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A STUDY OF BIODETERIORATION AND CHROMATIC ALTERATIONS OF PAINTED AND GILDED MUMMY CARTONNAGE AT THE SAQQARA MUSEUM STOREROOM, EGYPT*

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Microbial biofilms have developed on the surfaces and within the painted and gilded layers of mummy cartonnage at the Saqqara museum storeroom in Giza, Egypt. SEM–EDX, XRD and FT–IR–ATR techniques were applied to analyse the coloured and gilded materials, ground layer, textile support and binder used for the cartonnage. Aspergillus niger (24.8%), Penicillium chrysogenum (21.5%) and a novel cartonnage-biodegrading bacterium, Bacillus sonorensis (23.7%), were the most abundant microbes growing over the cartonnage surface. In addition, Aspergillus tamari (15.4%), A. fumigates (8.1%) and Fusarium solani (6.5%) were identified. The pigments comprised Egyptian blue (cuprorivaite), cinnabar (red), orpiment (yellow) and green pigment made from a mixture of cuprorivaite and orpiment. Gold leaf was used for the gilded layer, calcium carbonate and gypsum comprised the ground layer, gum arabic was the binding medium and the fibre base was a fine linen textile. Microbial colonization tests were performed on aged cartonnage replica samples made from linen and pigments of similar composition to ancient pigments found in the cartonnage. Each sample was inoculated separately with A. niger, P. chrysogenum and B. sonorensis. Yellow orpiment samples were the exception, as no colour change was detected after colonization by the examined micro-organisms.

KEYWORDS: BIODETERIORATION, CARTONNAGE, EGYPTIAN MUMMY, EGYPTIAN BLUE, CINNABAR, GOLD

INTRODUCTION

In ancient Egyptian funerary masks, cartonnage, a type of material made of layers of linen or papyrus and gesso, was used from the first Intermediate Period to the Roman era (Wright 1983; Scott *et al*. 2009). The painted layer of gesso—in some cases, partially gilded—with the organic materials (cotton, linen or wool); makes these masks vulnerable to microbial attack.

The support part of the mummy cartonnage consists mainly of cellulosic materials (textile or papyrus), and the binder material or the preparatory layer was manufactured from animal or plant glues (Abdel-Kareem 2010). Micro-organisms such as fungi (Abdel-Kareem *et al*. 1997; Szostak-Kotowa 2004) readily attack textiles, particularly those comprising natural organic fibres, such as cotton, linen and wool. Furthermore, the stratigraphic section of the technologies

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used to manufacture the materials in the ancient Egyptian cartonnage has been reported (Scott *et al*. 2003, 2009; Daniels 2007).

The pigments and binders of ancient Egyptian artefacts have been studied; for example, some pigments, grounds and media from Egyptian cartonnage fragments had been documented (Scott *et al*. 2003, 2004, 2009). The employed pigment colours for cartonnage [red (cinnabar or hematite), yellow (orpiment or goethite), blue (Egyptian blue), white (lead), black (charcoal black), green (Egyptian green or malachite—organic copper carbohydrate or proteinate green), gold etc.] have been reported (Green 1955; Nagashima *et al*. 1996; Goresy 1997; Uda *et al*. 2000; Yoshimura *et al*. 2002; Scott *et al*. 2004, 2009; Afifi 2011).

Previously, it has been suggested that alterations of Egyptian blue to a degraded green colour are related to the incorporation of organometallic compounds of copper, such as copper–proteinate, copper–carbohydrate and copper–wax pigments (Lee and Quirke 2000; Scott *et al*. 2003, 2004; Daniels 2007). Egyptian green may degrade to form another green-coloured compound comprising basic copper chloride (Schiegl *et al*. 1989; Lee and Quirke 2000).

The binding media of nine cartonnage samples were characterized and identified as egg white, animal glue and plant gum (Scott *et al*. 2009). Gum arabic was the most common binding medium used in ancient Egypt (Newman and Serpico 2000; Scott *et al*. 2004; Calza *et al*. 2007a,b). The use of beeswax as a binder for paint or as a protective coating has been reported from at least the 18th Dynasty onwards (Newman and Serpico 2000; Serpico and White 2000; Daniels 2007). Mixed media (gum arabic and coated wax surface) were also used in Egyptian art (Palet and Porta 1990; Scott *et al*. 2004).

Fruit (cherry or peach) and Egyptian acacia gums (gum arabic) are well documented as art materials readily available in ancient Egypt (Scott *et al*. 2009). Blue colourant was identified as cuprorivaite, yellow pigment as goethite and animal glue was used as a medium colouring for some cartonnage fragments obtained from Hawara, Egypt, in the Fayoum Excavation (Afifi 2011). The ground layers in the cartonnage were principally made up of calcite and gypsum, but mixtures of calcite and animal glue were also sometimes used as a preparatory layer in cartonnage painting (Lee and Quirke 2000; Heywood 2001; Eastaugh *et al*. 2004).

For cultural heritage conservators, fungal colonization of art remains a problem that requires treatment (Sterflinger 2010). Micro-organisms cause the biodeterioration of cultural heritage objects, ultimately resulting in aesthetic damage that includes pigment discolouration, staining and the formation of a biofilm on the painted surface (Gaylarde *et al*. 2011; Ljaljević-Grbić *et al*. 2013).

Cracking, disintegration of painted layers and the degradation of supported polymers or binders comprise structural damage that may lead to detachment of the painted layer from the cartonnage support (Ciferri 1999; Sakr *et al*. 2012, 2013). Deterioration may also be due to the acids and enzymes produced as a metabolic activity of micro-organisms (Abdel-Haleim *et al*. 2013). The poor coverage and transparency of Egyptian blue (cuprorivaite) contributes to the overall darkening that occurs (Daniels *et al*. 2003).

The presence of mitosporic fungi conidia in the indoor air of the museum (Florian 2002) might be attributed to the transport of their spores on the clothes of workers and visitors, through doors and windows (Niesler *et al*. 2000), or through the air-conditioning system (Ljaljević Grbić *et al*. 2008). Fungal infestation of art may affect all the objects in the museum and presents a health threat to conservators (Florian 2002; Ljaljević-Grbić *et al*. 2013).

Abdel-Kareem (2010) reported changes in the colour of linen textile samples following infestation by micro-organisms. Materials colonized by fungi usually exhibit changes in their chemical and physical characteristics (Florian 2002). In addition, the eventual formation of microbial biofilms leads to changes in reflectivity and texture after long-term exposure (Gaylarde *et al*.

2011). The random environmental conditions in the storage rooms of Egyptian museums ultimately stimulate fungal growth on textile objects (Florian 1997).

This study was aimed at identifying the fungi and bacteria colonizing painted and gilded mummy cartonnage at the Saqqara Museum Storeroom, Egypt, as well as the chromatic alterations as affected by the isolated micro-organisms. Various techniques, such as scanning electron microscopy – energy-dispersive X-ray spectroscopy (SEM–EDX), Fourier transform infrared spectroscopy – attenuated total reflectance (FT–IR–ATR) and X-ray diffraction (XRD) were used in the present study. Preservation treatment of the cartonnage included surface spraying with 70% ethanol as initial sterilization for 15 min, followed by cleaning and subsequent storage under the conditions of the Saqqara storeroom.

MATERIALS AND METHODS

Visual observation

During the excavation of Khalid Mahmoud in Gisr El-Mudir, Saqqara, Giza, Lower Egypt, in 2009, painted and gilded mummy cartonnage was found. This cartonnage mask was stored under the Saqqara Museum Storeroom conditions, with random temperature and relative humidity: it has the accession number 1 for 2009, and its dimensions are length 40 cm and width 25 cm.

Visual observation indicated extensive colour changes in the textile support with deteriorated fibres, which might be due to thermal degradation or biological infestation. Disintegration of the textile layers may result in severe damage to the layers of the colourful and gilded gesso (Figs S1 (a) and S1 (b)).

Microscopic examinations

The textile structure and degradation were examined by light microscopy (USB and Zeiss). SEM was used to determine the fibre thickness as well as the biodeterioration caused by the micro-organisms.

Isolation and identification of micro-organisms from the cartonnage

To identify the microbial genera that deteriorated the cartonnage pigment and support, samples from different five areas were taken by using sterile cotton swabs, as shown in Figure 1.

For fungi, microbial samples were cultured on Czapek–Dox agar plates (30 g sucrose, 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KC, 0.01 g $FeSO_4$, 15 g agar, distilled water 1000 ml at pH 7.3) and M40Y media (400 g sucrose, 20 g malt extract, 5 g yeast extract, 20 g agar). Streptomycin (10–50 µg L^{-1}) was used to inhibit the growth of actinomycetes and bacteria. The isolated bacterium was cultured on nutrient agar plates (5 g peptone, 3 g beef extract, 5 g NaCl, 20 g agar in 1 L distilled water, pH 7–7.1) and the plates were incubated for 3 d at 37 °C. The isolated bacterium was identified according to *Bergey's manual of systematic bacteriology* (Krieg and Holt 1984). Fungal isolates were identified morphologically according to previous reports (Raper and Fennell 1965; Ellis and Ellis 1997; Samson *et al*. 2004, 2010).

Pigment identification of cartonnage

Scanning electron microscopy was undertaken using a FEI Quanta 200 SEM FEG with an accelerating voltage between 10 and 15 kV, at various magnifications (Salem 2016).

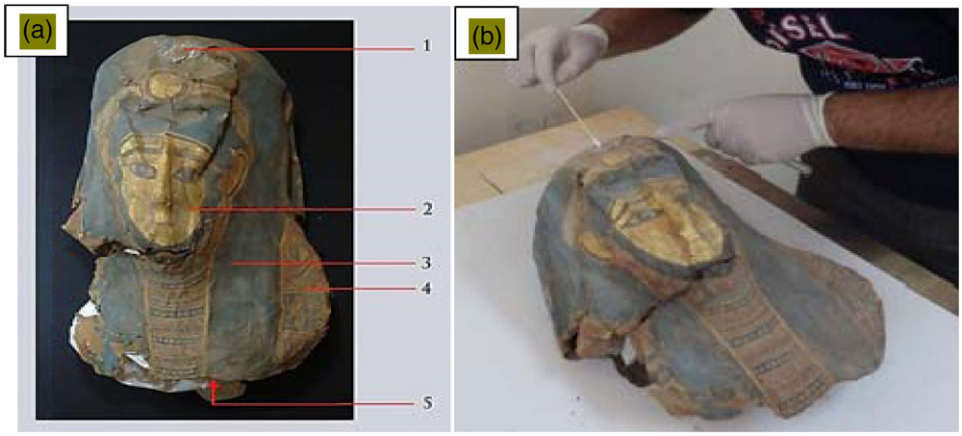


Figure 1 *Two views of the cartonnage sampling areas: 1, turquoise colour appears to be damaged; 2, gilded layer; 3, turquoise colour not damaged; 4, another colour (red); 5, inside the mask (textile). [Colour figure can be viewed at wileyonlinelibrary.com]*

Sample fragments were analysed by Fourier transform infrared spectroscopy – attenuated total reflectance (FT–IR–ATR), using a Bruker Vertex 70 FT–IR spectrometer equipped with a detector using an ATR crystal, which represents added scans (at 2 mm s^{-1}) in a spectral region ranging from 4000 to 500 cm^{-1}, with 4 cm^{-1} resolution.

The chemical compositions of coloured and gilded materials, the ground layer, the textile support, the binder and morphology of the pigments in the cartonnage fragments without damage were characterized by X-ray diffraction (XRD) (Afifi, 2011; Robador *et al*. 2016). Briefly, sample fragments were placed on glass slides and analysed using an X-ray diffractometer system (the PANalytical PW3040 pro model) with a Cu target tube and an Ni filter, at 40 kV and 30 mA (X'Pert HighScore).

Experimental samples designed to mimic the stratigraphic layers of the cartonnage were prepared. Samples were prepared using a linen support and a ground layer of calcium carbonate ($CaCO_3$) and gypsum ($CaSO_4.2H_2O$) in percentages of 63% and 14%, respectively, mixed with gum arabic. Pigments supplied by Kremer were used, including Egyptian blue ($CaCuSi_4O_{10}$, N.10060), cinnabar (HgS, N.42000) and orpiment (As_2S_3, N.10700). Gold leaf (2% gold), with the presence of silver and copper, was provided by the Nazionale Battitura Metalli s.r.l. in Italy. Gum arabic was provided by Sigma-Aldrich, Cairo.

Micro-organism inoculation of cartonnage samples

Under laboratory conditions, artificial cartonnage samples were prepared in 20 × 20 mm vortexes. Samples were sterilized by ultraviolet light exposure for 48 h ((Joseph *et al*. 2011). For the preparation of spore suspensions, 10 mL of sterilized distilled water was added to culture plates containing Czapek–Dox medium (7-day-old) and spores were spread using a camel hairbrush. Spore suspensions were then individually filtered through muslin and standardized to contain 1.2×10^6 spores per millilitre, using a hemocytometer slide.

The micro-organisms inoculated cartonnage samples separately, with the highest prevalence on the original cartonnage surface. Colonization was evaluated after 2 months in both standard and inoculated samples. Degradation and colour change on the surface were evaluated. SEM

was used to study the surface of the samples before and after colonization by micro-organisms. Changes in the elemental composition of the pigments were studied on both the inoculated and non-inoculated samples by EDX analysis: the micro-organisms use some elements, such as calcite, iron and copper, in order to grow (Mansour 2013). X-ray powder diffraction (Philips 1840, Cu tube) was used to study the chemical composition of the pigments.

Colorimetric measurements

Colour measurement was investigated using a NR-3000 Handy Colorimeter (Nippon Denshoku, Tokyo, Japan), calibrated with a standard whiteboard (D65/10, $X = 82.43$, $Y = 87.40$, $Z = 89.77$) to measure its $L^*a^*b^*$ values, and the mean of three replicates was calculated. The colour change values demonstrated by ΔL^*, Δa^*, Δb^* and ΔE^*, respectively, were measured according to the CIELAB equation (Bacci *et al*. 2003). The delta values (ΔL, Δa and Δb) indicate how much a standard and sample differ from one another in L, a and b, and are often used for quality control or formula adjustment (Bacci *et al*. 2003; Yusuf *et al*. 2012). Tolerances may be set for the delta values. Delta values that are out of tolerance indicate that there is too much difference between the standard and the sample.

***L* scale:** Light versus dark, where a low number (0–50) indicates dark and a high number (51–100) indicates light.

***a* scale:** Red versus green, where a positive number indicates red and a negative number indicates green.

***b* scale:** Yellow versus blue, where a positive number indicates yellow and a negative number indicates blue.

In the present study, a ΔE^* value of 5 was used as an initial threshold value, and was considered large enough to be detected by most observers. Changes in the colour values greater than 5 indicate an extreme colour difference (del Hoyo-Meléndez and Mecklenburg 2011; Yurdun *et al*. 2013).

RESULTS AND DISCUSSION

Technical study of the cartonnage

The type and structure of the fibre base or support textile, as well as the degree of damage, were recorded by light microscopy (Fig. S2). The textile structure can be observed in Figures S2 (b) and S2 (c). The structure, where the extracted flax fibres have thick secondary cell walls with tapered ends, has been identified previously (Borojevic and Mountain 2013). The severe damage on the surface of the fibre itself, as well as the beginning of the damage or splitting of the internodes, is shown in Figure S2 (d) (Abdel-Kareem and El-Nagar 2005), and causes disintegration of these technological layers. Changes in the textile colour from dark brown to black may be due to thermal degradation of the textile fibres or biological infection (Fig. S2 (a)) (Szostak-Kotowa 2004; Pekhtasheva *et al*. 2012). Previously, Szostak-Kotowa (2004) reported that microbial growth on textiles decreases the strength, and leads to elongation, discolouration and changes in appearance.

SEM examination

By means of the SEM examination, the thickness of the textile fibre was shown to range from 21 to 17 μm, which was similar to the characterized anatomical features of the flax plant (Fig. 2 (a)). The presence of acute microbial infection resulted in the emergence of spherical objects and fungal hyphae (Figs 2 (b) and 2 (c)) (Arshad and Mujahid 2011), and the characteristic cross-section that is distinctive for flax plants, as well as the appearance of biodeterioration of the fibre surface (Fig. 2 (d)).

FT–IR–ATR spectra

The FT–IR–ATR spectra of the ancient linen fibres, as well as those of the new linen fibres (experimental sample) (Fig. 3), revealed the presence of an OH group in the region of 3356.3 cm^{-1}, which represents an increase in the relative intensity of the OH group spectrum compared to the experimental linen sample (Abdel-Kareem and El-Nagar 2005). A C = O group at a wavelength of 1654.6 cm^{-1}, which was related to the stretching vibration of the carbonyl bonds, could correspond to soluble compounds with ketone or aldehyde functional groups (Ajuong and Breese 1998; Ajuong and Redington 2004; Salem *et al*. 2016). This wavenumber appeared with a strong

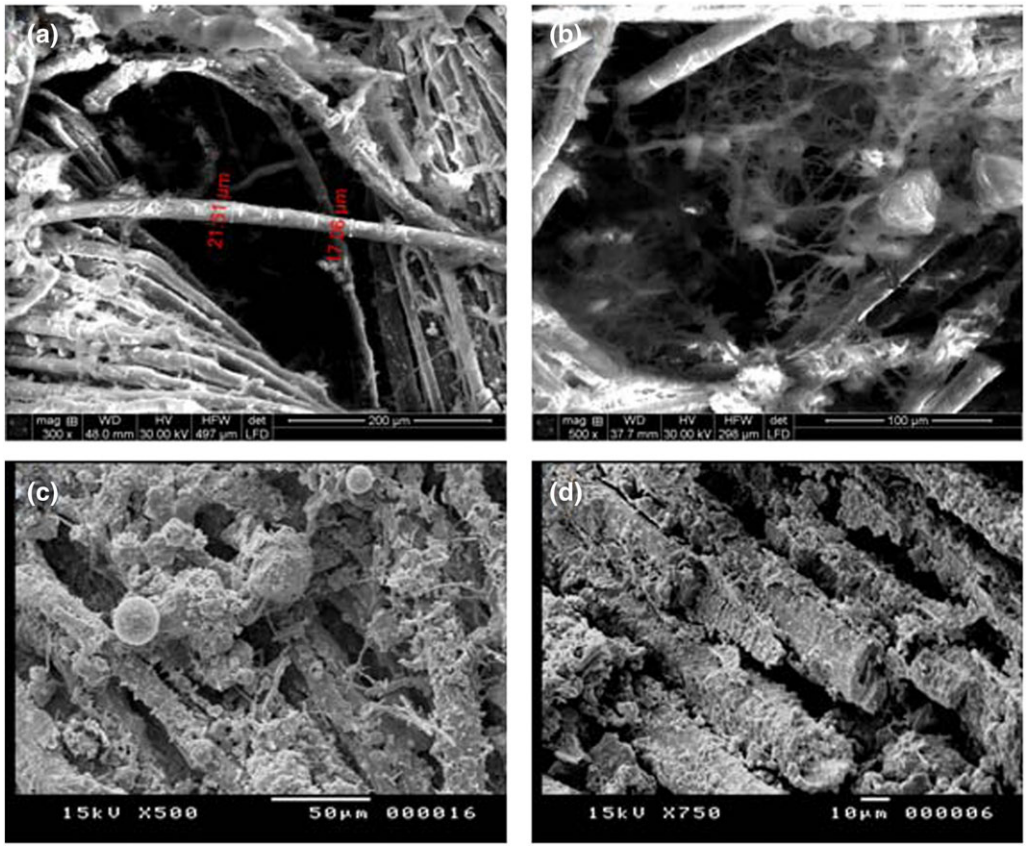
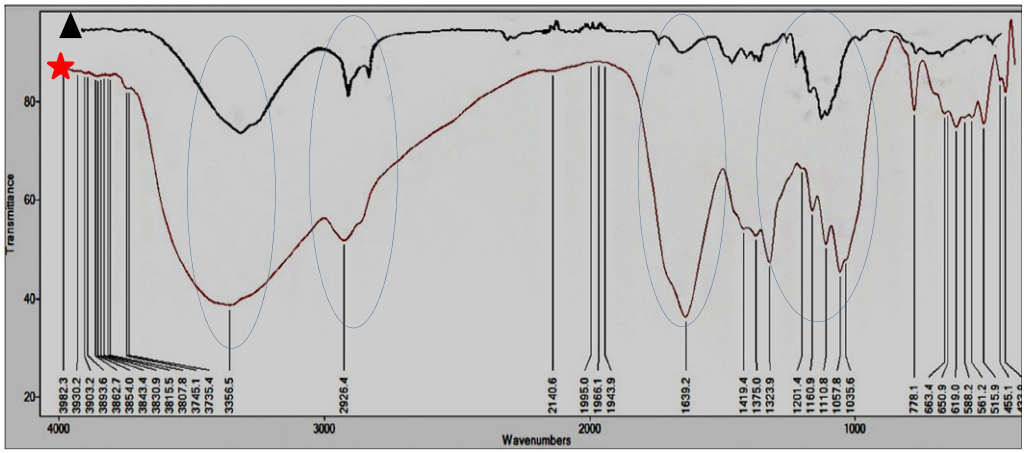


Figure 2 *SEM images of the linen fibre thickness (a) and the biodeterioration of textile fibres (b–d). [Colour figure can be viewed at wileyonlinelibrary.com]*



Ancient sample ★, Standard sample ▲

Figure 3 *FT–IR–ATR spectra of ancient and standard linen specimens. [Colour figure can be viewed at wileyonlinelibrary.com]*

intensity in the ancient damaged sample and this absorption band was also observed in the experimental sample, but in a relatively weak form, resulting in damage to the linen and degradation (Arshad and Mujahid 2011). The functional groups showed strong relative intensities at 1372 cm^{-1}, 1336 cm^{-1}, 1313 cm^{-1}, 1280 cm^{-1}, 1160 cm^{-1} and 1105 cm^{-1}, which indicated a shift in the cellulose polymer from high-crystalline to amorphous regions. Subsequently, these changes suggested that the archaeological samples were degraded compared with the experimental sample, and these results were consistent with those reported by Hulleman *et al*. (1994) and Arshad and Mujahid (2011). Our results indicated that linen textile is the fibre material used for the cartonnage layers, and this finding is consistent with those of Scott *et al*. (2009).

XRD spectra of cartonnage fragments

The results of the XRD spectra indicated that the studied cartonnage fragments comprised Egyptian blue pigment (cuprorivaite, $CaCuSi_4O_{10}$) (Bianchetti *et al*. 2000; Mazzochin *et al*. 2004; Scott *et al*. 2009) and red pigment (cinnabar, HgS), mixed with hematite (Fe_2O_3) and yellow pigment (orpiment, As_2S_3) (Scott *et al*. 2009), bound in gum arabic and applied to linen grounded with a mixture of calcite and gypsum. The XRD results showed the presence of cuprorivaite ($CaCuSi_4O_1$), calcite ($CaCO_3$), cuprite (CuO), silica (SiO_2) and atacamite [$(Cu_2Cl.(OH)_3$] as the main chemical components of the light blue pigment, Egyptian blue (Fig. S3 (a)). Cuprorivaite, calcite and silica were found in the dark blue pigment (Fig. S3 (b)). Calcite, anhydrite $CaSO_4$, HgS and hematite Fe_2O_3 were combined for the red pigment (Fig. S3 (c)). The yellow pigment consisted of calcite, orpiment As_2S, geothite and silica (Fig. S3 (d)). The green pigment (Fig. S3 (e)) comprised a mixture of Egyptian blue and orpiment, and silica, calcite and wollastonite were also found. Wollastonite ($CaSiO_3$) is the distinctive compound for Egyptian green pigment. Gold leaf was used for the gilded layer (Fig. S3 (f)) and consists mainly of gold and calcite.

The presence of cuprite here should be noted because Egyptian blue is a synthetic pigment of quartz, a copper source (cuprite used here), calcite and flux (alkali flux or wood ashes), made by

mixing and heating these substances together at around 850–950 °C, and the heating was not completed. Additionally, atacamite was found because the mask was discovered in soil that was rich with NaCl, which interacts with Egyptian blue to give atacamite. The green pigment was found here because the Egyptian blue compounds had been heated to a high temperature in excess of 1000 °C.

Isolation and identification of fungi and bacteria from cartonnage

The results indicated that the identified micro-organisms were related to the isolated fungi *Fusarium solani*, *Aspergillus fumigatus*, *A. tamarii*, *A. niger*, *Penicillium chrysogenum* and *Cladosporium* sp., and the isolated bacterium *Bacillus sonorensis* (Table 1 and Fig. S4). *A. niger* (24.8%), *P. chrysogenum* (21.5%) and the isolated bacterium *B. sonorensis* (23.7%) were the most common micro-organisms found on the cartonnage surface. *A. tamari* was found less frequently (15.4%), and the occurrence of *A. fumigates* (8.1%) and *F. solani* (6.5%) was low. These results support those of previous studies, where the most dominant fungi on the studied textile samples were *Alternaria*, *Aspergillus*, *Chaetomium*, *Penicillium* and *Trichoderma* species, which can grow in relatively dry conditions compared with other cellulolytic fungi (Abdel-Kareem 2010).

Previously, it was reported that the most dominant fungi found on textile fabrics from the Egyptian museum was *Aspergillus*, followed by *Penicillium* (Abdel-Kareem 2010). Also, Ljaljević-Grbić *et al*. (2013) reported that the prevailing fungal species documented in the air of the quarantine room of the Cultural Center of Belgrade was *A. niger* (62.5%), while the fungi presented on wooden substrata and photographs were *Absidia*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Neurospora*, *Penicillium*, *Rhizopus*, *Syncephalastrum*, *Trichoderma*, *Fusarium*, *Humicola*, *Paecilomyces* and *Ulocladium*.

In the present study, *A. niger* was found as most dominant fungi growing on the cartonnage surface, where this fungus produces small globose or sub-globose conidia (5 μm in diameter) that are easily dispersed through the air and settle on different surfaces (Florian 2002). *A. niger* is a common contaminant on various materials, which produces naphtho-γ-pyrones and malformins, toxic secondary metabolites (Florian 2002; Samson *et al*. 2004), as well as the allergens Asp n 14 and Asp n 18 (Knutsen *et al*. 2011).

Study of the colonization of laboratory samples

The experimental samples were inoculated separately with *A. niger*, *P. chrysogenum* and *B. sonorensis*, the most prevalent micro-organisms detected on the cartonnage surface. Visual

Table 1 *The identified fungal and bacterium isolates*

Micro-organism growth on Czapek–Dox agar media	*Average of frequent occurrence (%)*	*Micro-organism growth on M40Y media*	*Average of occurrence (%)*
Fusarium solani	6.5	*Aspergillus niger*	23.4
Aspergillus fumigatus	8.1	*Penicillium chrysogenum*	22.6
Penicillium chrysogenum	21.5	*Cladosporium* sp.	10.8
Aspergillus tamarii	15.4	*Aspergillus fumigatus*	15.3
Aspergillus niger	24.8	*Aspergillus terres*	8.2
Bacillus sonorensis	23.7	*Bacillus sonorensis*	19.7

examination revealed that the colonization by fungi on samples occurred after 2 months and affected colour changes in Egyptian blue, cinnabar and gold in comparison with control samples. Yellow orpiment samples were the exception, as no colour change was detected after colonization by the examined micro-organisms (Fig. 4).

Table S1 presents the elemental analysis performed using EDX to study the changes in chemical composition of the prepared pigment samples (Egyptian blue, cinnabar, orpiment and gold) with a composition similar to that of the cartonnage pigments for the colonizing test. All the samples were characterized by a predominant development of fungal biofilms and bacteria. The typical colour changes were observed over the surface of all cartonnage samples. The original subaerial biofilm, observed using the USB microscope, contained diverse microbial structures in close contact with the material of the paint layer. Fungal hyphae grew abundantly, in the form of a black pigmentation, and spread over the Egyptian blue, cinnabar and gold samples.

The painted layer surface contained numerous fungal filaments and fungal spores. In general, the colour changes and alterations of the painted layer surface were caused by a complex subaerial biofilm. Microscopic examination, however, revealed a consistent change in the colour of the sample surface. The chains of fungal spores remained attached to the material, suggesting the high propagative and spreading potential of the micromycetes.

Colonizing fungi, including *Aspergillus*, have long been considered the major deteriogens of painted surfaces (Winters and Guidetti 1976; Grant *et al*. 1986; Bravery 1988). *B. sonorensis* on the surface can be observed by the consistent colour changes in the surface sample. Pigments and binders of ancient Egyptian artefacts have been documented to deteriorate and change over time (Scott *et al*. 2009). The present investigation revealed that yellow orpiment (Fig. S5) resisted infection by micro-organisms in all samples, which may be due to the presence of

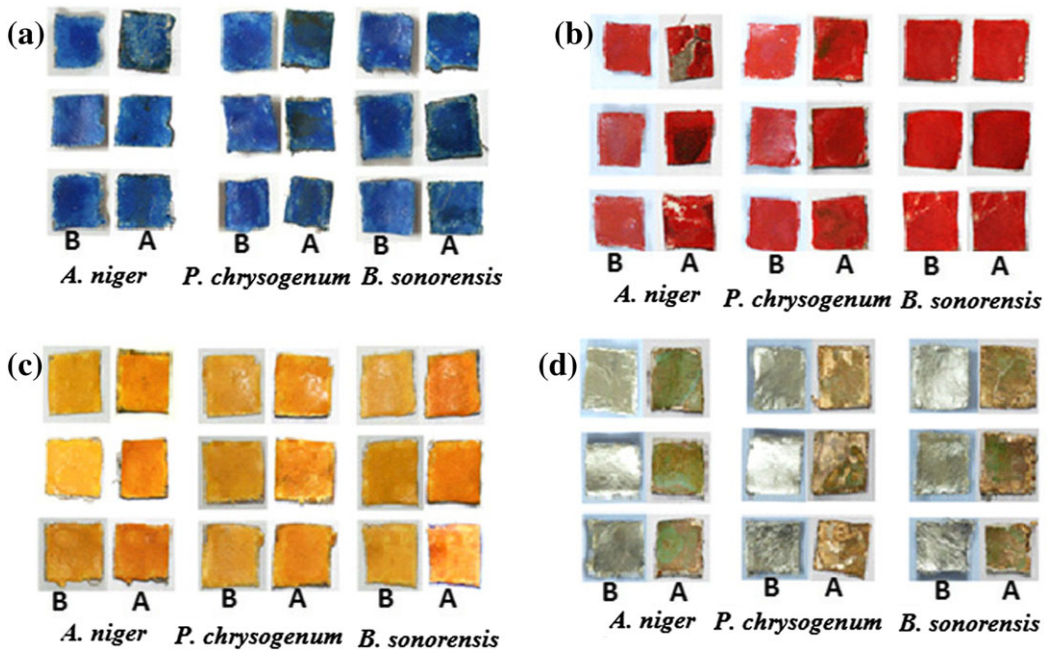


Figure 4 *Egyptian blue (a), red cinnabar (b), yellow orpiment (c), and gilded layer (d) samples before (B) and after (A) infection with fungi and the isolated bacterium. [Colour figure can be viewed at wileyonlinelibrary.com]*

arsenic ions that have a high toxicity to most micro-organisms (Garg *et al.* 1995; Albarracín *et al.* 2005).

Alterations of chromatic parameters caused by micro-organisms

Table 2 shows that the chromatic parameters of the Egyptian blue sample became darker due to colonization by the fungus *A. niger* (standard sample $L^* = 44$, $a^* = 22.9$, $b^* = -50.5$, inoculated sample $L^* = 33.7$, $a^* = 16.2$, $b^* = -33.9$, with $\Delta E = 5.8$). Furthermore, a shift towards green occurred in the samples colonized with *P. chrysogenum* (standard sample $L^* = 39.1$, $a^* = 23$, $b^* = -50.6$, inoculated sample $L^* = 38.2$, $a^* = 7.6$, $b^* = -20.6$, with $\Delta E = 6.8$). Additionally, the isolated bacterium *B. sonorensis* caused a change to a black colour (standard sample $L^* = 39.5$, $a^* = 67.2$, $b^* = -49$, inoculated sample $L^* = 37.3$, $a^* = 19.3$, $b^* = -44$, with $\Delta E = 3.3$). Decreases in blue colour for Egyptian blue pigment were observed (Δb^*) as affected by *A. niger* and *P. chrysogenum*.

The chromatic parameters of the red cinnabar sample were shifted to a darker colour with samples inoculated by *A. niger* (standard sample $L^* = 51.58$, $a^* = 71.9$, $b^* = 36.3$, inoculated sample $L^* = 39.9$, $a^* = 56$, $b^* = 25.3$, with $\Delta E = 6.5$). Samples inoculated with *P. chrysogenum* became more green (standard sample $L^* = 50.5$, $a^* = 66.1$, $b^* = 29.5$, inoculated sample $L^* = 45.6$, $a^* = 42.5$, $b^* = 27.6$, with $\Delta E = 5.2$), while the isolated bacterium *B. sonorensis* caused a change to a black colour (standard sample $L^* = 52$, $a^* = 67.2$, $b^* = 33.8$, inoculated sample $L^* = 52$, $a^* = 64.6$, $b^* = 29.7$, with $\Delta E = 2.4$). The black colour was due to inoculation with *A. niger* and the darkening of organic materials used as binder media (gum arabic), while the shift towards green was due to the green colour of *P. chrysogenum* (Holt and Macdonald 1967).

The yellow orpiment sample showed no change in the chromatic parameters, as it is resistant to fungal activity, but the bacterial damage was unverified. Orpiment is damaged by other factors such as the influence of light or particular environmental conditions. Gilded samples exhibited changes in the chromatic parameters towards a lighter tone, and a shift towards green occurred in samples infected with *A. niger* (standard sample $L^* = 75.1$, $a^* = -16.2$, $b^* = 26.1$, inoculated sample $L^* = 63.5$, $a^* = -23.3$, $b^* = 29.1$, with $\Delta E = 4.8$).

Table 2 *The chromatic parameters measured for the samples in the L*a*b* (CIE 1976) colour system*

		Micro-organisms											
		Aspergillus niger				*Penicillium chrysogenum*				*Bacillus sonorensis*			
Colour		*L**	*a**	*b**	*ΔE*	*L**	*a**	*b**	*ΔE*	*L**	*a**	*b**	*ΔE*
Egyptian	S*	44	22.8	−50.5	–	39.1	23	−50.6	–	39.5	22.5	−49	–
blue	E	33.7	16.2	−33.9	5.7	38.2	7.6	−20.6	6.8	37.2	19.3	−44	3.3
Red	S	51.6	71.9	36.3	–	50.5	66.1	29.5	–	52.	67.2	33.8	–
cinnabar	E	39.9	56.01	25.3	6.5	45.6	42.5	27.6	5.2	52	64.6	29.7	2.4
Yellow	S	78.9	19.3	70.1	–	79	19.9	68.7	–	78.5	20	66.8	–
orpiment	E	77.5	19.3	70.1	1	78.2	18.7	67.4	1	78.7	20.1	66.3	1
Gilded	S	75.1	−16.2	26.1	–	72.7	−13.2	20.1	–	78.2	−10.1	26.6	–
layer	E	63.5	−23.3	29.1	4.8	60.5	4.7	23.5	4.9	62.6	−17.2	30	5.2

*S, standard sample; E, infected sample.

A change in the redness of the gilded layer occurred in samples inoculated with *P. chrysogenum* (standard sample $L^* = 72.7$, $a^* = -13.2$, $b^* = 20.1$, inoculated sample $L^* = 60.5$, $a^* = 4.7$, $b^* = 23.5$, with $\Delta E = 4.9$). Furthermore, a change in the green colour was observed in samples inoculated with the isolated bacterium *B. sonorensis* (standard sample $L^* = 78.2$, $a^* = -10.1$, $b^* = 26.6$, inoculated sample $L^* = 62.6$, $a^* = -17.2$, $b^* = 30$, with $\Delta E = 5.2$), which could be related the corrosion of the lowest noble metals in the electrochemical series as well as the degradation of the gum arabic that was used as the paint binder. Gum arabic can discolour sufficiently to cause the paint to appear black.

By considering the variations in the L^*, a^* and b^* values in Table 2, it can be observed that the larger colour change was found with Egyptian blue and red cinnabar pigments as inoculated with *A. niger* and *P. chrysogenum*.

From the above results about the alterations of chromatic parameters caused by micro-organisms, the changes in colours could be related to deteriorations in linen textile after incubation with the micro-organisms (Abdel-Kareem 2010). The eventual formation of the thin microbial biofilm, whether thin (in the micrometre range) or thick (1 mm or more), leads to changes in colour and properties such as reflectivity and smoothness of the object over the long term (Gaylarde *et al*. 2011).

Preservation treatment

As previously stated, since the historical textiles in Egypt are more acidic due to the environment, which makes the conditions more favourable for fungal growth (Abdel-Kareem 2002), the surfaces of the mummy cartonnage were cleaned up by spraying with 70% ethanol as a sterilize agent for 15 min (Smith *et al*. 2011), prior to storage under the conditions of the Saqqara storeroom. This treatment does not affect the natural pigments and the mainly preventive environmental control that is recommended.

CONCLUSIONS

This study has examined colour changes that were caused by fungal infestation. In addition, micro-organisms isolated from archaeological cartonnage were examined to understand the colour changes that may occur in ancient cartonnage in Egypt. Artificial cartonnage samples inoculated separately with *A. niger*, *P. chrysogenum* or *B. sonorensis*, the most prevalent microbes on the cartonnage surfaces, exhibited colour changes in the surface pigments (Egyptian blue, cinnabar and gold) compared to control samples after 2 months. Yellow orpiment samples were the exception, as no colour change was detected after colonization of the identified micro-organisms.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. EDX Elemental analysis of different pigments from the investigated cartonnage

Figure S1. Showing the textile support from inside (a) and the degradation of the textile support and its effects on the gesso layers (b).

Figure S2. Textile support damage and darkened pigment (a) (Zeiss stereomicroscope 10X); the simple installation of textile structure (b) (USB Microscope 100X); the direction of the fiber, a trend twirl-twirl S (c) (USB Microscope 50X); The tissue was identified using a Zeiss stereomicroscope (250X) as linen fibers because of the cross markings, known as fiber "nodes", with serious surface damage to the fiber as well as the beginning of damage inside the fiber itself through the nodes (d).

Figure S3. XRD spectra of chemical composition of light blue pigments (a); dark blue pigments (b); Red pigment (c); Orpiment (yellow pigment) (d); Green pigment (e); Gold layer (f).

Figure S4. The identified fungal strains; (a) Cladosporium sp., (b) Fusarium solani, C- Aspergillus tamarii, (d) Aspergillus niger, (e) Aspergillus terres, (f) Aspergillus fumigatus, and (g) Penicillium chrysogenum.

Figure S5. USB microscope images (150X) of the surface of cartonnage containing red cinnabar, Egyptian blue, gilded layer, and yellow orpiment before (control) and after infection with Aspergillus niger, Penicillium chrysogenum, or Bacillus sonorensis.