The Bacteriological Contamination of Archaeological Ceramics: an Example from Pachacamac (Peru)

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Abstract
This paper concerns the efflorescence of bacteria on ceramics from archaeological excavations. Biodegradation was due to Streptomyces Sp., a bacterium of the Actinomycetale order. After analysis of each contaminated object, its location in the storeroom, and our conservation materials, it is suggested that the origin of the bacteria is in the soil of the archaeological site. Microclimatic conditions and organic nutrients from the soil create a favourable atmosphere for the development of bacteria inside the storeroom. With regard to conservation treatment, the desalination process does not prevent the emergence of bacteria but appears to restrict it considerably. The application of the biocide Biotin R at 2%, diluted in ethanol, has been successful: there has been no recurrence of the bacteria.

Fig. 1. Excavations of Cemetery 1 at Pachacamac (Photo: P. Eeckhout).

Introduction

Pachacamac is a monumental coastal site in the Central Andes that reached its apogee after being incorporated into the Inca Empire (figure 1). It is situated half a kilometre from the Pacific Ocean, near the mouth of the Lurín River. It covers about 600 hectares (2.31 square miles), of which one third is occupied by the monumental sector.

The U58' section of the Cemetery 1 excavated by the Ychsma Project was heavily utilised for interments from the 10th to the end of the 14th century A.D. (i.e. the Late Intermediate Period or LIP), leading to disturbance of earlier layers. In terms of stylistic/relative dating, we have funeral contexts displaying cultural markers that span the Middle Horizon (ca A.D. 700–1000) and part of the LIP, the latter corresponding to the Epigonal and Early/Middle Ychsma (Feltham and Eeckhout 2004; Vallejo 2004).

The Ychsma ceramic style includes several successive phases. Our typological classification is based on form, ware, and decoration. We distinguish three main local ware categories: Orange, Brown, and Black.

The surface of **Orange ware** has a few-mm-wide, moderate orange-pink (10R 7/4 on the Geological Society of America rock-colour chart [GRCC 2009]) paste next to a few-mm-wide, pale-red (10R 6/2) outer paste. Microscopical examination shows that the temper is polylithic with granite and fine-grained volcanics. A pale-coloured, very clean clay carries abundant, rounded to sub-rounded single grains of quartz, as well as zoned plagioclase, potassium feldspar including perthite, together with lesser amounts of zoned brown amphibole and clinopyroxene and trace amounts of sphene. The ware carries clasts from a medium-grained granite including quartz-plagioclase, potassium feldspar-plagioclase intergrowths. Internally fine-grained altered/weathered volcanics include much chert (some with quartz or feldspar microphenocrysts), 'trachyte', and felted feldspathic rocks, as well as rare sandstone. Voids in the clay are partially infilled with micritic carbonate.

The surface of **Brown ware** is a moderate brown (5YR 5/4) within a 1-mm-wide, discontinuous black (N1) rim. Microscopical examination shows that the temper is almost monolithic in its non-plastics. A dirty clay carries abundant, sub-rounded to sub-angular single grains of quartz, plagioclase (some altering to fine-grained white mica), and potassium feldspar, together with minor amounts of green and green-brown pleochroic amphibole and trace amounts of sphene and epidote. Other rock clasts are rare but include spherulitic rhyolite.

The surface of **Black ware** is a uniform medium-grey (N 5), with a darker rim that is less than 1 mm wide. The raw materials are similar to Orange ware, but black ware pots are made either of a fired untempered dirty clay or a polylithic sand temper added to a poorly cleaned/uncleaned clay.

Since 2005, the Ychsma Project's conservation team has been handling the treatment and preventive conservation of archaeological objects removed from the excavation. These are mainly ceramics, gourd, wood, metal, bone, and textiles. The team's tasks are to help on the excavation with the extraction of fragile pieces, to work in the laboratory on conservation and restoration treatment, and to make the storeroom at the Pachacamac Site Museum (MSPACH) fit for the long-term storage of objects. The conservation season lasts between one and three months.

Sampling

The selected group of samples includes 215 complete pottery vessels. These belong to the set of 254 archaeological objects that received conservation treatment between 2005 and 2011. 92.5% of the samples come from Unit 58'. The collection has been kept in the Ychsma Storeroom at the MSPACH.

After excavation, the archaeological material is taken to the registration area, where ceramic vessels (whole or fragmented) are separated from the remaining potsherds. The vessels pass to the conservation laboratory where they receive treatment; this consists in most cases of mechanical cleaning, desalination with deionised water, and the reassembling of

Fig. 2. Vessel storage in a cardboard box, Ychsma Storeroom 2010 (Photo: K. Colonna-Preti).

fragments. The remaining potsherds are washed with tap water, and then the diagnostic sherds are selected and receive a desalination treatment.
The vessels are kept on polyethylene foam stands and protected with bubble wrap; potsherds are stored in polyethylene bags (figure 2). All the pieces are placed in cardboard boxes, which are then arranged on the shelves of the storeroom with the rest of the archaeological material. Until 2011, the pieces were stored in 35 boxes. In that year, the ULB handed over the archaeological material to the MSPACH. At that time, the vessels were removed from the boxes, placed on shelves, and displayed in the same way as the rest of the pieces in the museum storerooms.

Biodeterioration

Description and Progression of Biodeterioration

It was in 2008 that we first observed an alteration to the surface of ceramic material; this consisted of small, white, round floccose spots, between 1 and 2 mm in diameter (figure 3). Despite the whitish colour, the spots can be distinguished from a saline efflorescence, which forms a more uniform veil whose crystals can be recognized. In that year, three vessels seemed to be affected. All of them came from the same archaeological unit (U58'), excavated in 2004 and 2005. They had been kept in individual bags and stored in two different boxes. None of the vessels had been desalinated. We carried out a desalination treatment and subsequent cleaning with ethanol (see table 1).
There was no conservation season in 2009, but an assistant monitored our collection. Six vessels were found to have been affected by the spots (figure 4), one of which had already been affected previously and been given treatment. All came from U58' and were excavated during the 2005 and 2008 field seasons. The vessels had been kept in three different boxes, one of which had already contained contaminated vessels. Five of the vessels had been desalinated. At that point, our investigations commenced with a view to identifying the micro-organisms responsible for the spots and to decide on the appropriate treatment.
The number of contaminated objects had already increased to 38 by the time we were able to start work on them in the following season. The vessels came from three units: U58', U89, and U94, excavated in 2004, 2005, and 2008. They had been kept in 11 different boxes, four of which already contained affected vessels. Two vessels had been desalinated. We washed the objects with tap water, desalinated them, and treated them with the selected biocide product.
In 2011, five new objects were affected: four came from U58', which had been excavated in 2004, 2005, and 2008. The vessels were kept in four different boxes, one of which

Fig. 3. Small white spots on ceramic observed in 2008 (Photo: K. Colonna-Preti).

Fig. 4. White spots observed on pottery in 2009 (Photo: K. Colonna-Preti).

had already been affected; none had been desalinated. We treated the affected vessels with the biocide as well as treating those that had been stored in the affected boxes.
In 2012, after monitoring the entire group of samples, it was observed that any vessel from the Ychsma Storeroom showed spots. Although the pottery of the 2012 season does not belong to the samples previously analysed, it is relevant to note that we found a bag with some diagnostic sherds with white spots (figure 5). These had not been previously analysed; however, they are very similar to the identified bacteria. The sherds come from U58', and they had only been washed with tap water. Following desalination, the fragments were treated with biocide. It is noteworthy that this 2012 material had never been in contact with the sample analysed previously, because in that year we installed our laboratory in Puente de Lurín, 5 km from MSPACH.

	2008	2009	2010	2011
Affected objects	3	6	35 (+6 already affected in 2009)	5
Boxes whose contents have been affected	2	3	8 (+3 already affected in 2009)	4
Desalinated objects	0	5	2	0
Treatment	ethanol	none (no conservation season)	Biotin R 2%	Biotin R 2%
Recurrence	-	1 (treated with ethanol)	1 (treated with ethanol)	0
Archaeological Unit	U58': 3	U58':6	U58':33, U89:1 U94:1	U58':4 A2:1
Field Season	2004, 2005	2005, 2008	1999, 2004, 2005, 2008	2004, 2005, 2008

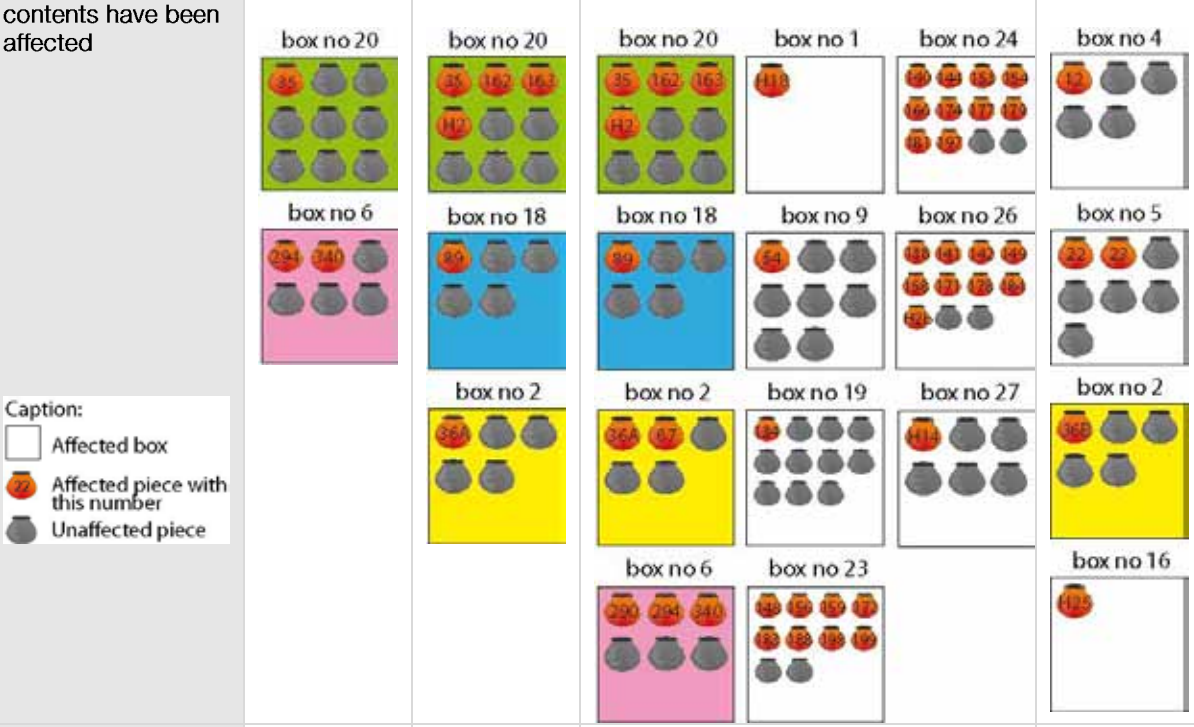

Table 1. Summary of the provenance of objects and biodeterioration progression.

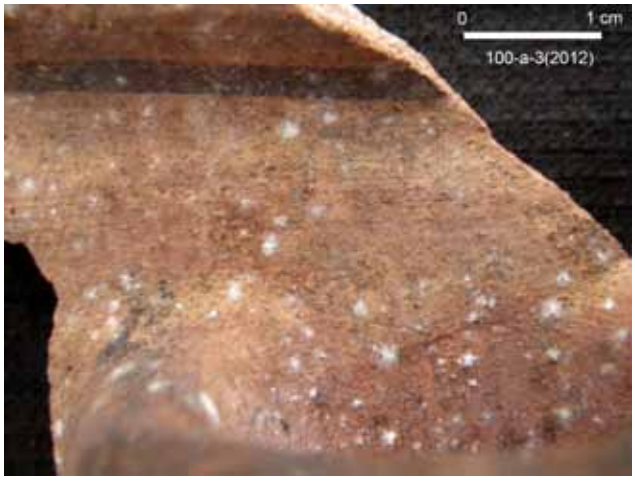

Fig. 5. Small white spots on ceramic observed in 2012 (Photo: K. Colonna-Preti).

Identification of Micro-organisms

Initially the appearance of the micro-organisms made us think of a fungal contamination. The microscopic observation of the samples in the Mycothèque of the Université Catholique de Louvain (MUCL), in 2009, determined that the colonies were not created by fungi, but exhibited the typical morphology of actinomycetes.

The Microbiology Laboratory at Ghent University (LMG) isolated five dominant colony types using the following method. The material was placed in an LMG medium no. 185 – TSA – and incubated aerobically at 28°C for several days. For the initial analysis, a fatty acid gas chromatography was used. The profiles were identified with the Microbial Identification System (MIDI Inc., Newark, DE, USA), using the TSBA50 database. The results indicate that three colonies, whose colour ranges from pale to dark beige, belong to the genus *Bacillus*, and one yellow colony belongs to the *Microbacteriaceae* family. The fifth type, a white colony similar to the spots observed on the original material, was identified in a second analysis after being isolated in actinomycetes isolation agar (AIA) and incubated at 30°C for several days. The results obtained with a partial 16S rDNA sequence analysis for the two strains indicate that they belong to the genus *Streptomyces*, of the Actinomycetale order of bacteria.

According to M. Pennincks (Unit of Microbial Physiology and Ecology – ULB), the principal biodeterioration is caused by those actinomycetes (personal communication 23/9/2009).

Biocide Treatment

With these results in mind, we carried out an investigation to determine the most appropriate treatment. Several practical limitations restricted our research: first, the difficulty of accessing the collection because of the short duration of our field seasons, which prevents us from making regular observations, from sampling at will, and from carrying out in situ research. Furthermore, because the next field season was almost upon us, it was urgent to find a speedy solution since we feared an uncontrolled contagion. Hence we concentrated on finding a biocide treatment to eliminate micro-organisms while making an in-depth study of contamination.

Searching through relevant literature, we found several biocides whose action is effective against bacteria (Borgioli, De Comelli, and Pressi 2006; Borgioli, Pressi, and Secondin 2003; Gabrielli and Zander 2007). Biotin S, a biocide from C.T.S. brand, seemed to be a good option because of its antibacterial properties, its broad spectrum of action and its resistance over time. This tin-derived compound contains heavy metals and is likely to be banned in the future because of its toxicity for the environment (Borgioli, De Comelli, and Pressi 2006). Upon the acquisition of the product, the supplier (C.T.S.) ceased to market it. They proposed a substitute: Biotin R, an iodopropynyl butylcarbamate (IPBC) and octylisothiazolinone (OIT)-based compound. The active ingredients of IPBC and OIT are known for their antibacterial and fungicidal efficacy and are used as preservatives for many products such as varnishes, plastics and wood. In conservation and restoration, they have been used against bacteria and other types of biodeterioration with good results (Bartolini, Pietrini, and Ricci 2007). The concentrated product is a thick yellowish liquid, not self-flammable, insoluble in water, and soluble in alcohol, ether, and aromatic and aliphatic hydrocarbons; it has been applied in a diluted form on numerous monuments (C.T.S. 2009).

In 2010 and 2011, after removing the white spots with water and a brush, and desalinating the pieces, we applied Biotin R at 2% in ethanol with a brush. So far we have not observed a recurrence of bacteria on the objects treated in that way.

Results and Discussion

After four years of study, we herewith propose an initial hypothesis about the origin of micro-organisms, the factors leading to their development, and the treatment that should be applied.

Origin

We have identified four possible sources of micro-organisms: the archaeological soil, the water used for washing and desalination, the cardboard boxes, and the air in the MSPACH storeroom.

With regard to the archaeological soil, it has been noted that the first three vessels with bacteria come from U58', as do 93.9% of the contaminated pieces. This high percentage is not surprising considering that 92.5% of the sample comes from this unit. In 2012, four years after the last excavation, we are still finding affected sherds from that same unit. Hence, an analysis of the soil in the unit U58' will be a priority for the next season.

With regard to the water, it has been noted that bacteria first appeared in three vessels that had been washed with tap water, but not desalinated. However, a minority (30.6%) of the affected vessels were washed with tap water in different seasons. If the contamination source comes from the water distribution system, it would mean that the water has been contaminated for several years and affects the MSPACH and Puente de Lurín. Yet it is unlikely that bacteria come from deionised water (coming from an external laboratory), since the first affected vessels and those from 2012 had not been desalinated. Interestingly, biodeterioration tends to appear on undesalinated vessels (81.6%). Ongoing water analysis will allow us to test these hypotheses.

With regard to the boxes, they were made to measure from re-used cardboard, as it is common in the Peruvian archaeological field. Until recently, we did not consider the boxes as a contamination source, which is why we did not replace them. In 2011, with the new display of the objects, the boxes were removed. As biodeterioration appeared in the material excavated in 2012 that was never stored in boxes, we think that the containers are not a source of contamination. Nevertheless, this hypothesis will have to be confirmed by the analysis of the remaining boxes.

In 2011 and 2012, we examined another storeroom within MSPACH, located in the same building as the contaminated Ychsma Storeroom. We did not observe any biodeterioration similar to the kind found in our collection. The fact that we find the same biodeterioration in material excavated in 2012, which has never been stored in the MSPACH, makes us think that the contamination does not come from the museum storerooms themselves, because it seems to be limited to the Ychsma Storeroom.

An examination of the literature reveals that actinomycetes, especially *Streptomyces*, have been identified in other items that are part of cultural heritage, such as wall paintings (Karbowska-Berent 2003; Karpovich-Tate and Rebrikova 1990) and stone (Abdulla and others 2008; May and others 2000). They often occur in a subterranean environment (Akatova, Gonzalez, and Saiz-Jiménez 2007; Groth and others 1999). However, up to now we have not found instances where they have been identified on ceramics.

The actinomycetes are a successful group of bacteria found in a multiplicity of natural and man-made environments. They are an important component of the microbial population in most soils. Among the genera, isolated *Streptomyces* are the most numerous. Regarding its presence in soil, Goodfellow and Williams (1983, p.195) mention: 'Streptomycetes spores can be dispersed above the soil when soil aggregates are disturbed by wind or rain, whereas dispersal within soil is assisted by water movement and arthropods'. Actinomycetes are also found in aquatic environments: 'There is little to suggest that any actinomycete has become specifically adapted to living in the freshwater ecosystem. However, there is some evidence that they can become active in such habitats in the presence of suitable substrates and favourable conditions for growth. Actinomycetes have also been detected in water distribution systems, where organic debris and well-oxygenated water can allow growth and production of taints' (Goodfellow and Williams 1983, p. 201).

At the present stage of our analysis, our conclusions suggest that the *Streptomyces* responsible for the biodeterioration come from the soil of U58', which has been excavated from 2004 onwards. This unit contains a cemetery with numerous organic remains. Its underground environment is conducive to bacterial growth, as has been observed in other cultural hypogea. However, at present we cannot rule out contamination from tap water, until we have made further analysis.

Growth Factors

Observations on the occurrence of micro-organisms in the Ychsma Storeroom indicate two possible methods for its transmission: either objects within the same box become contaminated and then the contamination is transferred from one box to another, or bacteria occur in a latent state in all the ceramic remains. Both possibilities might occur in tandem.

With regard to the reproduction and nutrition of actinomycetes, Goodfellow and Williams (1983, p. 195) indicate: 'It appears that streptomycetes exist for extended periods as resting arthrospores that germinate in the occasional pres-

ence of exogenous nutrients. Particulate organic substrates, such as root fragments and dead fungal hyphae, are rapidly colonized by mycelium, which soon produces spores above the substrate; several different strains may grow together in a restricted area'. Studies done on actinomycetes in decayed stone indicate that 'since the nutrients available in decayed stone are likely to be complex organic remains, the actinomycetes may dominate this environment as they have remarkable abilities to utilise a wide range of more complex and recalcitrant polymers such as proteins, polysaccharides and lignocellulose' (Abdulla and others 2008, p. 218).

An essential aspect for the growth of micro-organisms is the availability of water, which varies depending on the composition of the substrate and the environment. Bacteria are very demanding of water and can only be developed on substrates with high water availability (Cahagnier 2002).

Besides nutrition, several environmental factors influence the activity of actinomycetes in the soil. Studies conducted in the laboratory have shown that the optimum temperature for growth is between 25 and 30°C. Most grow with a pH between 5.0 and 9.0, with an optimum value around neutrality. However, there are acidophilic streptomycetes growing between pH 3.5 and 6.5 (Goodfellow and Williams 1983).

In the present case, the MSPACH is 3 km away from the sea, in an arid environment, which is deficient in rainfall throughout the year. The climate is semi-hot and humid (SENAMHI 2012). The MSPACH weather station indicates that moisture levels at the site are high, ranging from 65% relative humidity (RH) in the hottest months (January and February) to 100% in the coldest months (July and August). The average minimum temperature is 12°C during August; the average maximum temperature is 40°C in February (Pacheco and Uceda 2011).

Microclimatic conditions in the Ychsma Storeroom are conducive to the development of bacteria because of the warm temperature and high relative humidity outside. The fact that bacteria developed in 81.6% of cases in undesalinated vessels leads us to believe that the organic residue present on ceramics, even after they have received a preliminary washing with tap water, provides sufficient nutrients for the development of bacteria. Moreover, the presence of hygroscopic salts favours the availability of water (which the bacteria need for their growth). Further studies are needed to define better what values of temperature and relative humidity cause the growth of bacteria. For the moment, we cannot see if there is contagion from one piece to another, even though the growth of bacteria inside the boxes suggests it.

Alteration and Treatment

So far, examination of the objects was limited to visual observations of the surface with the naked eye. The alteration caused by the bacteria on ceramic sample that we observed is aesthetic in nature. The white spots on the surface hinder our appreciation of the ceramic paste and the surface finish.

Apart from the aesthetically disturbing effect, micro-organisms may cause further damage. Some bacteria can create discolouration and staining (Karbowska-Berent 2003; Portillo and González 2011). The chemical alteration produced by micro-organisms was initially disregarded, but some studies show that it has an effect on the substrate. Studies carried out by May and others (2000) indicate that the weight loss produced by actinomycetes in decayed stones is negligible. However, as seen under the scanning electron microscope, there was evidence of pitting and erosion troughs around the margins of colonies of bacteria.

With regard to the biocide treatment, if the bacteria have not recurred after a lapse of two years when Biotin R has been applied, it would be an indication of the product's efficacy. As a preventive measure against contamination, we applied the biocide on vessels stored in affected boxes. However, not all the objects have been treated, as we do not know how the product evolves in the long term. For this reason, and because chemical treatments may affect future analysis on ceramic, we limited ourselves to a minimal intervention. We are carrying out analysis to check chromatic variations of the ceramic after biocide application. Further investigation is necessary to check the chemical stability of the product, whether it is harmless for treated surfaces, and its long-term efficacy.

Although direct methods are one of the options, and sometimes the only option, for limiting the agents of biodeterioration, indirect means are to be recommended because their results are definitive. Limiting the source of nutrients and controlling the environmental conditions (i.e. humidity, light, temperature, and pH) are the best strategies for controlling the development of micro-organisms (Portillo and González 2011; Nugari and Salvadori 2003). Warscheid (2000) recommends keeping artefacts already contaminated at humidity levels lower than 55% of RH; non-contaminated objects will tolerate up to 65% RH, depending on the type of material. Because of its proximity to the sea and its structural features, the Ychsma Storeroom does not offer ideal conditions for controlling the microclimate. We have limited the supply of light and tried to promote better ven-

tilation; however, a long-term solution has to be found in collaboration with the MSPACH to control and maintain stable environmental conditions at low RH values. In addition, a periodic control and a simple maintenance work are essential to prevent biological growth.

Conclusions

Micro-organisms that appeared on archaeological ceramics from the Ychsma collection have been identified as bacteria from the genus *Streptomyces Sp.* and *Bacillus Sp.* It is the former that seem to be responsible for the macroscopic small white spots visible on the objects. The rapid development of these micro-organisms within the storage boxes has been impeded by the application of biocide Biotin R at 2% in ethanol.

Our own observations, as well as the analysis of the bacteria, have led us to conclude that the source of the micro-organisms lies in the excavation soil, particularly in U58' where many mummies and organic remains have been found. *Streptomyces* are common in soil and have been identified in other items of cultural heritage, such as mural paintings and stones, particularly in subterranean environments. Moreover, the tap water used to give a preliminary wash to the vessels is a source of possible contamination. Ongoing analysis will verify these hypotheses.

With regard to the factors that promote the growth of bacteria, we think that the organic residue already present on ceramics provides sufficient nutrients, even after a preliminary bath and desalination treatment. The presence of water within ceramics is essential for the development of bacteria, and the environment outside contributes to this development with its high humidity levels and microclimatic variations; the presence of hygroscopic salts inside the ceramics also participates. For this reason, desalination treatment appears to be an essential factor in limiting the supply of nutrients. At present, we are unable to determine if there is a contamination of micro-organisms within the boxes, and eventually from one box to another.

Biocide treatment has been successful in the short term. This direct method of eliminating biodeterioration must be combined with control of the microclimate within the Ychsma Storeroom in order to prevent bacterial growth. However, the reduced resources available locally for adapting rooms to the storage of archaeological material and climatic characteristics of this area is a real challenge for conservators. Further studies are needed to define better the chemical alteration produced by bacteria, to detect potential damage on the substrates, and to monitor the efficiency and stability of the biocide treatment.

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