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## **STICKY MICROBES AND DUST ON OBJECTS IN HISTORIC HOUSES**

Amber L. Tarnowski, Christopher J. McNamara, Kristen A. Bearce, and Ralph Mitchell

### **Abstract**

This research investigated the role microorganisms play in bonding dust to surfaces. Non-biological mechanisms of dust adhesion include molecular dispersive forces, electrostatic interactions, and capillary condensation. In addition, dust adhesion may result from contact with sticky exopolymers produced by microbial biofilms. Biofilms are communities of microorganisms, which are present on all surfaces. Biofilms are held together by exopolymers, which are created as products of microbial metabolism. Layers of dust, microorganisms, exopolymers, and substratum form a complex system that makes it difficult to clean delicate surfaces in historic interiors. Dust samples were collected from Knole House at Sevenoaks in Kent, and from Blickling Hall in Norfolk, England. Plate counting and nuclear staining were performed to qualify and quantify the microbes. Dust samples plated onto nutrient agar culture plates yielded high numbers of bacteria. Investigation of microbial metabolism revealed that in controlled humid environments, microorganisms utilized dust components as the sole source of nutrition. Exopolymers visible under the microscope were produced within days. Solvent extractions of dust samples were analyzed with gas chromatography-mass spectroscopy (GC-MS) to identify the hydrocarbon and fatty acid components in the dust that serve as nutrition for microbes. While these English country estate homes are relatively removed from the typical sources of hydrocarbon nutrients that can support microbial growth, such as smog and heavy traffic pollution, sufficient nutrients remain in the dust. To illustrate the interaction of microbial activity on textiles, thin biofilm samples were examined with electron microscopy. Bacterial isolates displayed preferences for breaks and ends of wool or silk fibers. The analytical techniques used in this study are standard in the field of microbiology, and can be used to analyze housekeeping practices of historic house interiors and their contents.

### **1. Introduction**

Biological colonization of outdoor sculpture, monuments, and architectural surfaces is a phenomenon that has been observed by conservators for years. Nutrients and moisture are available and renewed constantly in the outdoor environment, sustaining microbial growth on surfaces, which can eventually damage the substratum. The characterization and effects of biodeterioration on indoor art and heritage materials have been studied in recent years by microbiologists and conservators. These investigations have illuminated a variety of problems including fungal growth on cellulosic materials such as books and works of art on paper (Florian 1997; Hideo 1984; Szczepanowska 1994); deterioration of wool carpet and other textiles (Suwanarit 1995; Mahall 1982); and the susceptibility of paintings and silk to fungal growth (Seves et al. 2000). Fresco paintings, murals, and rock art in semi-closed environments have also suffered from biological attack. The closing of the Lascaux Caves to the public in 1963 serves as a reminder that biological bloom can be persistent and may require ongoing preventive measures (Ciferri 1999). These studies show that microorganisms can be problematic for objects

in both the indoor and outdoor environments. Since microorganisms can colonize almost any surface, no object is absolutely exempt from microbially-induced degradation (Gu et al. 2000 A).

In this study, the approach was to investigate possible biological causes of severe dust adhesion to delicate objects inside historic houses. In March of 2002, conservators of the National Trust of England and researchers at the University of East Anglia commenced a three-year interdisciplinary study to examine soiling processes on sensitive materials in historic properties [1]. Although a regular cleaning program is instituted in the houses of the National Trust, typical techniques for dust removal had in many cases become ineffective. This was especially true for sites with long winters and high humidity, like Knole (Lithgow 2004). The strong dust adhesion was suspected to be a result of environmental and biological factors. Using an approach combining microbiology and conservation, the role sticky biofilms play in dust adhesion was investigated.

### **1.1 Adhesion as a biological phenomenon**

Biological adhesion of microbes to surfaces is of the result of factors such as electrostatic forces, bacterial attachment structures, and the production of sticky polymers ('exopolymers') produced as a product of bacterial metabolism. Communities of microbes held together by exopolymers are called biofilms, and while the biofilm is primarily composed of polysaccharides, the exopolymer may contain proteins, nucleic acids, humic acids, lipids, and other carbohydrates (Roldan et al. 2003). Biofilms may include various microorganisms like bacteria, fungi, algae, and lichens (Flemming 2002; Varnam 2000). Investigations of microbial colonization on stone sculptures and fresco paintings exposed to the environment have revealed that a diverse community of microorganisms are able to colonize a surface, not just one type of microorganism (Albertano et al. 1991). Although some biofilms can serve as a protective patina, many microorganisms also produce acids as products of metabolism. For example, oxalic acid excreted from microbes has been observed to cause pitting and exfoliation on stone and glass (di Bonaventura, et al. 1999; Dornieden, et al. 2000).

Adhesion of dust particles is affected by factors such as the dynamics of molecular dispersive forces, electrostatic interactions, and capillary condensation (Phenix et al. 1990). Additionally, dust in itself is a key transporter of bacteria and fungal spores (Yoon et al. 2000), and a discussion of dust adhesion on non-sterile surfaces should therefore include consideration of the effects of biological adhesion [2]. Bacterial adhesion to a surface occurs when electrostatic forces, cell structures, and natural polymers bind the bacteria to a surface and work is required to separate them. Bacteria are able to adhere only after van der Waals and electrostatic forces bring the bacteria very close to a surface. When microbes sense short-range interactions with a substratum, there is a physiological response resulting in a modification of the cell surface leading to adhesion; the type of interaction with the surface depends upon the bacterial species present and the surface physicochemistry (Mozes et al, 1991). Although medical and environmental research is mainly focused on biofilms at solid-liquid and liquid-air interfaces, aerophytic biofilms can exist at solid-air interfaces (Gu et al. 2000 B). In this environment, the bacteria in the exopolymer matrix sequester nutrients from the substratum by excreting enzymes (Marshall 1996). Biofilms can form from just a few bacteria; the underlying bacteria multiply,

exude polymer, and die, while new ones are perpetually deposited on top from falling dust. The biofilm can grow on and around the dust particles, strongly securing themselves and the dust particles to surfaces.

## 1.2 The nature of dust

Indoors, microbial nutrition is dependent on available surfaces and dust particles. The term *dust* includes solid or liquid particles and aerosols. Indoor dust contains organic and inorganic soil particles tracked in from shoes of visitors, fibers from clothing and carpets, hair, dead skin cells, insects, salts and pollutants (Macher 2001). In Knoll House and Blickling Hall, the chief components of the dust were determined under magnification to be textile fibers, insect remains, and particles like quartz (see results). The wide range of nutrients is sufficient for bacterial and fungal growth. Because the contents of dust brought in from the outdoors will vary throughout the year, it is impossible to fully characterize the contents of the dust in a quantitative manner that represents the full picture. It is usually in a state of flux with direct relationships to other variables, such as the seasonal climate and precipitation, pollution, redeposition of particles from cleaning, admission of external air, and visitor numbers (Yoon et al. 2000). While the amounts of dust and dust contents vary, so do the microorganisms that survive from dust nutrients. Microbial population numbers indoors can equal or exceed the numbers found outdoors in common soil, which is 10 million per gram, including up to 10,000 different species (Ogram and Sharma 2002). Additionally, over 1.5 million fungi are estimated to exist on earth, only 80,000 of them are known, and about 1800 discovered and named every year (Bennett et al. 2002). Therefore, the investigation focused on the biological community as a whole, instead concentrating on the identification of individual species. The basic method for characterizing a biofilm community used here is to examine the environment and available nutrients.

## 2. General history of the case study sites

Two historic properties were selected for dust sampling. The first site was Knole in Sevenoaks, which lies 25 miles southeast of London. Knole is a Tudor mansion set in a 1000-acre deer-park, used for the deer-hunting activities of the Archbishops of Canterbury in the 15<sup>th</sup> century. Knole's history of royal ownership includes Edward VI and Henry VIII. It was given by Queen Elizabeth I to the Sackville Family in 1586. Vita Sackville West, friend of Virginia Woolfe, lived in Knole. Both Vita and Knole provided the inspiration for Woolfe's classic novel *Orlando*. Knole was given to the National Trust in 1947 (Brady 1839; Sackville-West 1958). Today Knole is home to a fine collection of art and furniture. Paintings by Van Dyke, Gainsborough, Holbein, and Reynolds, decorate the walls above 17<sup>th</sup> century royal Stuart furniture. Dust samples were gathered in two rooms: the Brown Gallery, and the Venetian Ambassador's State Bedroom.

Likewise, dust was collected from the Peter the Great Room in Blickling Hall. Blickling Hall is an early 17<sup>th</sup> century icon of England's Jacobean style country houses. It stands in the countryside of Norwich, inland from the sea by about 22 miles. It is surrounded by 4,777 acres of woodland, parkland, brick cottages, and farmland (National Trust 1987). Sir Henry Hobart owned Blickling during the reign of King James I, and rebuilt the house in 1619, replacing the earlier settlement that dated back to the first millennium. Throughout history the ownership of

Blickling changed many hands to include prominent figures and families, including Sir John Fastolfe, who was used as fertile material for a comic character by Shakespeare. Fastolfe sold the house to Sir Thomas Boleyn, so Blickling later became the accepted birthplace of Anne Boleyn. The house is also legendary because many of the original contents, gardens, and park remain preserved together, unlike other country houses that eventually lost their components due to high taxation. Blickling Hall was given to the National Trust of England in 1940, by the founder of the Country Houses Scheme, Philip Kerr, the 11<sup>th</sup> Marquis of Lothian. Philip Kerr (1882-1940) is famous for his political career, as he drafted the preface to the Treaty of Versailles in 1919 and helped unite America and England during World War II (National Trust 1987). Blickling's 18<sup>th</sup> century room furnishings include rare books and tapestries, furniture upholstery, and state bed textiles. The microorganisms harvested from dust collected at Blickling and Knole were used as the investigative biomaterial.

### **3. Experimental**

Vacuum cleaner bags were collected from two rooms in Knole, between June of 2001 and October of 2003. Three bags were collected from the Venetian Ambassador's bed: the upper right valance area, the upper left valance, and the foot valance. These are referred to as dust samples A-C, respectively. In the Brown Gallery in Knole, dust sample D was collected from the crewelwork chair covers. Sample E was collected from the seats of furniture in the Peter the Great Room in Blickling Hall in November of 2003. The contents of individual vacuum-cleaner bags were stored in sterile containers at 5° C. Particulate matter in the dust samples were analyzed with light microscopy to identify the majority of the dust components.

#### **3.1. Enumeration of microorganisms**

Dust from samples A-E were diluted with sterile deionised water and inoculated onto nutrient agar plates. The plates were incubated for 10 days at 28°C and 65% relative humidity (RH). The colony-forming units (CFU's) were counted.

The culture plate method is only a rough estimate of colonies able to grow on a particular medium. Total numbers of microbes in samples were counted by staining with a nuclear dye. Triplicate solutions of dust samples A-E were prepared for counting by diluting weighed amounts of dust into sterile deionised water and formaldehyde. Samples were concentrated by filtration (15 kPa vacuum) onto 0.22 µm pore size black polycarbonate membranes (Whatman Track-Etch Nucleopore with 0.2 µm pore size). This process trapped the microbes on the filter membranes, as bacteria and fungi are generally 2 to 4 µm in size or larger. Bacteria and filter membranes were stained with 4', 6-diamido-2-phenylindole (DAPI) and rinsed with deionized water (Gustashaw 1991). Filter membranes were air dried by vacuum suction and mounted onto glass slides, secured with Cargille non-drying immersion oil. An Olympus BX 60 epifluorescent microscope was used to view and count the cells on each filter.

### 3.2. Dust as microbial nutrition

To examine how microbes utilize dust for nutrition, the bacteria producing the most exopolymer in each dust sample were selected from the culture plates, and isolated as pure cultures by transferring cells from the culture plates with a sterile loop to tryptic soy broth (TSB). Cultures were agitated at 32 °C for 24 – 48 hours at 100 rpm. Cells were harvested from the solution by centrifugation, separating the pelleted cells from the TSB. The bacteria were resuspended in sterile deionized water. The bacteria were centrifuged a second time, the water was decanted, and bacteria were transferred to a sterile glass slide. An average of 6.4 µg of dust in 100 µg of sterile deionised water was added to each slide as the sole nutrient source. The slides were placed in sterile petri dishes containing a moisture reservoir of 100 µL of sterile deionised water. The culture plates were sealed with Parafilm wax to create a microchamber at a constant humidity. Each microchamber was incubated for 48 hours at 32°C. Bacteria on the slides were Gram-stained, which helped to make the bacteria visible under magnification, and to differentiate between gram (+) and gram (–) bacteria by their outer cell wall chemistry (Bartholomew 1958). The bacteria were visible under 1000x magnification (using a 100x oil immersion lens). Imaging was assisted with the SPOT RT Color version 3.0.4 software program connected to an Olympus BX 60 microscope.

### 3.3. Electron Microscopy analysis

The effect of microbial activity on textiles was examined. The process of placing culture isolates and dust on slides in a culture plate microchamber was repeated, and included several individual sterilized fibers of wool and silk. Modern wool and silk replica textile used to replace curtains, seat upholstery, and wall-hangings in the Peter the Great Room of Blickling Hall served as a substratum layer for microbial growth. Slides made from dust samples B, C and E were incubated at 28°C and 65% relative humidity for 9 days. They were examined in a FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM). This method allowed the biofilm to remain intact, as the sample preparation method did not require dehydration.

Biofilms were cultured with sterile textile fibers and dust in a microchamber a second time as described. Sterile Thermanox plastic cover slips (10.5 x 22 mm, NUNC Brand Products) served as the substratum for biofilm formation instead of glass slides. After incubating at 31°C and 100% humidity for 8 weeks, biofilms were visible on and around textile fragments. The biofilms were fixed overnight in 3% formaldehyde solution and dehydrated in a progressive series of ethanol and water solutions (from 40% ethanol to 100% ethanol in increments of 10%). Samples were prepared for the SEM with an Argon-ion sputter deposition system (Desk II Sputtering Unit, Denton Vacuum) after critical-point drying (Autosamdri-815, Tousimis). Samples were examined in a LEO (Zeiss) Field Emission Gun Scanning Electron Microscope (SEM). The sample preparation method may have caused distortion or loss of the biofilm during the dehydration process, but allowed for better resolution at higher magnification.

### 3.4. GC-MS

Gas chromatography-mass spectroscopy was used to determine the soluble components in the dust that may serve as microbial nutrition. Two extractions were performed on dust sample D. To extract hydrocarbons, 0.0216 g of the dust was placed onto Whatman GF/F 0.7  $\mu\text{m}$  filter paper and 1.5 mL of 98.5% pure hexane (85% n-hexane) was passed through. A total of 30  $\mu\text{L}$  of the extraction was used for GC-MS analysis. For extracting other compounds, 1.5 mL of a 1:1 solution by volume of sterile distilled water and ethanol was passed through 0.0582 grams of dust, using the same Whatman filter type. 15  $\mu\text{L}$  of the extraction solution was used for GC-MS. Both samples were analyzed by GC-MS on a HP 6890 GC System with a HP 5973 Mass Selective Detector and a HP 6890 Injector. The oven was heated from 50° C to 250° C with a rate of 13° C/min. Results were compared to the ChemStation Software database (Agilent Technologies).

To verify the ability of bacterial and fungal isolates to grow on hydrocarbons such as those identified in dust samples using GC-MS, bacterial and fungal isolates were inoculated into a minimal salt medium [3] containing 1% sterile filtered kerosene (n C6 – n C16 alkanes). The kerosene was passed through a syringe with a glass filter size 0.22  $\mu\text{m}$  to remove existing bacteria and other particles. Twenty-eight isolates were inoculated into the medium and shaken at 100 rpm for 106 days. Ability to grow using kerosene as the sole carbon source was assessed visually (i.e., increased turbidity of cultures resulting in an opaque appearance).

## 4. Results

Informal visual analysis by light microscopy revealed the major components of the dust samples to be textile fibers, insect remains, quartz, and other glass-like particles.

### 4.1. Enumeration of microorganisms

Numbers of bacteria in dust samples were determined using plate counts and DAPI staining. The average numbers of viable colonies of bacteria determined by plate counting were  $3.52 \times 10^6$  CFU/gram of dust for Knole, and  $1.03 \times 10^6$  CFU/gram of dust for Blickling. Numbers of CFU were variable among samples, with the highest numbers of CFU observed in samples A and E (Table 1). Numbers of bacteria determined using DAPI staining were one to three orders of magnitude greater than the plate counts, but numbers were much more similar among samples: Knole averaged  $2.25 \times 10^8$  bacteria per gram, and  $1.64 \times 10^{10}$  for Blickling.

Dust sample	Sample Location	CFU's/g dust (colony count)	Bacteria/g of dust (DAPI count method)
A	Venetian Ambass. Bed, Knole	$1.36 \times 10^7$ ( $\pm 1.3 \times 10^7$ )	$2.36 \times 10^8$ ( $\pm 5.7 \times 10^7$ )
B	Venetian Ambass. Bed, Knole	$1.23 \times 10^5$ ( $\pm 4.5 \times 10^4$ )	$1.83 \times 10^8$ ( $\pm 3.0 \times 10^7$ )
C	Venetian Ambass. Bed, Knole	$1.56 \times 10^5$ ( $\pm 6.9 \times 10^4$ )	$1.13 \times 10^8$ ( $\pm 1.6 \times 10^7$ )
D	Brown Gallery, Knole	$1.55 \times 10^5$ ( $\pm 5.4 \times 10^4$ )	$3.67 \times 10^8$ ( $\pm 7.1 \times 10^7$ )
E	Peter the Great Room, Blickling Hall	$1.03 \times 10^6$ ( $\pm 5.9 \times 10^5$ )	$1.64 \times 10^{10}$ ( $\pm 7.7 \times 10^9$ )

Table 1. Numbers of bacteria in dust samples from Knole and Blickling Hall (Mean $\pm$  se, n = 3).

#### 4.2. Dust as microbial nutrition

After 48 hours of incubation at 30°C, the microbes adhering to dust particles were visible with transmitted light microscopy with a 100x oil immersion lens. Half the samples had produced exopolymer visible without magnification, and the other half demonstrated initial stages of biofilm growth. Gram's Stain was used to determine the variety of gram (+) and gram (-) bacteria present in the dust samples, and to render the cells visible. In the dust samples analyzed, the dominant microbial species was bacteria. It was also evident that bacteria appeared to be adhered to the surfaces of dust particles

#### 4.3. Electron microscopy results

It was evident from the ESEM micrographs that microbes prefer to colonize on breaks or ends of the fibers (see Fig. 1). Individual isolates have definite nutritional preferences for either the dust, the wool, or the silk fibers. Isolates from dust sample B preferred the dust particles, and trails (or elongated colonies) of bacteria lead from one dust particle to the next (Fig. 2). Isolates from dust sample C preferred silk and did not agglomerate on wool. In this example, stringy exopolymer exuded by bacteria (larger than the fimbriae or flagella which would be 2 or 3 nm in length, not 2 or 3  $\mu$ m), is also visible (Fig. 3). Isolate E from Blickling Hall demonstrated a much higher bacterial population and preference for both types of textile fibers (Fig. 4). In Figure 5 from isolate E, the stringy polymer is visible on the silk. Intact biofilms from dust samples D and E were examined with SEM. Figures 6 and 7 demonstrate clusters of bacteria and polymer can cover fibers completely. In all examined biofilm slides, the presence of exopolymer was sufficient to secure the dust particles to the textile fibers and to the glass, even long after the biofilms were dehydrated for two weeks at 50% RH and below. Overall, observations of microbial activity were similar for ESEM and SEM (Figs. 6 and 7).



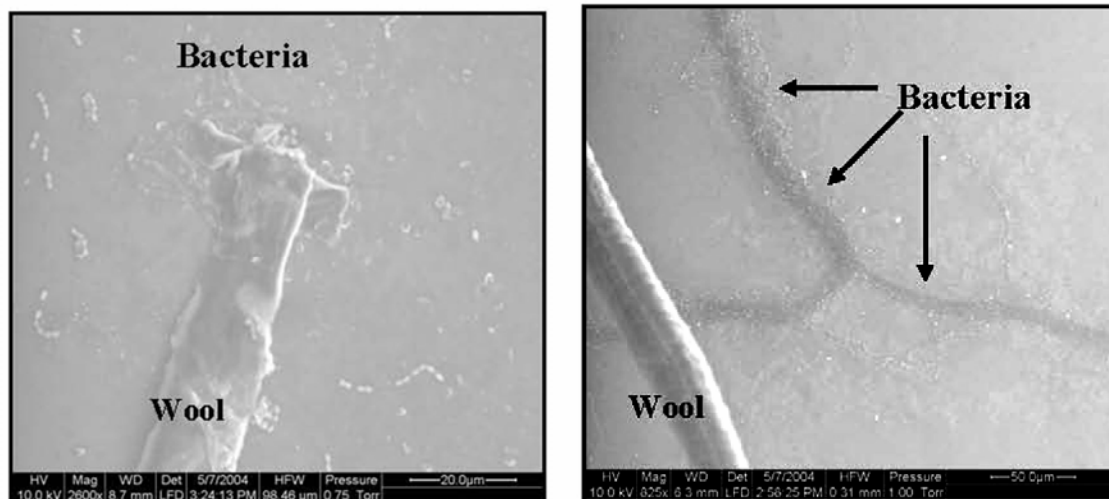


Figure 1 (above left). Isolate bacteria B with microbes colonizing on the ends of the fiber. The scale bar in the bottom right of the black information panel is 20.0 μm. ESEM micrograph.

Figure 2 (above right). Isolate bacteria B (from Knoles) has little affinity for the wool fiber. The darker 'trail' of microbes leads to dust particles. The scale bar is 50.0 μm. ESEM micrograph.

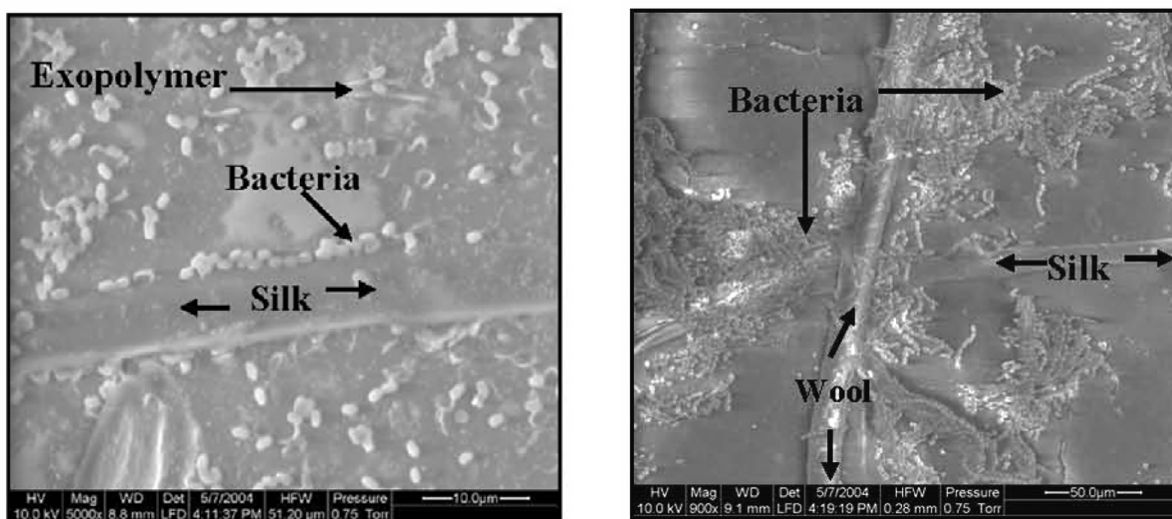


Figure 3 (above left). Isolate bacteria from dust sample C (from Knoles) clusters on the surface of the silk. The scale bar is 10 μm. ESEM micrograph.

Figure 4 (above right). Isolate E (from Blickling) shows an abundance of bacteria adhering to both wool and silk present in the sample. The wool fiber has scales and lies vertical. The silk fiber lies horizontally. The scale bar is 50 μm. ESEM micrograph.

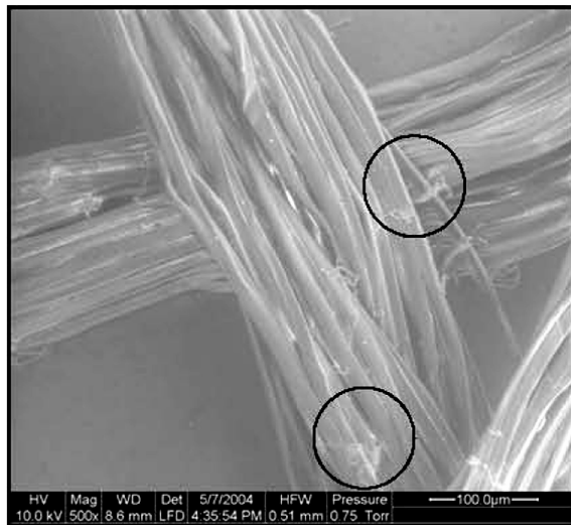


Figure 5 (above left). Isolate E (from Blickling) has formed stringy-looking trails of polymer (indicated with circles) over and around the silk. The scale bar is 100 µm. ESEM micrograph.

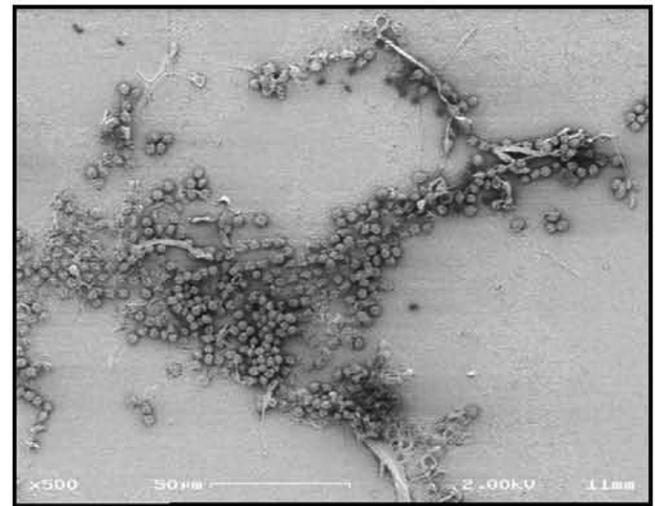


Figure 6 (above right). Fibers covered with microbes and polymer, isolate bacteria from dust sample D. The scale bar is 50 µm. SEM micrograph.

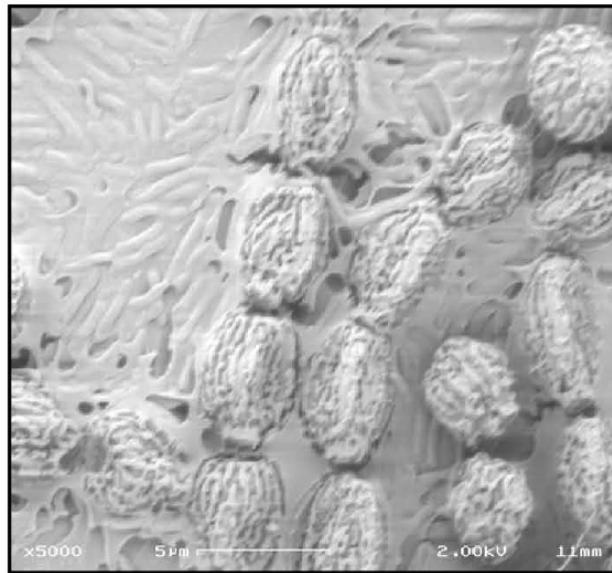


Figure 7. Exopolymer and microbes from isolate bacteria from dust sample D. The fibers are no longer visible because the biofilm has covered them. The scale bar is 5 µm. SEM micrograph.

#### 4.4. GC-MS

The compounds identified from the hexane extraction included diphenyl ether (commonly used as a textile flame retardant and pesticide) and pentaethylene glycol (an anti-icing agent in petroleum,  $C_{10}H_{22}O_6$ ). There were heavy hydrocarbons from fuel combustion and motor oil as well: octadecane ( $C_{18}H_{38}$ ), eicosane ( $C_{20}H_{42}$ ), and heptacosane ( $C_{27}H_{56}$ ) (CambridgeSoft Corp. ChemFinder 2004; Potter et al. 1998; see Fig. 8).

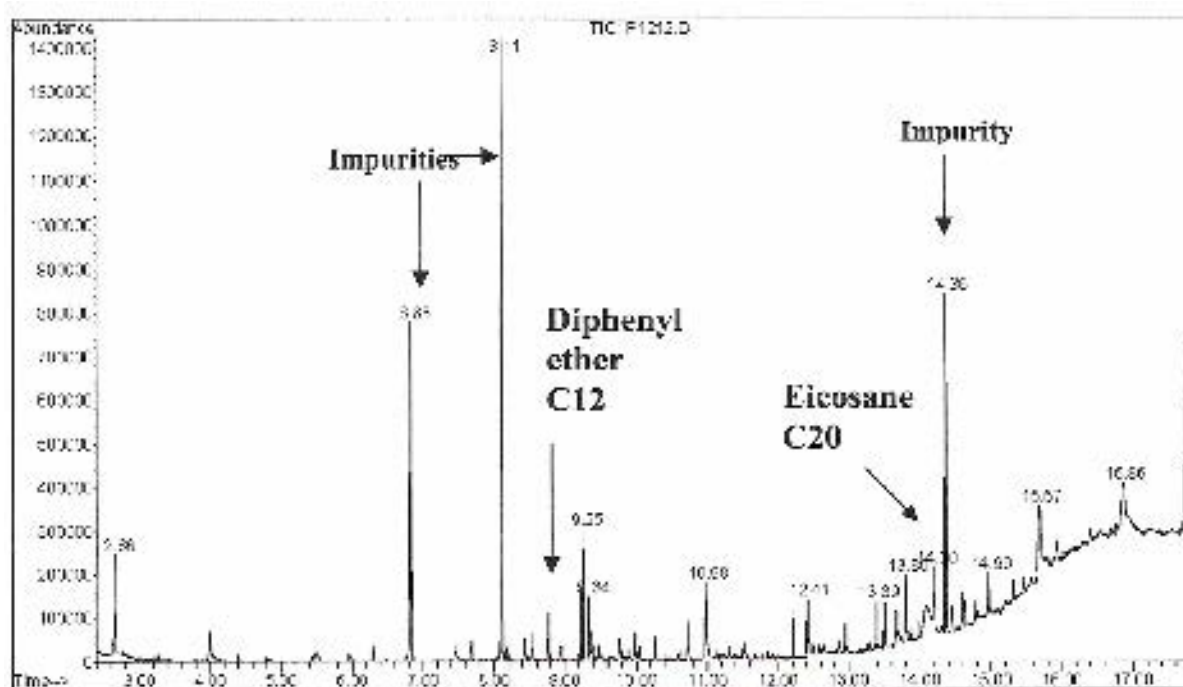


Figure 8. Chromatogram for the hexane extraction of dust sample D (Brown Gallery, Knole).

The 1:1 ethanol and water extraction yielded many fatty acids and chemicals: dimethylamine ( $C_2H_7N$ ), 1H-Pyrazole ( $C_3H_4N_2$ ), 4,5-dihydro-1-phenyl, acetic acid (ethanoic acid,  $C_2H_4O_2$ ), octanoic acid ( $C_8H_{16}O_2$ ), propanoic acid ( $C_3H_6O_2$ ), and 1,2-benzenedicarboxylic acid, butyl ( $C_{16}H_{19}O_4$ ). Although there could be many sources for these chemical compounds, these compounds had specific probable sources in common: insecticides, pesticides, herbicides, and fungicides. Chemicals commonly used in textile manufacturing and processing were also identified: glycerin ( $C_2H_4O_3$ ), hexanoic acid ( $C_6H_{12}O_2$ ), and adipic acid (hexanedioic acid,  $C_6H_{10}O_4$ ) (Merck Index 1996; CambridgeSoft Corp. ChemFinder 2004, Washington State U., 2002; PAN Pesticides 2004; Nettles 1983; Canadian C.O.H.S. Cheminfo 2004; U.S. EPA 2004). These are likely from the textile fibers in the dust (see Fig. 9).

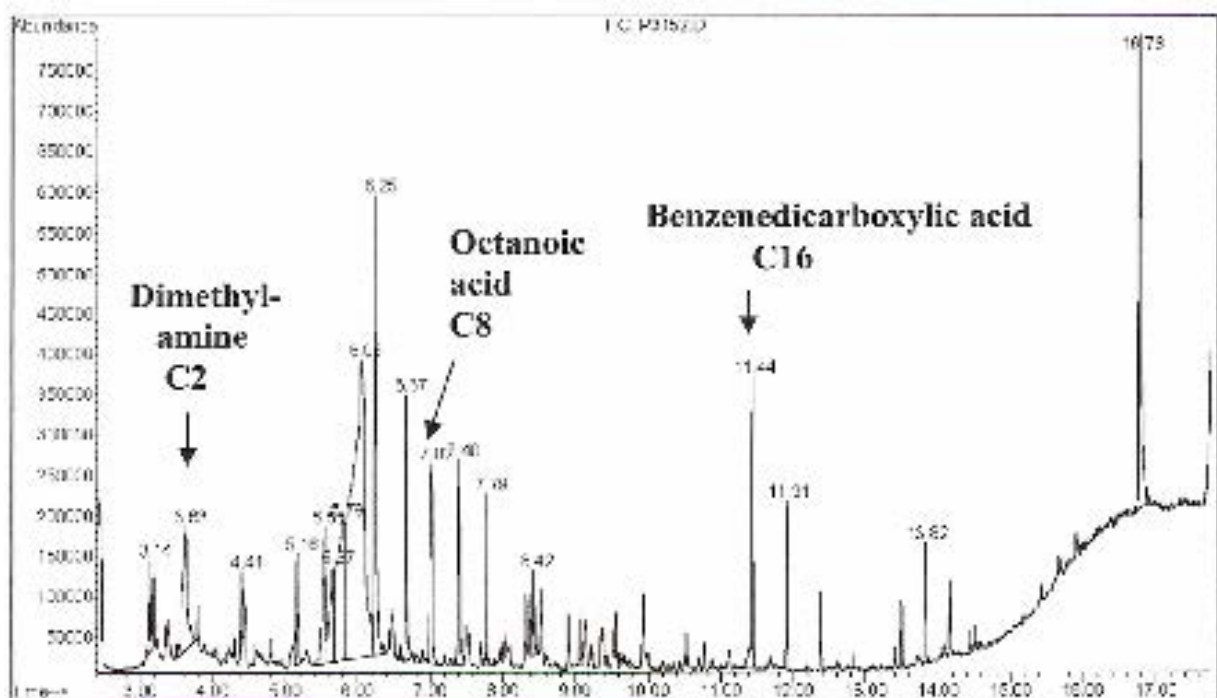


Figure 8. Chromatogram for ethanol and water extraction of dust sample D (Brown Gallery, Knole).

Of the 28 isolates in 1% kerosene and minimal salt solution, 82% displayed bacterial growth and 11% displayed fungal growth (Table 2). All of the fungal specimens grew from isolates harvested from Blickling Hall dust samples, and no fungal growth was observed in kerosene inoculated with dust from Knole. This confirmed that the hydrocarbons in the dust samples provided nutrients for microbial growth.

Dust sample	Sample Location	Percent growth
A	Venetian Ambass. Bed, Knole	50
B	Venetian Ambass. Bed, Knole	80
C	Venetian Ambass. Bed, Knole	100
D	Brown Gallery, Knole	86
E	Peter the Great Room, Blickling Hall	100

Table 2. Percent of isolates able to grow using kerosene as the sole energy source.

## 5. Discussion

These techniques illustrated that microorganisms play a role in dust adhesion. The prevention of dust adhesion due to microbial polymers in biofilms may be achieved by three-step process: identification of the microbial community and its environment; sanitation and cleaning; and prevention of new biofouling (Flemming 2002). The experiments described offer a model for achieving step one, identification of the microbial community. Collecting the vacuum cleaner bags allows for later investigation of the dust contents and the microbial population. Other models of collecting dust for examination include glass slides and sticky samplers (Yoon et al. 2001).

Characterizing the biological community in the dust revealed information on the type and population of microbes present. However, the two methods used to quantify the microbial population are not flawless; counting CFU's reflected only microbial colonies that were able to grow on a specific pure culture plate media. The dust on the objects at these two sites does not represent pure culture media, but could be a much more complex nutrition scenario. The DAPI stain method causes all microbes present (and sometimes other matter) to fluoresce and be counted; naturally the numbers will be higher with the DAPI method. Although imperfect, the methods do allow for valuable comparisons. High populations may signal that there are problems on the microscale level occurring on the dusty surface of an object. Non-commonalities and new developments in the dust can alert the conservator to possible risk for the objects due to the microbial activity observed. The polymers generated in combination with the dust can be difficult to remove with time; surfaces may eventually be damaged by prolonged contact with acids produced by metabolism. The degree of wool and silk textile deterioration due to prolonged exposure of dust particles and microbes remains unknown, as it would be unique to each set of historic house variables (i.e. dust accumulation, humidity levels, microbes present, available nutrition, etc.).

In the case of Knole, the compounds found in the dust by GC-MS analysis may indicate that past use of the permethrin-based insecticide 'Constrain' on the floorboard and wall interfaces may have left a residue that microbes can utilize for nutrition (Bullock 2004; Cornell University Extoxnet 2004). Further analysis on this issue would be beneficial for collections subjected to pesticides.

The other compounds found in the samples provided nutrition for biofilm growth, and microbial exopolymers were able to secure dust particles to textile fibers in a high humidity environment in a short period of time. Housekeeping practices like vacuuming cannot address or remediate this problem after a biofilm is established. Traditional means of sanitation and cleaning (step 2) can be employed along with new and innovative methods (Madigan et al. 2003). Although washing is avoided until necessary with historic textiles, one of the benefits of washing is removing dust and bacteria. Detergents effectively disrupt the outer protective lipid and protein membranes of bacteria (lysing), and detergents help break the bonds of exopolymer adhesion. Stable materials not affected by exposure to alcohol and water could be wiped or sprayed with an application of 80% ETOH in distilled and deionized water after routine cleaning, which can lyse most kinds of

bacteria (Helenius et al. 1979). When washing is not safe for the textile or affected object, testing known or innovative methods for remediation can be a fruitful source of alternatives to cleaning.

Frequent housekeeping may help to avoid formation of compacted dust and biofilms formation, but is not necessarily the only prevention strategy. When thinking in terms of controlling microorganism growth, the obvious solution is careful humidity control and application of biocides; but this may not deter bacteria as they exude *more* polymer when under stress conditions, and can fix oxygen from sources other than water. Stress conditions include periods of low humidity and nutrition following periods of high humidity with nutrition. Biocides have also not proven to be an effective means to control bacteria, because the biofilm matrix prevents deep penetration into the community of the biocide, and because bacteria can alter their genetic expression to be less susceptible to the biocide (Flemming 2002; Roldan et al. 2003). Regular sanitization and cleaning are preferable to the use of biocides that are increasingly restricted by the government.

Ultimately, prevention involves the development and implementation of a monitoring program where the dust components, microbial population, and humidity levels are evaluated together determine essential relationships. Avoiding even short spikes in humidity can slow or prevent the initial growth of biofilms. Yoon and Brimblecombe have monitored and analyzed dust in museums, and have found correlations between dust deposition and visitor numbers, the proximity of visitors to the objects, and local climate cycles (Yoon et al. 2000, 2001). Prudent preventive practice may include the addition of dust monitoring to an established program of temperature and humidity monitoring and pest management.

## 6. Conclusion

The study illustrated the nature of the relationship between bacteria and dust particles. The experiments have shown that the microbes in the dust samples are mainly bacteria with a population equal to or higher than outdoor soil samples. Bacteria create a sticky exopolymer as a result of metabolism that adhere dust and textile fibers together. Biofilms can grow from dust on textiles in laboratory conditions in 48 hours. The adhesive and selective nature of bacteria to dust and textiles was illustrated with electron microscopy. Sources of nutrition were identified with GC-MS and are likely deposits from petroleum combustion, textile processing, and localized use of pesticides. Since the interactions between biofilms and historic textiles remain largely unknown, amassing and comparing data on microbial growth on sensitive surfaces would be helpful for evaluating the frequency and adequacy of any cleaning program. Prevention of biofilm formation would ease the degree of difficulty in cleaning delicate objects and reduces exposure to acids excreted by microorganisms. Prevention is achieved with an understanding of the nutritional cycle and eliminating sources of microbial nutrition whenever possible, accomplished by limiting sources of dust and instituting regular cleaning. The study highlights the fact that perfecting strategies and methods for monitoring and preventing indoor biofilms on historic objects, as well as effective remediation measures, remains worthy of further investigation.

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## Endnotes

1. The three-year project (2002-2005) was organized by Helen Lloyd of the National Trust of England, and Dr. YH Yoon and Professor Peter Brimblecombe of the School of Environmental Sciences at the University of East Anglia, Norwich, UK.
2. There is disagreement in the scientific literature about the effect surface roughness has on bacterial adhesion. This is very important when examining bacterial adhesion of dust to textiles because textile surfaces can be rough or smooth, like comparing silk to wool. The variance in observations and conclusions is largely due to the technique used to examine this phenomena (Hilbert et al. 2003).
3. Rohan's Minimal salt media: 0.22 g  $(\text{NH}_4)_2\text{SO}_4$ , 1.20 g  $\text{K}_2\text{HPO}_4$ , 0.23 g  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ , 0.23 g dihydrate  $\text{CaCl}_2$  in 1 L distilled sterile  $\text{H}_2\text{O}$ .

## Suppliers

4',6-diamido-2-phenylindole (DAPI):  
Sigma-Aldrich. P.O. Box 14508, St. Louis, Missouri 63178. Tel.: 1-800-325-3010.  
<https://www.sigmaaldrich.com>.

DIFCO Nutrient Agar culture plate media, Tryptic Soy broth media, and Gram's stain: Becton Dickinson and Company, Sparks, Maryland 21152. Tel.: 1-800-675-0908. Worldwide to the US: 1-410-316-4000.

Thermanox Plastic Coverslips (NUNC Brand Products): VWR, West Chester, Pennsylvania 19380. Tel.: 1-800-932-5000.

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