Viable airborne and deposited microorganisms inside the Historical Museum of Crete

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Abstract

Measurements of viable ambient microbial levels and of microbial deposits on surfaces were performed in the Historical Museum of Crete for a period of two years. The concentrations of airborne microbes in museum rooms showed a considerable variability, which is mainly related to indoor activities (such as the number of visitors), the regulation of the indoor environmental conditions and air exchange rate, the chemical composition and conservation treatment of the exhibits, as well as the cleaning and storage conditions. An enrichment of acid producing bacteria, heterotrophic bacteria, and autotrophic chemo lithotrophic bacteria was encountered indoors. A considerable decrease of the measured viable microbes was measured after the deployment of photocatalytic ionizers in the different indoor sites ranging from 5.5 % to 76 %. The use of photocatalytic ionizers improved the air quality by reducing mainly the presence of acid producing bacteria, which may cause the deterioration of cultural heritage objects. The microbial colonization on 24 different painting materials and surfaces of model essays, without the use of any preservation treatment, was examined for an exposure period of 18 months. The results from the bacterial deposition on paintings showed a gradual colonization, specific to the materials and surfaces, in concentrations ranging from 10 to 300,000 CFU cm⁻². Although airborne fungi were measured in the exhibition rooms (yearly average concentration: 143 ± 115 CFU m⁻³), no growth of fungi could be detected on the majority of the used painting materials of the model essays. Very slow fungal surface colonization could be only determined on tempera, charcoal, wax pastel, and carton board in low concentrations ranging from 70 to 200 CFU cm⁻².

Keywords: bioaerosols, airborne bacteria and fungi, indoor air quality, photocatalytic ionizers, museums.
1 INTRODUCTION

The factors, which determine the degradation of cultural heritage objects inside museums, have been the focus of the scientific community due to their importance for the preservation of historical objects (Brimblecombe, 1990; Camuffo, 1998). Microclimatic conditions (e.g. temperature, relative humidity, light), air pollutants, and microbiological load are key parameters, which have to be studied for determining the suitability of the indoor environment for the preservation of the cultural heritage objects (Camuffo, 1998; Anaf et al., 2013; Ruga et al., 2015).

Microorganisms have been shown to present an important risk for cultural heritage objects, especially organic objects such as wood, woollen fabric, leather, and paper (Brimblecombe et al., 1999; Gauzere et al., 2013; 2014, López-Miras et al., 2013; Lazaridis et al., 2018). In addition, an important factor for the preservation of organic materials indoors in collections is the proper maintenance and climatic conditions of the museum microenvironment. Orlandi et al., (2015) found a high variability of fungal bioaerosols in the crypt of St. Peter in Perugia with higher concentrations during summer. Biodegradation of art objects has been studied in the scientific literature and specific fungal and bacterial strains have been identified to deteriorate organic materials (Gallo, 1993; Lazaridis et al., 2015; Lazaridis et al., 2018; Pasquarella et al., 2015; Di Carlo et al., 2016). The deterioration depends on the structure of the material itself and the chemical composition of the substrate. Organic materials are mainly hygroscopic and sensitive to degradation processes (Nielsen et al., 2010; López-Miras et al., 2013). An extensive scientific literature exists in respect to the biodegradation of organic materials such as paper, organic coatings and binding agents for paintings (McNamara et al., 2004; Bogomolova et al., 2007) but also in respect to degradation of inorganic objects (Cataldo et al., 2005).

The use of heating, ventilation, and air-conditioning (HVAC) systems is related with the accumulation of dust and microorganisms in museums (Camuffo, 1998). A previous study showed
that high density of visitors in a museum resulted to accumulation of airborne bacteria and fungi in the air handling unit of an air condition system for both constant and variable air volume (Li et al., 2016). Furthermore, in the study by Li et al. (2016) observed an increase of the microorganism ambient concentration with an increase of the relative humidity. The concentration of airborne microorganisms in museums may also affect the health of the museum’s personnel, consequently, a detailed assessment of microbial hazards has to be implemented specifically in the cultural heritage conservation laboratories (Gorny et al., 2016).

The concentration and variability of airborne microbes inside museums can be affected by several factors, such as the infiltration of the outdoor air, the human presence and activities (e.g. dust resuspension from visitors walking), bioaerosol emission by human activities like talking or coughing (Stelzenbach, 2002), indoor microclimatic conditions, the construction materials of the building itself, as well as, the composition of the cultural heritage objects.

The reduction of the microorganism concentration indoors at cultural heritage buildings is a challenge for the museum’s personnel, and the protection of the exhibits. Disinfection of books using sprays enriched with benzalkonium chloride was investigated as a treatment for the reduction of fungi inside a public library (Micheluz et al., 2018).

In the current work, a study was performed to determine the concentration of viable airborne microbes for a period of two years in different indoor sites of the Historical Museum of Crete as well as outdoors, before and after the installment of air purifiers (photocatalytic ionizers). In addition, the microbial colonization on the surfaces of different painting materials and model essays was examined to evaluate their deposition and abundance on the surface of cultural heritage objects.

2 METHODS
2.1 Sampling Locations

Monthly measurements of the airborne microorganisms (bacteria and fungi) were performed for a period of two years (March 2014 – February 2016) at the Historical Museum of Crete. The first year (March 2014 to February 2015) measurements were conducted before the use of air purifiers (photocatalytic ionizers, Daikin air purifier MC70L, flow rate 170 m$^3$h$^{-1}$, horizontal air inlet, vertical air outlet, 0.013 kw), whereas the second year (March 2015 to February 2016) measurements were performed after the placement of the air purifiers. Continuous measurements of meteorological data were performed both indoors and outdoors (Glytsos et al., 2018). The Historical Museum is located in the center of the city of Heraklion close to the sea (Lazaridis et al., 2015). The museum collections consist of mainly organic materials, including cotton, wood, paper and paintings. The number of visitors can reach 1,500 persons per month during summer and about 300 persons per month in the winter, giving an average value of about 500 visitors per month. The museum is a mechanically ventilated building during visiting hours with an average air flow of 1,509 m$^3$h$^{-1}$ (70-100 % of recirculation) in the whole museum, whereas the average natural infiltration rate is 220 m$^3$h$^{-1}$. Table 1 presents the natural infiltration and mechanical recirculation conditions at the different measurement sites. The mechanical ventilation system uses 100 % recirculation of air, and was operated only during the opening hours of the museum. Under standard conditions, 30 % of the recirculated air was obtained from outdoors. The average natural infiltration in the whole museum was measured to be equal to 0.5 h$^{-1}$.

Measurements in air were performed outdoors (site A) and at four sites indoors. The indoor sites include site B (second floor, Ethnographic collection room), site C (ground level, main exhibition room close to the main entrance, A. Kalokerinos room), and sites D I and D II (first floor, rooms of periodic exhibitions, Z. Portalakis I and II rooms) (see Fig. 1). The arrows depict also the...
location of the placement of the instruments. In Fig.1 was also depicted the position of the
photocatalytic ionizers, and the inlet and outlet of the mechanical ventilation system.

The indoor sites correspond to different microclimatic conditions and were selected to determine
the effects of mechanical ventilation, heating, ventilation and air conditioning, and air infiltration
from the outdoor environment (Table 1). One sampling location was selected for each site, except
site D, where two locations (D I, D II) were chosen due to different mechanical ventilation settings.
In room D I there is sufficient ventilation through the roof and the floor, whereas in room D II there
is only ventilation on the floor level. In addition, site B (Ethnographic collection room) was chosen
as an example of a museum room with efficient mechanical ventilation (Table 1), where all exhibits
are placed inside showcases. Pictures of the measurement sites A, B, and C are presented also in
Fig. 1.

The indoor climatic conditions in the Historical Museum of Crete were well controlled during
the opening hours, but the central heating and air conditioning system was not in use after the
closing of the museum. Continuous meteorological measurements (temperature (T) and relative
humidity (RH)) were performed indoors (sites B, C, and D) and outdoors (site A) for the whole
measurement period. The average values and standard deviation were: outdoors: T= 19.2 ± 3.5 °C
and RH= 67.9 ± 13.1 %; indoors: T= 23.7 ± 1.1 °C and RH= 52.8 ± 3.8 %. The average indoor
yearly values are similar to average seasonal values due to the controlled environmental conditions
indoors during the museum opening hours. The temperature variation indoors was lower than
outdoors, confirming the microclimate controlling conditions inside the museum. As reported
before (Glytsos et al., 2018), the average monthly variations of temperature and relative humidity
between outdoors (site A) and indoors (site B) in the Historical Museum of Crete showed smaller
difference during summer and larger deviations during winter. Although the temperature and
relative humidity values indoors are close to the recommended conditions for cultural heritage
buildings (50% ± 5 RH) and 21°C (ASHRAE, 2013), they exceed the proposed limit values for the paper conservation as described by ASHRAE (2003, 2011, 2013). Only the measurements during March comply with the Standards. This could be due to the fact that the central HVAC system was not used after the closing of the museum.

The effectiveness of the use of photocatalytic electrostatic precipitators (plasma ionizers) in the museum for improving the air quality in respect to the concentration of viable airborne microbes was further examined. The placement of photocatalytic ionizers (one Daikin air purifier MC70L per 65 m³ of room space) was performed to determine their efficiency to remove airborne microbes (Table 1).

2.2 Bioaerosol sampling and measurements

Measurements were performed one day per month, during the working hours of the museum, using the MAS 100 one stage sampler with flow rate of 100 L min⁻¹ (Merck, Germany) which collects viable particles larger than 1.1 μm. The sampling methodology is described in detail by Lazaridis et al., (2015). The collected air volume by the MAS 100 sampler varied from 50 to 250 L. The referred air volumes of the collected samples using the MAS 100 sampler were optimized in experiments before, so that, for reliable results, the colony number per plate (90 mm diameter agar Petri dishes) not to exceed the number of 80. Each aluminum orifice stage was disinfected using wipes containing 70% isopropyl alcohol between collections of different samples.

Different selective microbiological growth media were used for the cultivation of viable airborne microbes (Lazaridis et al., 2015; Lazaridis et al., 2018; Katsivela et al., 2017). Two sequential repetitions on each specific growth medium were done at each site. The present study is focused on the collection and analysis of the following airborne microorganisms: i) opportunistic pathogenic, heterotrophic bacteria, ii) autotrophic chemolithotrophic bacteria, iii) acid producing
bacteria, and iv) fast-growing mesophilic fungi. Opportunistic pathogenic, heterotrophic bacteria were measured due to their possible health effects, whereas autotrophic chemolithotrophic bacteria were chosen to be analyzed as representatives of the autochthonous airborne microbes. In addition, acid producing bacteria were examined because of the possibility to contribute to the deterioration of exhibits due to formation of acidic deposits, while mesophilic fast-growing fungi were determined due to their degradation potential.

The methodology of analysis for the viable, cultivable, airborne microorganisms is based on the cultivation of the air sampled microorganisms on specific microbiological growth media. The heterotrophic bacteria were cultivated in Tryptone Soy Broth (Merck, Germany) containing 1.5% (w/v) Agar at 37 °C in the dark for 48 h. Incubation temperature of 37 °C was chosen for the determination of the airborne, heterochthonous (not indigenous to the area of present occurrence), opportunistic pathogenic, heterotrophic bacteria due to health-related interests. The autotrophic chemolithotrophic bacteria were cultivated in Minimum Mineral Tris Phosphate Agar (MMTPA; Leibniz Institute DSMZ, No. 457 Mineral Medium (Brunner) without any carbon source at 37°C for 8 days. The acid producing bacteria were cultivated in the Gluconobacter oxydans Medium (Leibniz Institute DSMZ–No. 105 Gluconobacter oxydans Medium) at 37°C in the dark for 8 d. Acid producing bacteria were counted only when form clear zones below the colonies. In parallel, viable mesophilic, fast-growing fungi were cultivated in Malt Extract Broth (Lab M, England) containing 1.5% (w/v) Agar at 20 °C in the dark for 72 h. Air samples were collected in two sequential repetitions on each specific growth medium in all indoor locations of museum (sites A, B, C, D I, D II), and outdoors using a single sampler. The counted number of colonies was precisely corrected using the positive hole conversion tables, supplied by the manufactures of the used sampling instruments. Concentrations of airborne microorganisms were expressed as colony forming units per cubic meter (CFU m⁻³).
2.3 Microbial deposition on painting surfaces

Microbial deposits on painting surfaces were determined. Therefore, different painting materials and model essays were exhibited for eighteen months inside the museum. Two boards (a colour board and a blackboard) with the following 24 different materials were used: textile, marker pen, pen ink, watercolours, aluminium foil, magazine, oil colours, flo-master pen, ink, coloured tissue paper, tempera, acrylic colours, aquarelle, oil pastel, carton board, newspaper, chalk pastel, wax pastel, charcoal pencil, pencil, cotton undercoat with acrylic coating, charcoal, colour pencil and unused cotton undercoat (Fig. 2). The boards were not treated using any preservation (e.g. toping of varnishes) to facilitate a more efficient deposition and colonization of airborne microbes. Varnishes have been used for the preservation of oil paintings against deterioration of the painting materials and surfaces from the 15th century until today. The existence of viable microbial deposits on the surface of the essays was examined for each exposed material. Pieces from the boards with a surface area of 2.25 cm² (1.5 × 1.5 cm²) were cut using a sterile titanium scrapple, and were aseptically placed in 3 mL of sterile phosphate buffered saline, pH 7.2 (Sigma-Aldrich) in glass test tubes with screw caps. After two days of equilibration under shaking at 200 rpm at 30 °C, 100 μL of the serial diluted puffer was used for inoculation by the spread plate technique on the above-mentioned solid growth media. Tryptone Soy Agar was used for the cultivation of heterochthonous, opportunistic pathogenic, heterotrophic bacteria, and Malt Extract Agar for the cultivation of fast-growing fungi. Concentrations of viable microorganisms on surfaces were calculated in colony forming units per square centimeter of essay (CFU cm⁻²).

3 RESULTS AND DISCUSSION

3.1 Airborne bioaerosol measurements without the use of photocatalytic ionizers
The concentration levels of the airborne microbes before the installation of the photocatalytic ionizers during the one-year sampling (March 2014 – February 2015) is shown in Fig. 3. A high variability in the measured microorganism concentrations was observed at each site. The range of concentration values varied from 215 to 5,345 CFU m$^{-3}$, 10 to 1,612 CFU m$^{-3}$, 37 to 3,545 CFU m$^{-3}$ and 0 to 290 CFU m$^{-3}$ regarding the airborne heterotrophic bacteria, the fast-growing fungi, the autotrophic chemolithotrophic bacteria, and the acid producing bacteria, respectively. In comparison, relatively lower bacterial concentrations and variability were detected outdoors (range of concentrations from 40 to 508 CFU m$^{-3}$, 37 to 985 CFU m$^{-3}$, and 0 to 10 CFU m$^{-3}$ regarding the airborne heterotrophic bacteria, the autotrophic chemolithotrophic bacteria, and the acid producing bacteria, respectively). However, the airborne fast-growing fungi showed outdoors concentrations in the same range as indoors (71 to 1,490 CFU m$^{-3}$). According to the Italian Ministry of Cultural Heritage and Activities and Tourism (MiBAC) (1998) threshold values for the protections of cultural heritage objects were proposed. The recommended concentrations of heterotrophic bacteria and fungi have to be below 750 and 150 CFU m$^{-3}$, respectively. These recommendations agree with the studies presented by Parchas (2008), and Flieder and Capderou (1999) which recommend concentrations of airborne fungi lower than 120 CFU m$^{-3}$. Gorny et al. (2016) presented a detailed discussion concerning threshold limit values. These proposed threshold values were exceeded for heterotrophic bacteria and fungi inside the measurement sites of the Historical Museum of Crete during some of the measurement periods as shown in Fig. 3. The average one-year indoor concentration of cultivable heterotrophic bacteria was 999 ± 821 CFU m$^{-3}$, whereas the average one-year indoor concentration of cultivable fungi was 184 ± 210 CFU m$^{-3}$. In comparison, the average one-year outdoor concentration of the heterotrophic bacteria and fungi was 218 ± 159 CFU m$^{-3}$ and 303 ± 393 CFU m$^{-3}$, respectively. Due to reported exceedances of the recommended values a further study for the reduction of the observed values of airborne microorganisms was
performed. This study was elaborated using air cleaning systems, such as photocatalytic ionizers as described in the materials and methods section.

The measured average sum of one-year microbial concentration measurements at all indoor sites were in the same range varying from $2,144 \pm 1,591 \text{ CFU m}^{-3}$ (minimum average one-year concentration at site D I) to $2,719 \pm 1,561 \text{ CFU m}^{-3}$ (maximum average one-year concentration at site D II). In accordance in another reported study in the Louvre Museum, the indoor airborne bacterial community remained stable over time with a common microflora of more than 50% (Gauzere et al., 2014). The relatively higher concentrations measured at site D II (room II), may be due to lower infiltration rate (natural ventilation equal to $450 \text{ m}^3 \text{ h}^{-1}$ and mechanical ventilation with recirculation equal to $1,000 \text{ m}^3 \text{ h}^{-1}$). In comparison at site D I the infiltration rate was higher (natural ventilation equal to $120 \text{ m}^3 \text{ h}^{-1}$, and mechanical ventilation with recirculation equal to $1,920 \text{ m}^3 \text{ h}^{-1}$) (see Table 1). At site D I the total air movement (natural and mechanical) was $2,040 \text{ m}^3 \text{ h}^{-1}$, whereas for D II was $1,450 \text{ m}^3 \text{ h}^{-1}$ (see Table 1). Specifically, the mechanical ventilation of D I was almost two times higher than D II. The average sum of one-year microbial concentration measurements was considerably lower outdoors (site A) and equal to $968 \pm 907 \text{ CFU m}^{-3}$.

A seasonal variability was observed at the different sites indoors, which is however different for each site (Fig. 4). This highlights the variability of the microorganism concentration in air. High seasonal variability of airborne microbial concentrations was measured both indoors in the different exhibition rooms and outdoors. Furthermore, the indoor airborne bacterial concentrations were higher than outdoors in the majority of cases, except during the summer period in the A. Kalokerinos and Z. Portalakis I and II rooms, where the concentration of chemoautotrophic bacteria was higher outdoors. In contrast, a relatively low concentration of airborne fungi was measured indoors. These results agree with previous measurements in the Historical Museum of Crete as well as in other cultural heritage collections (Lazaridis et al., 2015; Lazaridis et al., 2018).
Although Orlandi et al. (2015) found higher concentrations of airborne fungi in summer in the crypt of St. Peter in Perugia, in the present study, the summer period showed lower microorganism concentrations indoors in respect to other seasons (Fig. 4). As shown, the highest concentrations during spring and summer were measured in the Ethnographic collection, whereas the Z. Portalakis room II (site D II) and the A. Kalokerinos room (site C) showed the highest microbial concentrations during autumn and winter, respectively. Although, similar concentrations were observed between site D I and site C during spring and summer, differences were measured during autumn and winter. In comparison, the highest outdoor microbial concentrations were measured during the summer period, whereas the lowest concentrations were observed during the winter period.

The enrichment of airborne microorganisms indoors was studied also in respect to other activities, which may result to reduction of the emission sources such as cleaning and restriction to museum visitors. Fig. 5 shows the effects of room cleaning and visitor presence in site D I. A considerable reduction of the concentration of airborne microbes was observed during the cleaning work performed from May to June 2014, when no visitors were allowed to access the site. Contrary, an enrichment of airborne microbes was measured during the opening hours of the museum from 11.30 a.m. to 3.30 p.m. at the 17th of July 2014 due to the increase of visitor’s presence. However, no statistically significant correlation between airborne microbes and number of daily visitors could be found in any of the indoor exhibition sites, although higher concentrations of viable microbes were generally encountered during the opening hours of the museum when visitors are present. An implication of these measurements is the necessity of the control of the intensity of the flow rate of the mechanical ventilation system in the museum. The intensity has to be increased during noon when a major part of visitors has been entered in the museum as well as during the occurrence of other activities indoors.
As reported before (Stelzenbach, 2002), building occupants are a major source of bacterial aerosols indoors. Human activities like talking, coughing, and walking are some of the possible emission sources of microbes in the air. In accordance, the common bacterial airborne microbial community in the Louvre Museum was related to human-associated microflora (Gauzere et al., 2014). In addition, building materials, components, and surfaces can serve as indoor sites of microbial colonization and subsequent dispersal into the air though the natural and mechanical ventilation (Lightthart and Stelzenbach, 1994; Buttner et al., 1999), and the occupant’s activities (Reynolds et al., 1990; Buttner and Stelzenbach, 1993; Stelzenbach, 2002).

The enrichment of airborne microbes can be also depicted from their average seasonal Indoor-to-outdoor (I/O) concentration ratio as shown in Fig. 6. As seen, the seasonal average I/O ratios varied among the different groups of microbes. The acid producing bacteria showed the highest I/O ratios (I/O ratio: 13 – 92) in all indoor sites during autumn, spring and summer. The potential pathogenic heterotrophic bacteria were the next group of bacteria that showed the next high I/O ratios (I/O ratio: 2 -21), followed by the autotrophic chemolithotrophic bacteria (I/O ratio: 1-14) in all indoor sites. The lowest enrichment of airborne microbes was observed during winter. The enrichment of acid producing bacteria indoors may result to the deterioration of exhibits in the museum due to acidic deposits on the exhibits. It is known that acids are intermediates or dead-end metabolites of acid producing bacteria (Maier et al., 2000). Absence or very low enrichment of airborne fast-growing fungi (I/O ratio: 0.1 - 3) could be detected inside the Historical Museum of Crete during the measurement period. These results are in accordance to measurements in other cultural heritage collections where the microclimatic conditions are well controlled by HVAC systems, probably, due to absence of fungal emission sources indoors and the low outdoor air supply (Gauzere et al., 2014; Lazaridis et al., 2015; Lazaridis et al., 2018). Petushkova and Kandyba (1999) found also that the majority of the airborne microbes inside the Moscow Kremlin
cathedrals were capable of producing acids. In addition, biodegradation of porous inorganic materials is reported to be caused by the formation of carbonic acid (formed by the dilution of carbon dioxide in water), and by other acids being metabolites of microbes (Karyś and Ważny, 2007). Increased enrichment of airborne fungi (Orlandi et al., 2015, Lazaridis et al., 2018) and bacteria (Brągoszewska et al., 2018) in naturally ventilated rooms was observed in comparison to rooms equipped with HVAC systems.

3.2 Airborne bioaerosol measurements during the use of photocatalytic ionizers

Fig. 7 (a) presents the one-year average values of the measured microbial concentrations before and after the installation of photocatalytic ionizers inside the museum. In general, the use of photocatalytic ionizers improved considerably the indoor air quality in respect to the presence of viable airborne microbes. The indoor conditions were kept constant during the experimental campaign, temperature and relative humidity were controlled indoors and the visitors were not allowed to enter the measurement site 30 min before the measurements started. The photocatalytic ionizers had a mean air flow rate 170 m$^3$ h$^{-1}$ and the ventilation conditions were kept constant (Table 1).

However, differences in the reduction of airborne microorganisms were encountered after the deployment of the photocatalytic ionizers in the four indoor sites. The one-year average decrease of the sum concentration of the cultivable airborne microbes varied from 5.8 to 76 %. The microbial removal efficiency of the photocatalytic ionizers depends, probably, not only on the installation of the correct number of air purifiers (one ionizer per 65 m$^3$ room space, Table 1) but also on the total aeration rate of the room, as well as, on the emission rate of the airborne microbes (visitor presence and occupant’s activities, as reported before in the section Airborne bioaerosol measurement...
without the use of photocatalytic ionizers). However, these findings are limited to the measurement period and have to be treated with caution.

As shown in Fig. 7, the best air quality conditions were achieved at site B (Ethnographic collection room), where the sum concentration of airborne microbes was even lower than the outdoor level. Thus, the highest decrease of airborne viable microbes (76 %) was encountered at site B, where besides the placed ionizers, the aeration rate is the highest in the museum (natural ventilation equal to 750 m$^3$ h$^{-1}$, and mechanical ventilation with recirculation equal to 2,500 m$^3$ h$^{-1}$) (see Table 1). In addition, as mentioned before, all exhibits at site B are placed in closed showcases. In contrast, the lowest decrease of viable airborne microbes (5.5 %) was measured at site D II (Z. Portalakis, room II), which had the highest concentration of airborne microbes in the whole museum before the deployment of the ionizers and the lowest aeration rate (Table 1). However, two sites with similar aeration rate (site D I, and site C, see Table 1) showed large deviations regarding the reduction of the sum concentration of viable microbes. At site D I was encountered microbial removal efficiency of 39 %, whereas at site C was measured only a reduction of 10 %. This implies the need of an optimum control of the flow rate of the mechanical ventilation system in the museum for the reduction of the enrichment of microbes in respect to human activities as well as the use of showcases for the protection of artworks.

Fig. 7 (b) shows also the seasonal average concentrations of viable, cultivable airborne microorganisms in the museum after the placement of photocatalytic ionizers. Even though the placement of the photocatalytic ionizers resulted to the reduction of the concentration of microorganisms indoors, it did not affect the indoor variability at the different sites, as well as, the seasonal variability.

Furthermore, the calculation of the one-year average I/O concentration ratios of each category of microbes at every site, before and after the placement of photocatalytic ionizers, was performed.
for a better evaluation of their efficiency (Fig. 8). Significant reduction of the one-year average I/O ratio from 26.4 to 3.0 was observed for acid producing bacteria. Since acid producing bacteria are a risk factor for the degradation of organic objects, the use of photocatalytic ionizers improved the air quality by reduction of their concentration (see Fig. 8). This was achieved by decreasing the average I/O concentration ratios of the acid producing bacteria by a factor of 8.8 in all rooms. In comparison, a moderate reduction of the one-year average I/O concentration ratios by a factor of 1.5 was obtained after the application of the photocatalytic ionizers for all the other measured viable airborne microbes (heterotrophic bacteria, fast-growing fungi, and autotrophic chemolithotrophic bacteria).

In agreement, Park et al. (2016) reported differences on the killing efficiency of the microbial population of different bacterial species by air purifying ionizers. The authors showed that ionizers cause bactericidal effect due to oxidative damage of cells and DNA by the generated negative and positive ions. Pushpawela et al. (2017) also observed a reduction of ultrafine particles with the use of ionizers in indoor environments. The authors concluded that air ionizers are more effective in removing ultrafine particles than high-flow air filters in rooms larger than 25 m³. In addition, it was also observed that the particle removal efficiency is decreased as the room volume is increased.

### 3.3 Microbial colonization on different painting substrates

Two boards (a colour board and a blackboard) consisting of different essays painted with different materials, without the use of any preservation treatment, were placed on a wall at the site D II (Z. Portalakis room II) in the museum for an exposure period of 18 months (16/07/2014 - 06/01/2016) (see Fig. 2). The objective was to determine the microbial deposits on the different materials during the placement of the boards in the museum. The colonization and growth of microbes on the surfaces can be an important source of emission of bioaerosols, such as spores,
cellular debrides, toxins, allergens), that can be potentially dangerous for human health (Stelzenbach, 2002). On the other hand, microbial surface colonization can cause deterioration of artworks (Urzi et al., 2001; López-Miras et al., 2013). A sample from each material was cut at the beginning (control), after 7, 10, 16, and 18 months of exposure to determine the gradual enrichment of the materials and surfaces with viable, cultivable microbes (heterotrophic bacteria and fungi).

The results from the bacterial colonization on paintings showed a clear correlation between the colonization and survival of viable heterotrophic bacteria on the exhibits surfaces and the chemical composition of the used painting materials of the model essays. The deposition of viable heterotrophic bacteria on the 24 different painting materials and surfaces of model essays showed a gradual colonization, specific to the materials and surfaces, mostly followed by a reduction of microbial population after 18 months of exposition (Fig. 9). The data analysis has shown, that the bacterial colonization can be divided in three main categories:

1. Fast colonization and growth in high concentrations up to 300,000 CFU·cm⁻²
2. Slow colonization and growth in concentrations lower than 15,000 CFU·cm⁻²
3. Very slow colonization (earliest detection after 10 months of exposition) and growth in concentrations lower than 5,000 CFU·cm⁻²

Specifically, fast colonization and high concentrations of viable heterotrophic bacteria (up to 300,000 CFU cm⁻²) were measured on newspaper, magazine, textile, aluminum foil, oil colours, ink, coloured tissue paper oil pastel, carton at the colour board, as well as, at surfaces covered with oil pastel, flo-master pen, charcoal, and color pencil at the blackboard.

In comparison, slow colonization and bacterial deposits in concentrations lower than 15,000 CFU cm⁻² were determined on watercolors, flo-master pen, and wax pastel at the color board, as well as on marker pen at the blackboard, and on linen coated with gesso primer.
Contrary, it was not measured significant enrichment of viable heterotrophic bacteria (earliest
detection after 10 months of exposition, and growth in concentrations lower than 5,000 CFU cm\(^{-2}\))
on surfaces painted with marker pen, pen ink, tempera, acrylic colours, aquarelle, chalk pastel of
the colour board, as well as, on surfaces painted with pen ink, charcoal pencil, pencil, ink and on
cotton undercoat with acrylic coating of the blackboard.

Surprisingly, the analysis of the microbial colonization showed that there were not determined
viable fungi deposits on 20 different essays and painting materials placed, although the yearly
average concentration of the airborne fungi was measured 143 ± 11 CFU m\(^{-3}\) in the exhibition
rooms. Low concentrations ranging from 70 to 200 CFU cm\(^{-2}\) were only determined on tempera
and carton essay of the colour board, as well as on charcoal and wax pastel of the blackboard after
18 months’ exposure. The ability of fungi to colonize surfaces depend on different factors, such as
bioreceptivity, spatial orientation of sedimentation surfaces, low requirement of nutrients and
energy, and environmental parameters (Urzi et al., 2001). A future study may concentrate also on
the microbial colonization evaluation of different painting substrates after the deployment of air
purifying ionizer devices in order to determine their efficacy of reducing the surface concentration
of microorganism deposits.

In agreement to our colonization results, studies of microbial communities adhering to the
surfaces of an oil painting on canvas (López-Miras et al., 2013) reported that the dominant
microbial community on the painting surface was shown to be directly related to the indoor air
quality where the painting was exposed. In addition, the authors confirmed a near absence of
actively growing microbes on areas of paintings showing no visible damage.

4 CONCLUSIONS
Viable, cultivable airborne bacteria and fungi were measured inside the Historical Museum of Crete as well as outdoors in the ambient air to study their concentrations before and after the placement of air purifiers, and to investigate the colonization of microbes on the surfaces of model essays and painting material exposed inside an exhibition room.

High seasonal variations of the airborne microbial load were observed inside the selected measurement sites in the Historical Museum of Crete. Higher concentrations of viable microbes were encountered during the opening hours of the museum when visitors were present. Even though, the enrichment of the different categories of airborne microorganisms was not the same at the different indoor sites, similar average sum concentrations were measured at all sites of the museum.

The average I/O ratio for the different categories of the viable airborne microbes ranged from 1 to 92, indicating the enrichment of microbes indoors. In all measurement sites was observed an enrichment of acid producing bacteria, which may result to the deterioration of the cultural heritage objects of the museum’s collections.

The use of photocatalytic ionizers in the museum improved the air quality in respect to reduction of the concentration of viable airborne microbes. The measurements of viable microbes at all sites showed that the reduction of their concentration in comparison to the values measured before the deployment of the ionizers was effective. The concentrations of cultivable airborne microbes at sites (site B), with sufficient aeration where all exhibits are stored into showcases, resemble the values measured outdoors. However, the efficiency of bacterial removal using photocatalytic ionizers as air purifiers was also depended on the total aeration rate, and the emission rates of the microorganisms indoors. Thus, it is recommended a sufficient regulation of the aeration, and the use of showcases for improving the indoor air quality with the use of air purifiers as well as for protecting exhibited artworks. However, these findings have to be treated with caution since they
were derived from a specific measurement period and may other factors influence the results such as the variability of microorganisms present indoors.

Finally, the study of microbial deposits on different painting model essays, which were exposed in the Historical Museum of Crete for a period of 18 months, did not show any colonization of viable fast-growing fungi on the surfaces of a large number of painting substrates and materials. This result depicts the toxicity of the painting colours, even without the use of any treatment of varnishes, and explains the preservation of paintings for long periods without always the optimum storage conditions. Contrary, a considerable enrichment of high concentrations of viable heterotrophic bacteria was observed on the surfaces of some materials (such as e.g. newspaper, magazine), probably, due to the chemical composition of these materials as well as the possible nutrient supply for the growth of the microbes. Surprisingly, no colonization of fungi could be determined on the same materials. Enrichment of low concentrations of fungi were detected only on tempera, charcoal, wax pastel, and carton board. We assume, that the measured results of the surface colonization of bacteria and fungi are directly related to the indoor microbial community and the air quality, where the painting materials and the model essays were exposed. The reason of the bacterial colonization on all exposed materials, in low or high concentrations, could be their dominance in the indoor air of the museum in relatively high concentrations. In comparison, the absence of fungal colonization on the majority of exposed materials is caused, probably, due to the low airborne fungal concentrations in the Historical Museum of Crete. Furthermore, the storage of the exhibits in showcases, with well-regulated microclimate, can act positively to the protection of the cultural heritage collections.

Summarizing, the increased enrichment of airborne microbes, mainly potential pathogenic, heterotrophic bacteria and acid producing bacteria, inside museums through numerous anthropogenic activities, can be avoided in exhibition rooms equipped with air purifying systems.
(such as the used photocatalytic ionizers) and microclimatic showcases. The efficiency of these relatively new technologies and practices can be improved by a proper regulation of HVAC systems, so that to improve the air quality in cultural heritage collections for both, humans and artworks.

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DISCLAIMER

The authors declare that they have no conflict of interest.

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heritage environments: Experience at the Palatina Library in Parma. Sc. of the Total Env. 536, 557-567.


Table 1. Natural infiltration, heating, ventilation and air conditioning, mechanical recirculation, and number of placed photocatalytic ionizers at the corresponding measurements sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Floor</th>
<th>Total room volume (m³)</th>
<th>Natural infiltration rate (m³ h⁻¹)</th>
<th>Heating, Ventilation and Air Conditioning (m³ h⁻¹)</th>
<th>Recirculation rate (m³ h⁻¹)</th>
<th>Number of placed photocatalytic ionisers (one per ~ 65 m³ with mean air flow rate 170 m³ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnographic collection room (Site B)</td>
<td>second</td>
<td>476</td>
<td>750</td>
<td>2,500</td>
<td>2,500</td>
<td>7</td>
</tr>
<tr>
<td>A. Kalokerinos room (Site C)</td>
<td>ground</td>
<td>264</td>
<td>150</td>
<td>1,920</td>
<td>1,920</td>
<td>4</td>
</tr>
<tr>
<td>Z. Portalakis room I, (site D I)</td>
<td>first</td>
<td>218</td>
<td>120</td>
<td>1,920</td>
<td>1,920</td>
<td>4</td>
</tr>
<tr>
<td>Z. Portalakis room II, (site D II)</td>
<td>first</td>
<td>189</td>
<td>450</td>
<td>1,000</td>
<td>1,000</td>
<td>3</td>
</tr>
</tbody>
</table>
(b) First Floor

Periodical Exhibition
Z. Portalakis Rooms (Site D)

Z. Portalakis Room II (Site D II)

Z. Portalakis Room I (Site D I)
Fig. 1. Location and pictures of the sampling sites where measurements were performed: (a) ground level—outdoor (site A), A. Kalokerinos Room (Site C); (b) first floor—Periodical Exhibition Z. Portalakis Rooms (Sites D I, and D II); (c) second floor—Ethnographic Collection (Site B). The position of the photocatalytic ionizers indicated with a green color, whereas the inlet and outlet of the mechanical ventilation system were shown with blue and orange arrows, respectively.
Fig. 2. Boards with painting materials and model essays exposed in the museum that used for the determination of microbial deposits inside the Historical Museum of Crete (holes indicate sampled parts). Blackboard on the left and colour board on the right.
(a) Acid producing Bacteria
Chemolithotrophic Bacteria
Fungi
Heterotrophic Bacteria

Sampling date

(b) Acid producing Bacteria
Chemolithotrophic Bacteria
Fungi
Heterotrophic Bacteria

Sampling date

(c) Acid producing Bacteria
Chemolithotrophic Bacteria
Fungi
Heterotrophic Bacteria

Sampling date
Fig. 3. Monthly measurements of viable airborne microorganisms (March 2014 – February 2015) at the Historical Museum of Crete (a) outdoors (site A) (b) Ethnographic collection room (site B) (c) A. Kalokerinos room (site C) (d) Z. Portalakis room I (site D I), and (e) Z. Portalakis room II (site D II).
Fig. 4. Seasonal average concentrations of viable, cultivable airborne microorganisms in the Historical Museum of Crete at the different measurement sites.
Fig. 5. Enrichment of airborne microorganisms in Z. Portalakis room I (site D I) associated with the visitor’s presence and room occupation.
Fig. 6. Seasonal average Indoor-to-outdoor (I/O) concentration ratio of viable airborne microorganisms in the different indoor sites of the museum.
Fig 7. (a) One-year average decrease of the ambient concentration of microbes (CFU m⁻³), and (b) Seasonal average concentrations of viable, cultivable airborne microorganisms at the different measurement sites after the placement of photocatalytic ionizers (Spring 2015-Winter 2015-2016).
Fig 8. Indoor-to-outdoor (I/O) concentration ratio at the different measurement sites with and without the use of ionizers for different categories of microbes.
Fig 9. Colonization of heterotrophic bacteria (CFU·cm$^{-2}$) on different model essays and painting materials after an exposure period of 18 months in the museum.