diseases such as asthma and bronchitis (Douwes et al., 2003; Fung et al., 2003; Kurup et al., 2000, 2003; Ring et al., 2001; Ruzer et al., 2005). Emissions of volatile organic compounds from microorganisms such as fungi can also cause human health problems such as irritation of mucous membranes and damage to the central nerve system (Fung et al., 2003; Ruzer et al., 2005). Glucose polymers in the cell walls of most fungi such as (1-3)-β-D-glucans can induce inflammatory responses and cause respiratory symptoms (Douwes et al., 2003; Shelton et al., 2002).

ACPD
10, 27725–27758, 2010

The adverse responses to fungi depend on their type and quantity. Since dose response is highly individual, the sensitivity of an exposed person is an important consideration (Ruzer et al., 2005). The abundance of bioaerosols in the ambient air and their health impacts depend on the season and on the environmental conditions. For example, the release of spores and their allergenic effects depend on water content (Ruzer et al., 2005).

Estimating the concentration of fungi in the atmosphere is commonly conducted using viable samplers or spore traps. With viable samplers it is possible to enumerate only live species, while spore traps followed by microscopic examination can provide total spore counts regardless of their viability. Both approaches provide number concentrations and species identification, but they are labor intensive, time consuming and do not provide. The contribution of fungal spores to atmospheric aerosols in terms of mass loading or impacts (Lau et al., 2006). To quantify and identify fungi contribution on atmospheric aerosols and the impact to public health, it has been suggested to use biomarkers in chemical analysis of collected aerosols. A widely used biomarker for determining the fungal biomass is ergosterol (Lau et al., 2006), which is a primary fungal membrane sterol shown in Fig. 1a. Ergosterol is almost exclusively found in fungi and is therefore an efficient biomarker. Photochemical degradation can cause a significant decrease of ergosterol content in living fungi, therefore it is necessary to avoid light exposure once the fungi are collected (Mille-Lindblom et al., 2004). Gas Chromatography-Mass Spectrometry (GC-MS) is the most efficient mean for determination of ergosterol (Miller et al., 1997). Trimethylsilyl derivatization of ergosterol is often used for increasing the detection sensitivity. The derivatization occurs by replacing the hydrogen from the OH group with a trimethylsilyl group (Saraf et al., 1997).

Recently, Bauer et al. (2008a) suggested that mannitol and arabitol (Fig. 1b and c) concentrations are correlated with the fungal spore counts in atmospheric PM_{10} (mass of airborne particles that have an aerodynamic diameter smaller then 10 µm). These sugar alcohols (polyols) are common storage substances in fungal spores. Bauer et al. (2008a) suggest that using these polyols for spores simplifies sampling, analytical

ACPD
10, 27725–27758, 2010

analysis and evades the need for parallel aerosol collection. Mannitol, although common in fungi, is also a common sugar alcohol in plants; it is particularly abundant in algae and has been detected in at least 70 higher plant families. This suggests that mannitol may not be a specific biomarker for fungi as ergosterol (Loescher et al., 1992).

Inorganic cations (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}) and anions (Cl^-, NO_3^-, SO_4^{2-}) are common markers for identifying aerosol sources and processes. Inorganic ions can often provide important information about the origin of the aerosols and are often used for source identification (Hays et al., 2002).

To date, limited research on the abundance and sources of biogenic aerosols in the Mediterranean basin in general and in Israel in particular has been conducted. While there is pioneering research on the occurrence of bioaerosols in this region (Schlesinger et al., 2006; Waisel et al., 2008), the biomarkers approach has not been applied yet. Fungi biomarkers in aerosols have not yet been studied and therefore the seasonal fungi abundance and the potential health effects, apart from dust storms (Schlesinger et al., 2006), are not well known.

In this study, ergosterol, arabitol, mannitol and inorganic ions were quantified in ambient aerosols in the Eastern Mediterranean region. Several goals were set:

1. Quantify ergosterol concentrations in ambient PM_{10} aerosol in order to study the annual and seasonal behavior of fungi-containing bioaerosols.

2. Test whether arabitol and mannitol are good predictors of ergosterol and hence of fungi containing aerosols.

3. Explore correlations between the tracers' abundance with inorganic ions, humidity, temperature, and synoptic data in order to identify dominant sources of fungal spores.

ACPD
10, 27725–27758, 2010

2 Experimental methods

2.1 Sample collection

Samples were collected using a high volume sampler (Ecotech High volume sampler model ECO-HVS3000 with PM_{10} inlet, rate of drawing 68 $m^3\,h^{-1}$) on the roof of a building at the Weizmann Institute of Science, Rehovot, Israel (31°54′2″ N, 34°48′33″ E). About sixty samples were collected on a semi weekly basis, between December 2008 and December 2009, each sampling was during 72 h. Quartz microfiber filters (Whatman 20.3 × 25.4 cm) were used, they were prebaked prior to use at 450 °C for 5 h in order to burn off organic matter that might contaminate the samples. All samples were kept at −20 °C from collection until analysis in order to inhibit fungal growth.

2.2 Analytical procedure

2.2.1 Ergosterol

Dark tubes and vials were used to avoid photochemical degradation, and the sampled filters were wrapped in aluminum foil. 1/8 of each filter was hydrolyzed in glass tubes containing methanol (Merck, > 99.9%) and placed in an ultrasonic bath containing 50 °C water for 45 min to break the fungi cell walls and release ergosterol to the solvent. Ergosterol extraction was performed twice using de-ionized water and hexane (Sigma-Aldrich 97.0%). 100 ng of an internal standard, 7-Dehydro cholesterol (DHC) (Sigma, > 98.0%), was added to each sample in order to estimate sample stability. DHC is a stable internal standard with a chemical structure very close to that of ergosterol. Division of the ergosterol peak area by the DHC peak area gave the relative response factor (RRF) which expresses the real amount of ergosterol and takes into account possible variations in sample preparation and extraction.

The excess water and hexane were evaporated under a gentle stream of nitrogen. The samples were re-dissolved in hexane-dichloromethane solution (1:1 v/v)

ACPD
10, 27725–27758, 2010

(Sigma-Aldrich, 99.8%) and were applied to disposable silica gel columns (Phenomenex 100 mg/1.5 ml 100 pk) pre-conditioned with hexane:dichloromethane (1:1 v/v) mixture that decreased the analysis error by filtering unwanted components present in the sample. Extraction was performed with 2 ml hexane:dichloromethane mixture (1:1 v/v) followed by 2 ml ethyl acetate (Sigma-Aldrich, 99.9%), the samples were again evaporated under a stream of nitrogen. Ergosterol and DHC derivatization was performed using Bis (Trimethylsilyl) trifluoro-acetamid (TMS) (Aldrich, 99.0+), derivatization was found to improve the ergosterol signal to noise ratio (Lau et al., 2006; Saraf et al., 1997) Pyridine was used as a catalyst. Derivatization improves the ergosterol signal to noise ratio (Lau et al., 2006; Saraf et al., 1997). After reacting at 65 °C for 45 min the samples were injected to a Varian GC-MS Saturn 2000 equipped with a Restek column (RXi – 5 ms 5% diphenyl 95% dimethyl polysiloxane 30 m, 0.25 mm ID, 0.25 µm). A sensitive GC-MS/MS procedure was developed for obtaining highest sensitivity. The second MS stage was used to detect the ergosterol at 363 *m/z* and DHC at 351 *m/z*. Injector split ratio was adjusted to the splitless mode to enhance sensitivity. Column flow rate was constant at 1.4 PSI except for a pressure pulse at 40 PSI for the first minute. Ergosterol retention time was between 25.6 to 26.1 min.

2.2.2 Mannitol and arabitol

Mannitol and arabitol were detected and quantified by ion chromatography using the following procedure: The sampled filter was weighted and for each sample, a 3 cm^2 piece was weighed separately. All samples were analyzed in duplicates. 6 ml of de-ionized water was added to each sample and put in an ultrasonic bath for 45 min at room temperature. The samples were filtrated in 0.45 µm syringe filters (Acrodisk) and were injected to the Ion Chromatograph (Dionex) with a CarboPac column (Dionex, MA1; 4 × 250 mm and guard). The column temperature was set to 30 °C, eluent flow (NaOH) was set to a rate of 0.4 ml s^{-1} and the injection volume was 50 µl. An electrochemical detector was used with a gold electrode as the work electrode and silver-chloride as

ACPD
10, 27725–27758, 2010

the reference electrode (Dionex). Arabitol (Sigma, > 98.0%) and mannitol (Riedel de Haen, extra pure) were used as standards.

2.2.3 Inorganic ions

A 3 cm^2 piece of the filter was used for each analysis; all samples were analyzed in duplicates. 8ml of de-ionized water were added to each sample. The samples were put in an ultra sonic bath for 45 min at room temperature; they were then filtrated with a 0.45 µm syringe filter (Acrodisc) and injected to an Ion Chromatograph (Dionex) All laboratory glassware was washed carefully with de-ionized water prior to use.

Anions were detected using the following conditions: The flow rate of the mobile phase, NaOH 13 mM, was 1 ml min^{-1} with an injection volume of 50 µl and suppressor current of 40 mA. An IonPac column (AS11A 4 × 250 mm and guard IonPac AG11A 4 × 50 mm) was used and the column temperature was set to 25 °C. A suppressor (ASRS 300 4 mm self regeneration), a conductivity detector (Dionex) and an automated autosampler (Dionex) were utilized.

Cations were detected using the following conditions: flow rate of the mobile phase, Methanesulfonic Acid 20 mM (Fluka) was 1.2 ml min^{-1} with injection volume of 50 µl and suppressor current of 59 mA. An IonPac column (CS12A 4 × 250 mm and guard IonPac CG12A 4 × 50 mm) was used and the column temperature was set to 30 °C. A suppressor (CSRS 300 4 mm self regeneration), a conductivity detector (Dionex) and an automated autosampler (Dionex) were utilized. Calibration curves (Fig. 3) were prepared according to the protocol above with: Ammonium Nitrate (Sigma Aldrich); Magnesium Sulfate (Baker); Calcium Chloride (Baker); Sodium Chloride (Fluka); Potassium Chloride (Merck); Ammonium Sulfate (Fluka); Sodium Nitrate (Riedel de haein). The following anions and cations were quantified: Cl^-, NO_3^-, SO_4^{2-}, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}.

ACPD
10, 27725–27758, 2010

2.3 Meteorological information

Humidity and temperature data was obtained from the meteorological stations at the Hebrew University in Rehovot and in Nes-ziona on a daily basis. Recorded dust storm events were taken from the ministry of environmental protection.

2.4 Back trajectory analysis

Back trajectories were calculated for each sampled filter using the Hybrid Single Particle Lagrangian Integrated Trajectory Model (HYSPLIT) (Draxler et al., 2010) developed by the United States National Oceanic and Atmospheric Administration (NOAA). The model produced back trajectories for 3 days for the sampling dates at 3 different heights (500, 1000, 1500 m) providing estimate of the source direction for each sample separately with the model vertical velocity, coordinates of the Weizmann institute are 31°54′2″ N and 34°48′33″ E. The HYSPLIT model was commonly used in different occasions to estimate PM_{10} sources (Dayan et al., 2005; Escudero et al., 2006; Falkovich et al., 2001; Waisel et al., 2008).

3 Results and discussion

3.1 Calibration curves for Ergosterol, arabitol, mannitol and ions

The analytical tools used in this study were all verified and calibrated with standards in concentration ranges relevant for the samples. Calibration curves for ergosterol, arabitol, mannitol and selected ions are shown in Figs. 2 and 3 respectively. For ergosterol (Fig. 2) each peak area was divided by the DHC peak area giving a relative response factor (RRF). An internal standard was not needed for mannitol and arabitol, because they are stable species. Figures 2 and 3 indicate that the species were measured with high precision.

ACPD
10, 27725–27758, 2010

3.2 Ergosterol, arabitol and mannitol concentrations during 2009

In Figs. 4 and 5, the variation of ergosterol, mannitol and arabitol in our sampling site from December 2008 to December 2009 are shown. The gaps in the data were caused by technical issues. The data covers 46% of the year. Table 1 provides detailed concentrations. The data contains 14 winter samples (December, January, February), 8 spring samples (March, April, May), 17 summer samples (June, July, August) and 17 autumn samples (September, October, November) totaling 56 samples. The ambient concentrations of ergosterol, arabitol and mannitol range between 0 and 2.73 ng m^{-3}, 1.85 and 58.27 ng m^{-3}, 5.57 and 138.03 ng m^{-3}, respectively. A visible correlation is noticeable between arabitol and mannitol during this year; the highest levels for all biomarkers are seen in the autumn when the vegetation decomposes and fungal population increase.

The concentrations of the three biomarkers were in the same order of magnitude and range to those reported for Hong Kong and Vienna. Ergosterol concentrations in Hong Kong were between 30.7 to 407.3 pg m^{-3} (Lau et al., 2006), sugars levels in Vienna during the autumn months were between 7 and 63 ng m^{-3}, and between 8.9 and 83 ng m^{-3} for arabitol and mannitol respectively (Bauer et al., 2008a). The slight difference in the biomarkers' concentrations between Rehovot, Hong Kong and Vienna may be attributed to the difference in the types of fungi species collected, the climatic regions and amount of surrounding vegetation.

3.3 Seasonal correlations between the biomarkers

Table 2 details the Pearson correlation coefficient (r) between mannitol and arabitol during the different seasons. Apart from the winter months, arabitol and mannitol are highly correlated throughout the year. Ergosterol is highly correlated with arabitol and moderately correlated with mannitol during spring and autumn but shows no correlation with either of the sugars during summer and winter.

ACPD
10, 27725–27758, 2010

Ergosterol is an established biomarker for fungi (Gessner et al., 1996; Lau et al., 2006; Lee et al., 2007; Mille-Lindblom et al., 2004; Miller et al., 1997; Saraf et al., 1997). According to Lau (2006) highest ergosterol levels were observed in Hong Kong during the autumn months (September, October, November and December) as was observed in Rehovot. Although slightly different in climate, it can be seen that in both regions the fungal spores exhibit similar behaviors.

Although Bauer et al.'s (2008a) suggestion that arabitol and mannitol can serve as fungi markers, with the advantage of the easier detection and quantification, our data show that in our region, these sugars correlate with ergosterol during spring and autumn but not during winter and summer. Bauer's measurements were conducted only during the autumn, when high concentrations of the sugars were observed in both sites and a significant correlation was found between them and the fungal spores (Bauer et al., 2008a). Although there are differences in environmental conditions between Israel and Austria, the arabitol levels detected were usually lower than mannitol levels in Israel, similarly to the observations in Austria.

Mannitol and arabitol, although common in fungi, are most frequently occurring sugar alcohol in plants; mannitol is particularly abundant in algae and has been detected in at least 70 higher plant families (Cheng et al., 2009; Lafay-Ette et al., 2004; Loescher et al., 1992). Hence, we conclude that mannitol and arabitol are not specific biomarkers for fungi. The observed correlation is confusing and might be attributed to high levels of vegetation during spring blossoms and autumn decomposition do not necessarily have a direct relation with fungi levels. Therefore we suggest that further studies should be conducted in order to establish whether they are good predictors of fungal spores.

3.4 Effects of ambient conditions on biomarkers concentration

We have investigated the correlation between the temperature and relative humidity (at the sampling point) and the biomarkers. Figures 6–7 show the correlation between the biomarker's concentration with relative humidity and temperature. The highest ergosterol concentrations were observed when the relative humidity was below 58%

ACPD
10, 27725–27758, 2010

and between 18–21 °C. At higher temperatures and RH, ergosterol concentrations decrease. During the measurements, average relative humidity in our site was between 55 and 70% (Fig. 7). Lau (2006) reported that the highest ergosterol levels were detected when the RH was below 75% (Lau et al., 2006). For Vienna the relative humidity was not reported. Arabitol and mannitol exhibit high levels between 18–21 °C, as ergosterol. Relative humidity levels did not show significant influence on arabitol, while mannitol levels are the highest when the RH is between 62–64%. The fact that the various biomarkers do not respond the same way to RH may be further indication that they originate from different sources.

Humidity was previously found to be a significant factor in effecting fungi concentrations. It was found that fungi are more abundant when the humidity is high in both indoor and outdoor environments (Arundel et al., 1986; Cox et al., 1995; Elbert et al., 2007; Li et al., 2010). However, ergosterol was negatively correlated with relative humidity in Hong Kong (Lee et al., 2007), our results show the same trend, meaning that high humidity levels do not have a linear relationship with high biomarker concentrations, which can be explained by the hydrophobic character of fungal spores (Wessels et al., 1996).

3.5 Biomarkers concentrations by geographical sectors

72 h back trajectories were calculated for each sample using HYSPLIT model (Draxler et al., 2010). The back trajectory model ran for 72 h back for each sampling date in order to capture the 72 h time frame of the sampling. In a research conducted recently in Maryland, the transport and characterization of ambient biological aerosol were studied using the back trajectories model. They found that the back-trajectory analysis indicate that samples that originated from both the ocean and land mass, were consistent with genetic material found in the samples, meaning that the trajectories model provides reliable information about the source of the samples (Santarpia et al., 2010).

ACPD
10, 27725–27758, 2010

The trajectories were broadly separated into 4 sectors (Dayan et al., 2005), each sector was categorized according to the major origins common in Israel: Southern Europe and Mediterranean Sea, Turkey and Central Western Asia, Arabian Peninsula and North Africa and Sahara (Fig. 8). Typical trajectories for each of the sections are shown in Fig. 9.

We found that the higher concentrations of mannitol and arabitol are associated with terrestrial trajectories or by mixed trajectories, which are trajectories that contain both terrestrial and marine sources (Fig. 10), while ergosterol has the highest concentrations only when the trajectories are from terrestrial source (Fig. 11). This was also observed in Hong Kong (Lee et al., 2007).

When investigating the trajectories according to sectors and seasons division (Fig. 12), generally, all biomarkers have the highest values when arriving from North Africa – Sahara region and Turkey. Mannitol and ergosterol show high levels when coming from the Turkey region mainly during autumn. The same trend is observed when investigating 24 or 48 h trajectories.

3.6 Statistical analysis for biomarkers and inorganic ions

The Pearson χ^2 test was chosen for its simplicity in explaining the relations between several inorganic ions and biomarkers coordinated with seasonality.

During 8 dust storm events that were sampled during 2009 we observed higher than average Ca^{2+} levels, as was previously reported (Falkovich et al., 2001; Schlesinger et al., 2006). The biomarkers levels in those dusty sampling days had very low correlation with the Ca^{2+} levels measured (Table 3). The correlations suggest that the average concentrations of biomarkers are low at most dust events, suggesting that ergosterol and hence fungi concentrations are not related to dust storms events. This finding may indicate that the biomarkers origin is not from remote desert regions but is probably local, possibly from the southern plain area, a region with ample agricultural activity and vegetation. These conclusions partially contradict the conclusion of Schlesinger et al. (2006) in which higher than average concentrations of fungi were found during dust

ACPD
10, 27725–27758, 2010

storms. However they agree with the conclusions of Lee et al. (2007) who did not find a distinct correlation between ergosterol and Ca^{2+}.

A negative or negligible correlation can be seen between SO_4^{2-}, NH_4^+ and NO_3^- with all markers for the entire period of measurements. SO_4^{2-}, NH_4^+ and NO_3^- are associated with aged aerosols (Lee et al., 2007) or with industrial emissions. This observation may indicate a low survival time of biogenic materials in the atmosphere, and the sensitivity of the airborne organisms to air pollution.

During the spring season, all biomarkers are correlated with all marine-indicating ions: Na^+, Cl^- and Mg^{2+}. However, we cannot conclusively determine the source of the biomarkers. It is difficult to assess whether the prevailing western winds carried biological material from algae sources in the Mediterranean Sea or from close by terrestrial sources.

4 Summary

Ergosterol, mannitol and arabitol were measured during the year 2009 in order to quantify fungal distribution in the eastern Mediterranean region, understand the seasonal behavior, and estimate possible sources. For this purpose we established analytical methods to quantify the biomarkers. Back trajectory analysis and inorganic ions have been investigated in order to obtain source identification. We find that ergosterol, which is an established biomarker for fungi, correlates with arabitol and mannitol only during the spring and autumn months. This correlation might be related to high levels of vegetation during spring blossoms and autumn decomposition and not necessarily have a direct relation with fungi levels.

Ergosterol concentrations were found to be the highest in air masses arriving from local terrestrial sources during the autumn with an average of 0.94 ng m^{-3}, while arabitol and mannitol have the highest levels in air masses arriving from local terrestrial and mixed sources during the autumn with an average concentration of 18.90 ng m^{-3} and 49.15 ng m^{-3}, respectively.

ACPD
10, 27725–27758, 2010

Although ergosterol is an established biomarker for atmospheric fungal spores (Lau et al., 2006), specific conversion factors should be determined for each geographic region, fungal species and environmental conditions for further use of ergosterol as a biomarker and for establishing the actual fungi concentration. Ergosterol abundance is dependent on the fungal species present (Pasanen et al., 1999) therefore further investigation is needed in order to establish these factors for our region.

Acknowledgements. The authors acknowledge the help given by Ilan Levy and Noam Levin. We also gratefully acknowledge the NOAA Air Resources Laboratory (ARL) for the provision of the HYSPLIT transport and dispersion model used in this publication. Y. R. acknowledges support by the Helen and Martin Kimmel Award for Innovative Investigation.

References

Arundel, A. V. S. E. M., Biggin, J. H., and Sterling, T. D.:Indirect Health-Effects of Relative-Humidity in Indoor Environments, Environ. Health Persp., 65, 351–361, 1986.

Bauer, H., Schueller, E., Weinke, G., Berger, A., Hitzenberger, R., Marr, I. L., and Puxbaum, H.: Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol, Atmos. Environ., 22(42), 5542–5549, 2008b.

Bauer, H. C., Vermeylen, M., Schueller, R., Weinke, E., Berger, G., Puxbaum, H.: Arabitol and mannitol as tracers for the quantification of airborne fungal spores, Atmos. Environ., 3(42), 588–593, 2008a.

Brock: Biology of Microorganisms, 774–775 pp., 1997.

Cheng, F. Y. Z., Guo, W. W., Pharr, D. M., and Williamson, J. D.: Salicylic acid stimulates secretion of the normally symplastic enzyme mannitol dehydrogenase: a possible defense against mannitol-secreting fungal pathogens, Planta, 6(230), 1093–1103, 2009.

Cox, C. S. W.: Bioaerosols handbook, crc press, 5–6 pp., 1995.

Crook, B. S.-H.: Sampling and assay of bioaerosols in the work environment, Journal of Aerosol Science Sampling and Rapid Assay of Bioaerosols, 3(28), 417–426, 1997.

Dayan, U. and Levy, I.: The Influence of Meteorological Conditions and Atmospheric Circulation Types on PM_{10} and Visibility in Tel Aviv, J. Appl. Meteorol., 44, 606–619, 2005.

ACPD

10, 27725–27758, 2010

Douwes, J. T., Pearce, P., Heederik, N.: Bioaerosol Health Effects and Exposure Assessment: Progress and Prospects, Ann. Occup. Hyg., 3,47, 187–200, 2003.
Draxler, R. R. A. R.: HYSPLIT – HYbrid Single-Particle Lagrangian Integrated Trajectory, Model access via NOAA ARL READY, http://ready.arl.noaa.gov/HYSPLIT.php, last access: August 2010, NOAA Air Resources Laboratory, Silver Spring, MD, 2010.
Elbert, W., Taylor, P. E., Andreae, M. O., and Pöschl, U.: Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions, Atmos. Chem. Phys., 7, 4569–4588, doi:10.5194/acp-7-4569-2007, 2007.
Escudero, M. E. A.: Determination of the contribution of northern Africa dust source areas to PM_{10} concentrations over the central Iberian Peninsula using the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT) model, J. Geophys. Res., 111, D06210, doi:10.1029/2005JD006395, 2006.
Falkovich, A. H. G., Levin, E., Formenti, Z., and Rudich, P.: Chemical and mineralogical analysis of individual mineral dust particles, J. Geophys. Res.-Atmos., 106(D16), 18029–18036, 2001.
Fung, F. A. H.: Health Effects of Indoor Fungal Bioaerosol Exposure, Appl. Occup. Environ. Hyg., 18(7), 535–544, 2003.
Gessner, M. S.: Use of Solid-Phase Extraction To Determine Ergosterol Concentrations in Plant Tissue Colonized by Fungi, Appl. Environ. Microbiol., 2(62), 415–419, 1996.
Hays, M. D. G., Linna, C. D., Smith, K. J., Schauer, N. D.: Speciation of gas-phase and fine particle emissions from burning of foliar fuels, Environ. Sci. Technol., 11(36), 2281–2295, 2002.
Hoose, C.: ATMOSPHERIC SCIENCE Biological ice formation, Nat. Geosci., 6(2), 385–386, 2009.
Kurup, V. P.: Fungal allergens, Curr. Allergy Asthm. R., 5(3), 416–423, 2003.
Kurup, V. P. S. and Banerjee, H. D.: Respiratory fungal allergy, Microbes Infect., 9(2), 1101–1110, 2000.
Lafay-Ette, P. R. K., Phan, R. M., and Parrott, B. H.: Arabitol dehydrogenase as a selectable marker for plants, In Vitro Cellular and Developmental Biology-Animal, 40, 17A, 2004.
Lau, A. P. S., Lee, A. K. Y., Chan, C. K., and Fang, M.: Ergosterol as a biomarker for the quantification of the fungal biomass in atmospheric aerosols, Atmos. Environ., 40(2), 249, 2006.

ACPD
10, 27725–27758, 2010

Lee, A. K. Y. L., Arthur, P. S., Cheng, J., Fang, Y. W., Chan, M., and Chak, K.: Source identification analysis for the airborne bacteria and fungi using a biomarker approach, Atmos. Environ., 13(41), 2831–2843, 2007.
Li, D. W. L.: Airborne fungi associated with ornamental plant propagation in greenhouses, Aerobiologia, 1(26), 15–28, 2010.
Loescher, W. H., Tyson, R. H., Everard, J. D., Redgwell, R. J., and Bieleski, R. L.: Mannitol Synthesis in Higher Plants: Evidence for the Role and Characterization of a NADPH-Dependent Mannose 6-Phosphate Reductase, Plant Physiol., 98(4), 1396–1402, 1992.
Mille-Lindblom, C., von Wachenfeldt, E., and Tranvik, L. J.: Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death, J. Microbiol. Meth., 59(2), 253, 2004.
Miller, J. D. A. Y.: The use of ergosterol to measure exposure to fungal propagules in indoor air, Am. Ind. Hyg. Assoc. J., (58), 39–43, 1997.
Möhler, O., DeMott, P. J., Vali, G., and Levin, Z.: Microbiology and atmospheric processes: the role of biological particles in cloud physics, Biogeosciences, 4, 1059–1071, doi:10.5194/bg-4-1059-2007, 2007.
Pasanen, A. L. Y.-P., Pasanen, K., Kalliokoski, P., Tarhanen, J.: Ergosterol content in various fungal species and biocontaminated building materials, Appl. Environ. Microbiol., 1(65), 138–142, 1999.
Pei-Chih, W., Huey-Jen, S., and Chia-Yin, L.: Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons, Sci. Total Environ., 253(1–3), 111, 2000.
Ring, J.: Allergy and hypersensitivity – Allergies on the increase – Editorial overview, Curr. Opin. Immunol., 6(13), 699–700, 2001.
Ruzer, L. S.: Aerosols handbook: measurement, dosimetry, and health effects, crc press, 291–342 pp., 2005.
Santarpia, J. L., Cunningham, D., Gilberry, J., Kim, S., Smith, E. E., Ratnesar-Shumate, S., and Quizon, J.: Transport and characterization of ambient biological aerosol near Laurel, MD, Biogeosciences Discuss., 7, 6725–6747, doi:10.5194/bgd-7-6725-2010, 2010.
Saraf, A., Larsson, L., Burge, H., and Milton, D.: Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography-mass spectrometry: comparison with fungal culture and determination of endotoxin by a Limulus amebocyte, Appl. Environ. Microbiol., 63(7), 2554–2559, 1997.

ACPD
10, 27725–27758, 2010

Schlesinger, P. M. Y. and Grishkan, I: Transport of microorganisms to Israel during Saharan dust events, Aerobiologia, 22, 259–273, 2006.
Shelton, B. G., Kirkland, K. H., Flanders, W. D., and Morris, G. K.: Profiles of Airborne Fungi in Buildings and Outdoor Environments in the United States, Appl. Environ. Microbiol., 68(4), 1743–1753, 2002.
Taha, M. P. M., Drew, G. H., Longhurst, P. J., Smith, R., and Pollard, S. J. T.: Bioaerosol releases from compost facilities: Evaluating passive and active source terms at a green waste facility for improved risk assessments, Atmos. Environ., 40(6), 1159, 2006.
Vali, G., Kulmala, M., and Wagner, P.: Ice nucleation – A review in: Nucleation and Atmospheric Aerosols, Pergamon Press, 1996.
Waisel, Y., Ganor, E., Epshtein, V., Stupp, A., and Eshel, A.: Airborne pollen, spores, and dust across the East Mediterranean Sea, Aerobiologia, 24(3), 125, 2008.
Wessels, J. G. H.: Fungal hydrophobins: Proteins that function at an interface, Trends Plant Sci., 1(1), 9–15, 1996.
Womiloju, T. O., Miller, J. D., Mayer, P. M., and Brook, J. R.: Methods to determine the biological composition of particulate matter collected from outdoor airment, Atmos. Environ., 37(31), 4335, 2003.

ACPD
10, 27725–27758, 2010

Table 1. Biomarkers levels were detected in each of the sampling dates (ng m^{-3}). Each date shown represents the starting date of the sampling period during 72 h. Averages are shown for each biomarker in all seasons. Arabitol, mannitol and ergosterol show the highest average concentrations during the autumn season, the lowest levels are seen during summer for ergosterol and mannitol and during winter for arabitol.

Season	Starting date	Arabitol (ng m^{-3})	Mannitol (ng m^{-3})	Ergosterol (ng m^{-3})	Season	Starting date	Arabitol (ng m^{-3})	Mannitol (ng m^{-3})	Ergosterol (ng m^{-3})
Autumn	6 Sep 2009	1.85	15.37	2.73	Summer	14 Jun 2009	10.76	12.98	0.14
September,	7 Sep 2009	16.29	100.38	0.95	June,	18 Jun 2009	18.06	16.49	0.22
October,	22 Sep 2009	25.50	102.63	0.28	July,	28 Jun 2009	27.86	28.86	0.24
November	27 Sep 2009	3.90	55.96	1.65	August	2 Jul 2009	9.91	11.03	0.12
	10 Oct 2009	6.09	16.90	0.47		5 Jul 2009	6.07	15.15	0.36
	15 Oct 2009	26.38	43.60	0.58		9 Jul 2009	7.41	8.67	0.01
	19 Oct 2009	9.23	27.89	0.03		12 Jul 2009	14.40	16.02	0.12
	22 Oct 2009	4.86	18.12	0.34		19 Jul 2009	9.37	19.52	0.02
	26 Oct 2009	11.31	28.91	0.00		23 Jul 2009	12.39	17.41	0.07
	29 Oct 2009	41.50	33.82	0.94		27 Jul 2009	8.10	12.94	0.90
	5 Nov 2009	22.63	37.24	2.17		30 Jul 2009	10.89	16.67	0.23
	9 Nov 2009	19.23	38.76	1.04		5 Aug 2009	9.45	14.72	0.42
	12 Nov 2009	58.27	138.03	1.92		8 Aug 2009	3.35	7.47	0.33
	19 Nov 2009	11.70	35.21	0.69		11 Aug 2009	6.63	13.22	0.34
	22 Nov 2009	27.15	69.39	0.70		16 Aug 2009	5.44	11.34	0.54
	26 Nov 2009	9.51	29.08	0.67		20 Aug 2009	7.14	12.57	0.35
	30 Nov 2009	25.92	44.28	0.86		23 Aug 2009	12.73	21.09	0.42
Average $n = 17$		18.9	49.15	0.94	Average $n = 17$		10.59	15.07	0.28
Winter	14 Dec 2008	12.77	21.39	0.83	Spring	27 Apr 2009	11.69	15.87	1.56
December,	18 Dec 2008	6.00	14.32	0.79	March,	30 Apr 2009	20.05	19.95	1.92
January,	1 Jan 2009	6.54	13.97	0.64	April,	4 May 2009	20.67	32.79	2.04
February	8 Jan 2009	11.63	15.92	0.24	May	7 May 2009	5.56	13.48	0.36
	11 Jan 2009	8.23	12.57	0.46		14 May 2009	13.45	12.69	0.62
	22 Jan 2009	6.87	9.37	0.51		18 May 2009	5.79	5.57	0.70
	25 Jan 2009	5.94	26.35	0.32		21 May 2009	8.69	17.80	0.25
	29 Jan 2009	9.51	21.06	1.11		28 May 2009	11.73	18.37	0.36
	1 Feb 2009	13.58	18.03	0.04					
	5 Feb 2009	3.95	8.66	0.04					
	8 Feb 2009	7.12	12.70	0.07					
	12 Feb 2009	6.30	34.89	0.18					
	10 Dec 2009	17.49	32.00	0.20					
	17 Dec 2009	2.06	65.94						
Average $n = 14$		8.43	21.94	0.42	Average $n = 8$		14.03	17.71	0.76

ACPD
10, 27725–27758, 2010

Table 2. Pearson coefficient (r) was used in order to determine the correlation between the markers during each season. Arabitol and mannitol are highly correlated during the year apart from the winter season. Ergosterol shows moderate correlation with both arabitol and mannitol during spring and autumn.

	Arabitol vs. mannitol	Mannitol vs. ergosterol	Arabitol vs. ergosterol
Winter	−0.1	−0.0	−0.0
Spring	0.78[1]	0.60[2]	0.81[1]
Summer	0.83[1]	−0.1	−0.2
Autumn	0.66[2]	0.41[3]	0.66[2]

[1] ± 0.70–1.00 – high correlation

[2] ± 0.50–0.70 – moderate correlation

[3] ± 0.30–0.50 – low correlation

ACPD
10, 27725–27758, 2010

Table 3. Pearson correlation coefficient (r) between Ca^{2+} levels and the measured biomarkers as was calculated for 8 dust events sampled during 2009. Low correlation is observed suggesting that the biomarkers are from a local source.

	Arabitol	Mannitol	Ergosterol
Ca^{2+} levels during dust events	0.34	0.36	0.07

ACPD
10, 27725–27758, 2010

Table 4. Inorganic ions and their correlation with the biomarkers were examined. Pearson coefficient (r) was used in order to determine the correlation between the measured biomarkers and the inorganic ions during each season. Arabitol (1), Mannitol (2), Ergosterol (3).

Season	Winter			Spring			Summer			Autumn		
	1	2	3	1	2	3	1	2	3	1	2	3
Cl^-	−0.01	−0.09	−0.04	0.76[1]	0.82[1]	0.79[1]	0.33[3]	0.20	−0.15	0.34[3]	0.24	0.12
NO_3^-	0.58[2]	0.00	−0.14	−0.22	−0.33[3]	−0.48[3]	0.02	0.08	−0.22	0.05	−0.31[3]	0.12
SO_4^{2-}	0.23	−0.51[2]	0.10	−0.56[2]	−0.47[3]	−0.71[1]	−0.55[2]	−0.39[3]	0.05	−0.04	−0.13	−0.05
Na^+	0.08	−0.22	−0.11	0.42[3]	0.58[2]	0.71[1]	0.10	0.02	−0.32[3]	0.48[3]	−0.25	0.13
NH_4^+	0.37[3]	−0.33[3]	0.10	−0.50[2]	−0.50[2]	−0.76[1]	−0.45[3]	−0.40[3]	0.02	−0.07	−0.07	0.21
K^+	0.07	−0.37[3]	0.21	0.50[2]	0.15	0.54[2]	−0.24	0.00	0.65[2]	0.27	0.19	−0.05
Mg^{2+}	0.10	−0.27	−0.18	0.49[3]	0.68[2]	0.72[1]	−0.44[3]	−0.12	0.06	0.30[3]	−0.15	0.12
Ca^{2+}	0.01	−0.38[3]	−0.31[3]	0.11	−0.10	0.18	−0.03	0.15	0.41[3]	−0.01	0.24	0.12

[1] ± 0.70–1.00 – high correlation

[2] ± 0.50–0.70 – moderate correlation

[3] ± 0.30–0.50 – low correlation

ACPD
10, 27725–27758, 2010

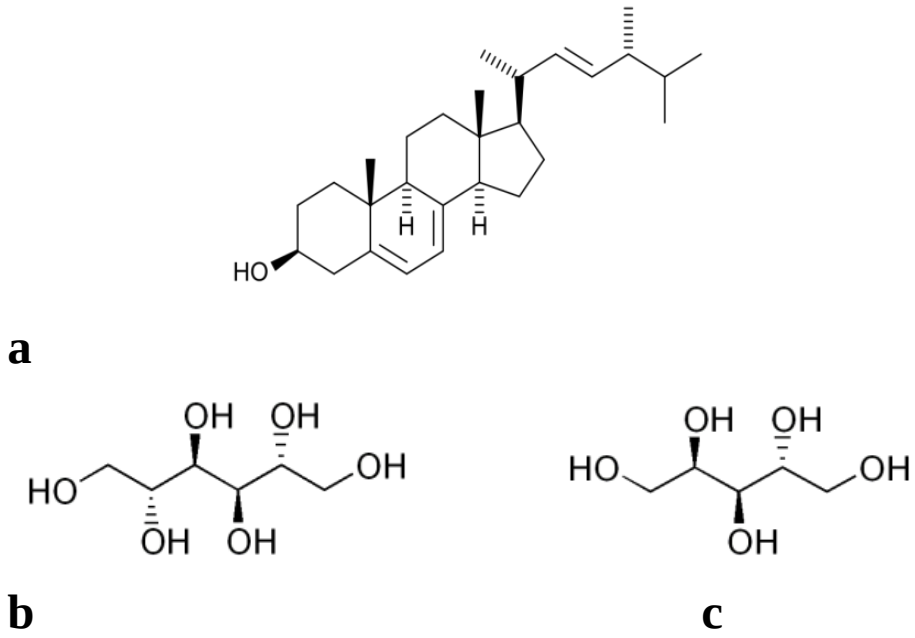

Fig. 1. Molecular structures of Ergosterol **(a)**, Mannitol **(b)** and Arabitol **(c)**.

ACPD
10, 27725–27758, 2010

Ergosterol, arabitol and mannitol as tracers for biogenic aerosols

N. Burshtein et al.

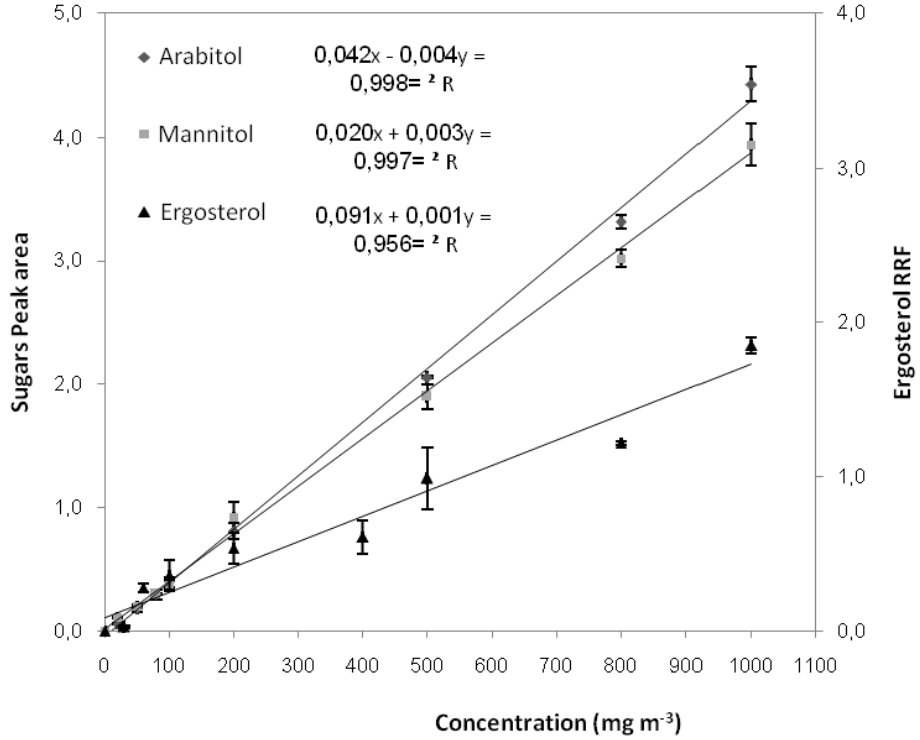

Fig. 2. Calibration curves for arabitol, mannitol and ergosterol. Peak area is shown (arbitrary units) versus marker concentration (gr m^{-3}).

ACPD
10, 27725–27758, 2010

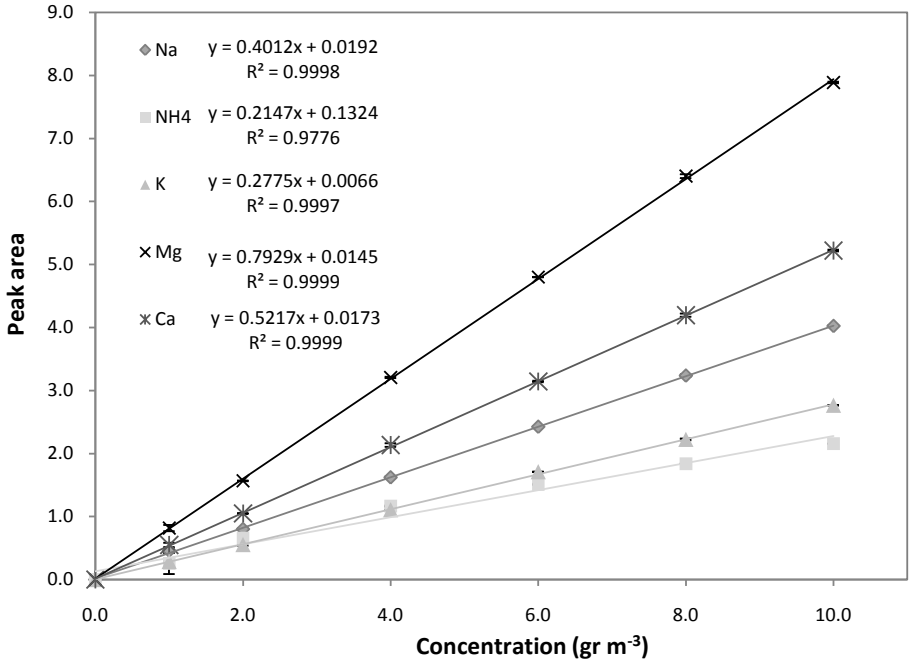

Fig. 3. Calibration curves for selected inorganic ions (Na^{+}, NH^{+4}, K^{+}, Mg^{2+}, Ca^{2+}) peak area is shown (arbitrary units) versus ion concentration (gr m^{-3}).

ACPD
10, 27725–27758, 2010

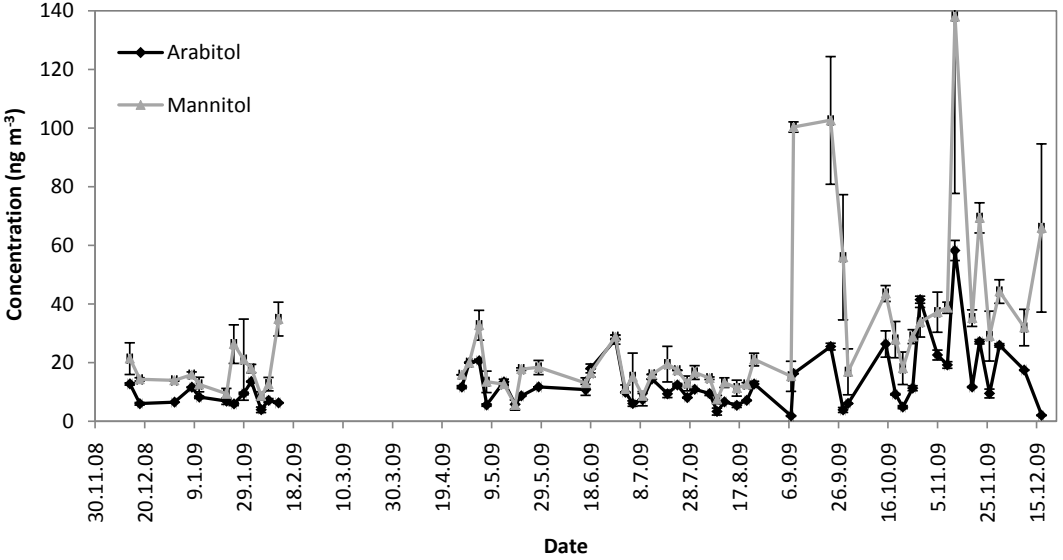

Fig. 4. Timeline of arabitol and mannitol concentrations during 2009. Missing samples are due to technical failures of the high volume sampler. High concentrations of the biomarkers are seen during the fall season.

ACPD
10, 27725–27758, 2010

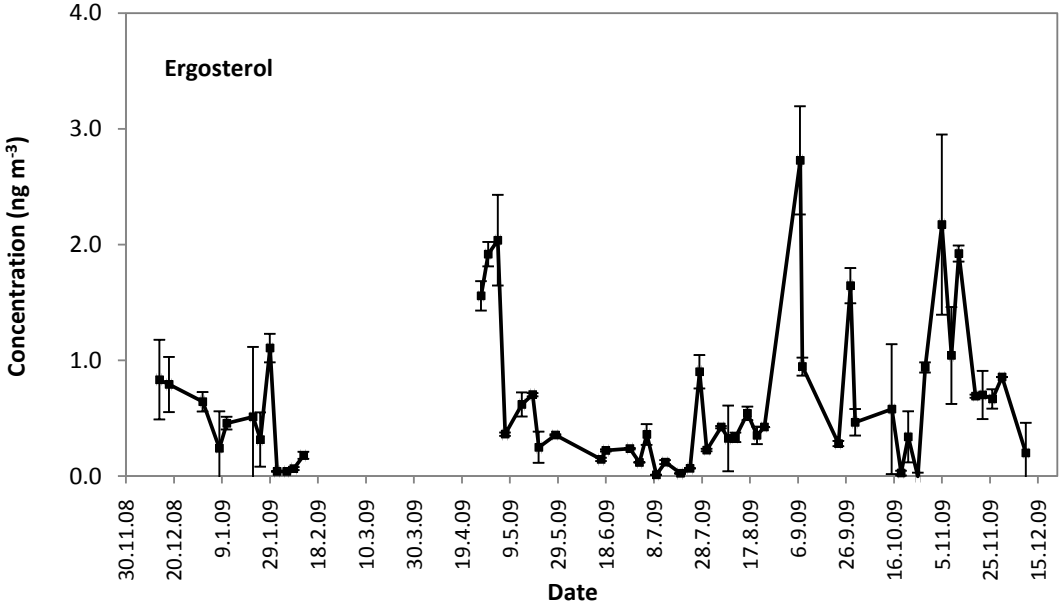

Fig. 5. Timeline of ergosterol concentration during 2009. High concentrations are seen during the fall season.

ACPD
10, 27725–27758, 2010

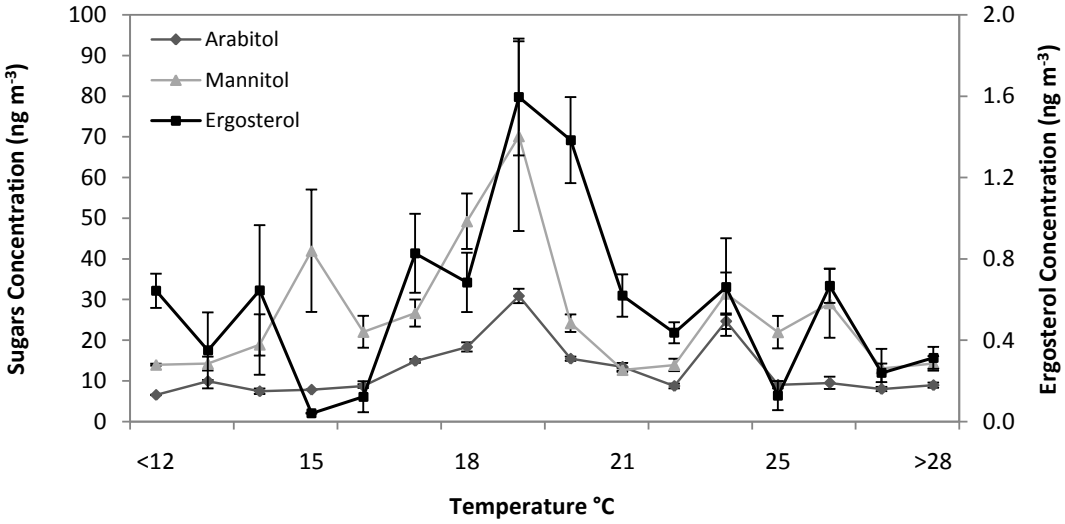

Fig. 6. Correlation between ambient ergosterol, arabitol and mannitol levels and temperature at the sampling site. The maximum values of all biomarkers appear between 19–20 °C which are typical average temperatures for the spring and autumn seasons.

ACPD
10, 27725–27758, 2010

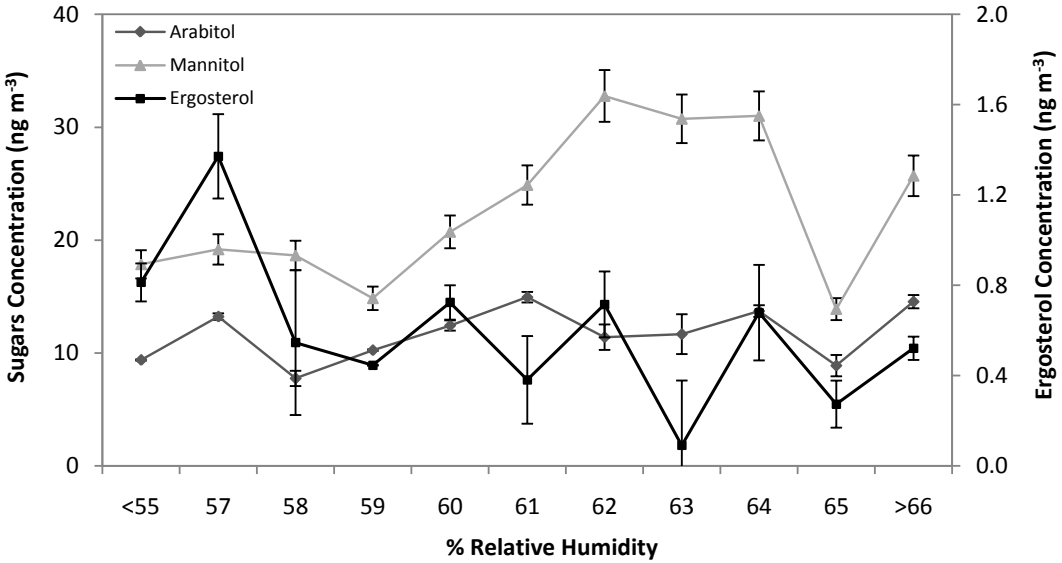

Fig. 7. Correlation between the ambient ergosterol, arabitol and mannitol concentrations with average relative humidity at the sampling site. Ergosterol shows high levels at low %RH while mannitol levels rise when average %RH between 62–65%. Arabitol does not show a distinct relationship with the relative humidity.

ACPD
10, 27725–27758, 2010

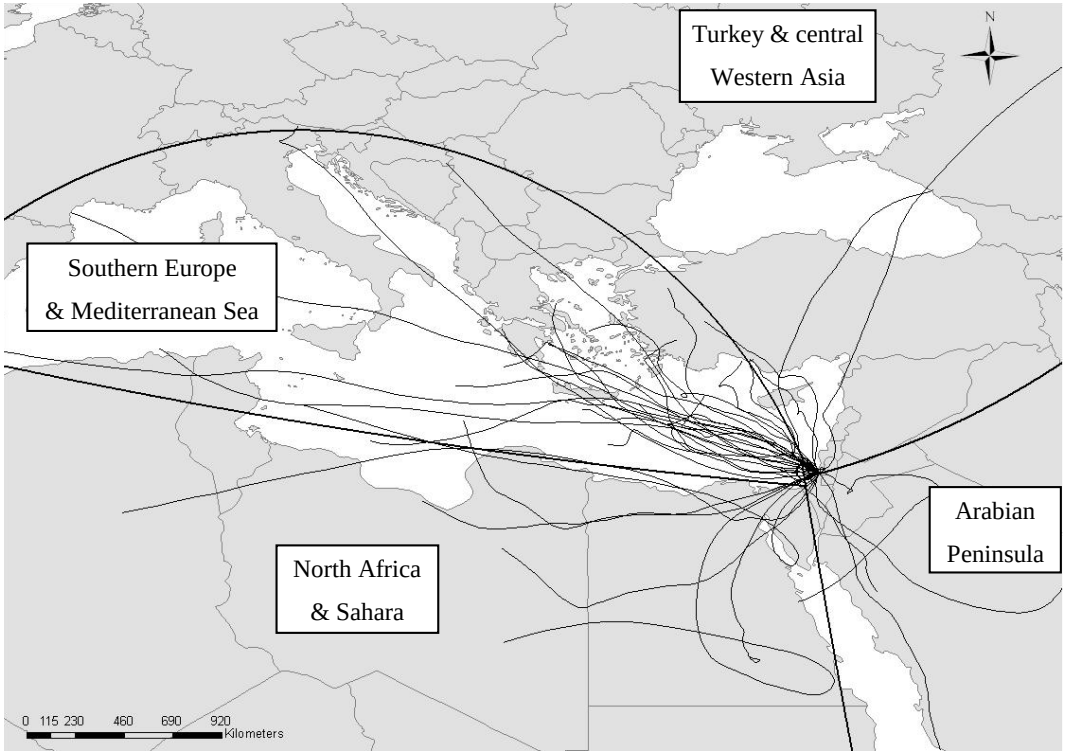

Fig. 8. Distribution of the trajectories according to air directions typical to Israel region. The trajectories are separated into 4 sectors (Dayan et al., 2005) that were categorized according to the main air directions common in the area. The major sources are: Southern Europe and Mediterranean Sea, Turkey and Central Western Asia, Arabian Peninsula and North Africa and Sahara.

ACPD
10, 27725–27758, 2010

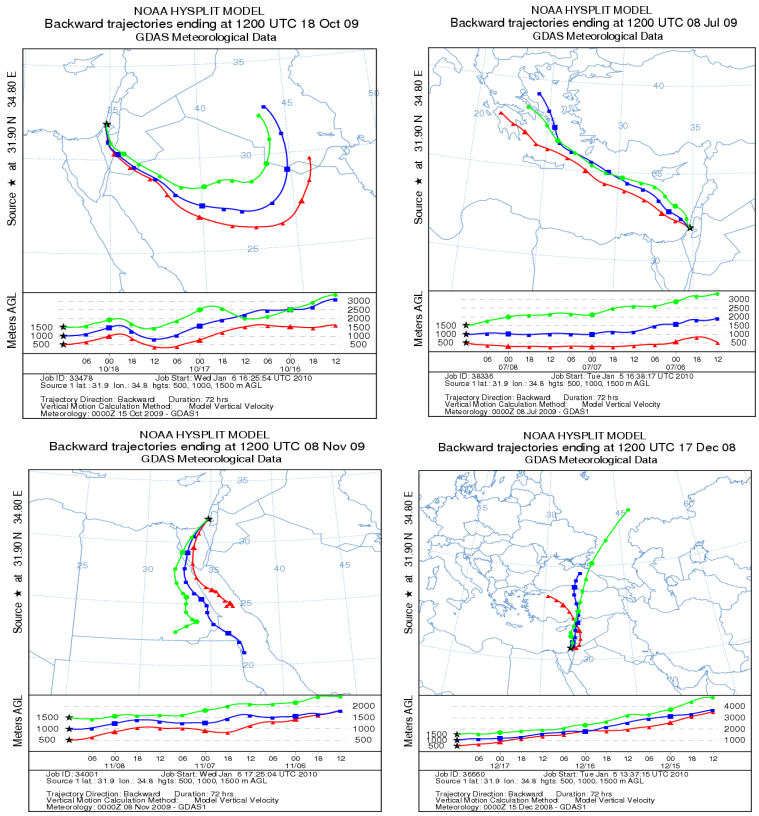

Fig. 9. Typical trajectories found for each sample with clockwise direction: Arabian Peninsula Southern Europe and Mediterranean Sea, North Africa and Sahara and Turkey and Central Western Asia. In most cases the trajectories obtained in the different heights were similar to each other. In cases at which trajectories at the different heights deviated from each other, the trajectory plotted at the average height of 1000 m was used to classify the sample to the sectors shown in Fig. 8.

ACPD
10, 27725–27758, 2010

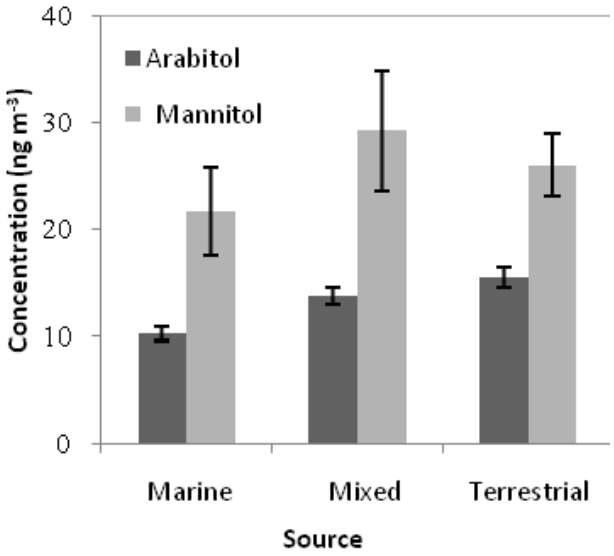

Fig. 10. Biomarkers concentrations according to source, broadly characterized to: marine environment (air mass that originated from the Mediterranean Sea), terrestrial sources (air mass from inland environments) and mixed sources (air mass that are not classified as marine or terrestrial). Ergosterol and arabitol have the highest concentrations when the samples are from terrestrial sources while mannitol behaves different with highest concentrations from mixed environment.

ACPD
10, 27725–27758, 2010

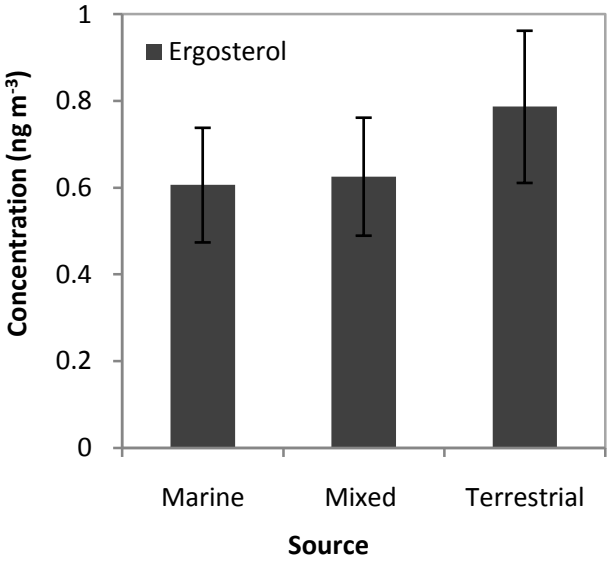

Fig. 11. Same as Fig. 10.

ACPD
10, 27725–27758, 2010

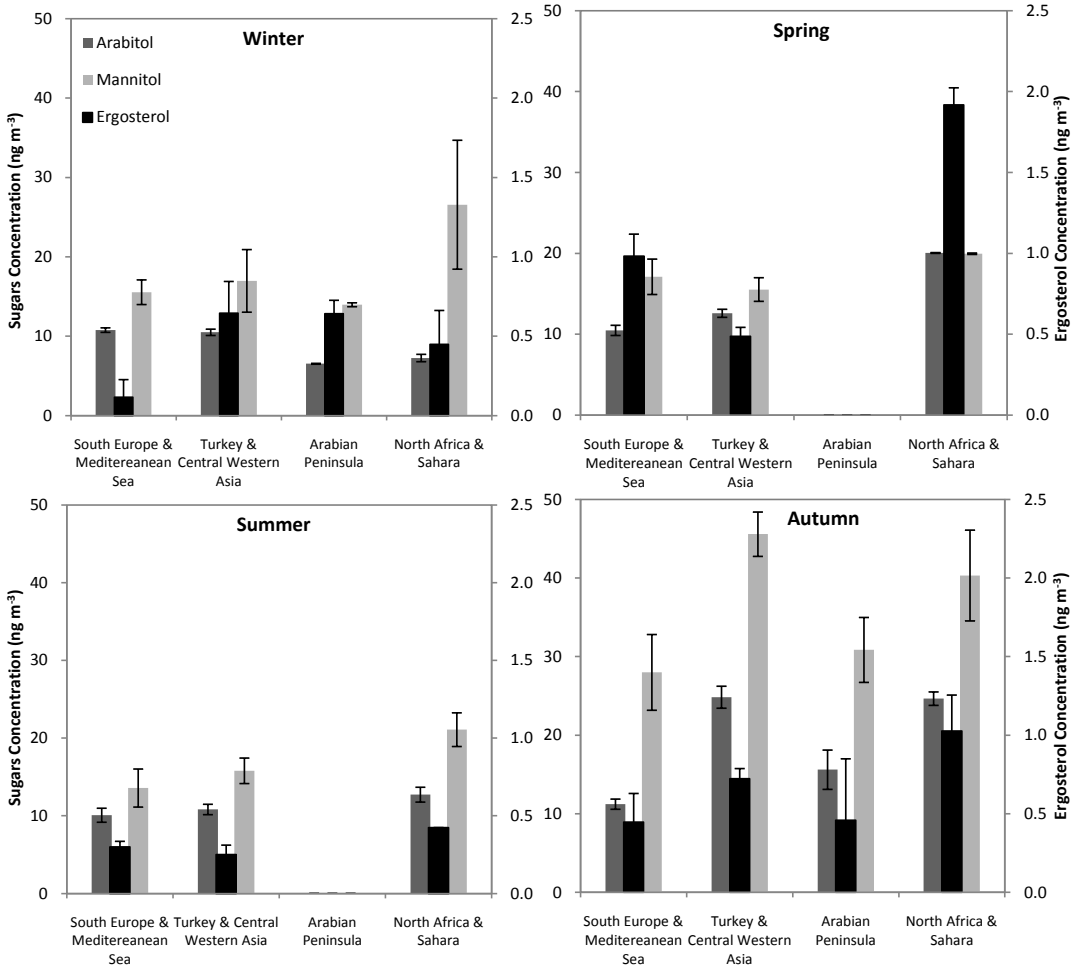

Fig. 12. Average concentrations of the biomarkers divided to the typical air mass sectors, according to seasons.

ACPD
10, 27725–27758, 2010

Ergosterol, arabitol and mannitol as tracers for biogenic aerosols

N. Burshtein et al.