Final publishable summary report

- An executive summary

The BAHAMAS planned project plan has been drawn and implemented with the aim to contribute to innovative biological methodologies for conservation through the development and application of new conservation methods where innovative techniques based on the use of fungi have been experimented in the laboratory and then validated in-situ.
The activity had a substantial impact on the field and is expected to remarkably improve the quality and quantity of future development of biological treatments. Advantages and limits of new conservative treatments on metal artefacts made by copper/bronze, iron and silver have been established, comparing the new with traditional methods.
Expected outcomes were the assessment of the efficacy of traditional and new protective agents for metal artefacts and the assessment of appropriate strategies for in situ preservation, to be distributed among operators in conservation. For all the new treatments taken into, the following working step sequence has been followed:
- selection and characterisation of samples (Task 1),
- study of the action mechanism of treatments and identification of the best conditions of application (Task 2),
- sample treatments and comparison of performances, with particular attention to the efficacy, compatibility, and impact on color (Task 3),
- study of the behaviour of the treatments to severe ageing with particular attention to the durability, color changing, re-treatability, etc (Task 3),
- test of the experimented treatments in-situ, on ancient metal artefacts, chosen as prototypes, such as the bronze statue from Jean-Michel Folon “la pluie” exposed in the Olympic museum in Lausanne (CH).

Through BAHAMAS, new treatments for the conservation-restoration of metal artefacts, based on innovative biological procedures, have been developed, tested, and compared with other methods. The obtained results upgraded the existing know-how on the behaviour of microorganisms in presence of metals. The research was also interconnected with networking activities, such as participation to conferences, where these conservation procedures have been presented in the perspective to diffuse their practices.
The main achieved result concerned the effectiveness of the fungal treatment developed for copper and bronze in the possible protection of the corrosion. Furthermore, if compared with the actual protective systems, the tested biological treatment shows the great advantage of causing minimal change to the appearance of the corrosion patina. So that the tested materials act not just as simple coating, with no chemical interaction with the patina components, but resulted to create a layer from the patina which makes the treatment more durable and stable. The EIS results have clearly demonstrated a different behaviour of the biological treatment respect to the others.

- A summary description of project context and objectives.

The nature of the corrosion products present on the surface of artistic and archaeological metal artefacts is intrinsically related to the environmental context (atmospheric or burial). In order to effectively protect and inhibit the corrosion of such metal objects, the practices adopted should take into account the nature of the patina and its corrosion behaviour. However, the treatments so far employed are organic protective coatings, which do not consider the difference in terms of patina composition and corrosion products stability and simply create a barrier against aggressive environments in a non-selective way. A

multidisciplinary project BAHAMAS was proposed for developing an alternative biological treatment as a novel approach for the conservation-restoration of metal artefacts. In order to achieve this innovative research issue in the field of conservation science, a unique interdisciplinary team was created based on the collaboration between the Swiss National Museums, the University of Neuchâtel and the researcher.
The research activity aimed at chemically and specifically modifying existing corrosion products into more stable and less soluble compounds while maintaining the surface's physical appearance. Some species of fungi have been already reported for their ability to transform metal compounds into metal oxalates, which are known to be insoluble and high protective compounds towards corrosion. Thus, the participants exploited this potential for the transformation of existing corrosion patinas into metal oxalates. The research work investigated the formation mechanisms and adhesion properties of the newly formed metal oxalates on different metal substrates (copper, iron and silver), which are frequently found in cultural heritage artworks. Particular attention was devoted to the efficacy, compatibility and impact on colour of the developed treatments to overcome the problems associated with the treatments in use nowadays. Finally, real samples were also included in the studies in order to validate the new methodology.

- A description of the main S&T results/foregrounds.

The different activities of the BAHAMAS project were implemented in order to develop innovative treatments which create protective patinas on three different types of metal, frequently found in cultural heritage artworks: copper, iron and silver. The newly developed protective systems were required to be efficient and durable but also harmless.
Based on the very promising results obtained within the framework of the 6FP EU-ARTECH project (Joseph et al. 2007, Di Francesco et al. 2007, Mazzeo et al. 2008a), the BAHAMAS project started in June 2010 for two years. The efficiency of the innovative treatment developed on bronze monuments during the EU-ARTECH project was further investigated. In particular, the newly formed copper oxalates were in-depth characterized in order to define their properties (formation mechanisms, adhesion...) and optimize the application procedure. The crystals aggregates were characterized through ESEM, FTIR and Raman microscopies, either on copper-enriched media or on corroded coupons (Joseph et al. 2011, Joseph et al. 2012a, Joseph et al. 2012b). Cross-section examination suggested that the first micrometers of the urban natural patina are completely converted into copper oxalates, as showed in figure 1.

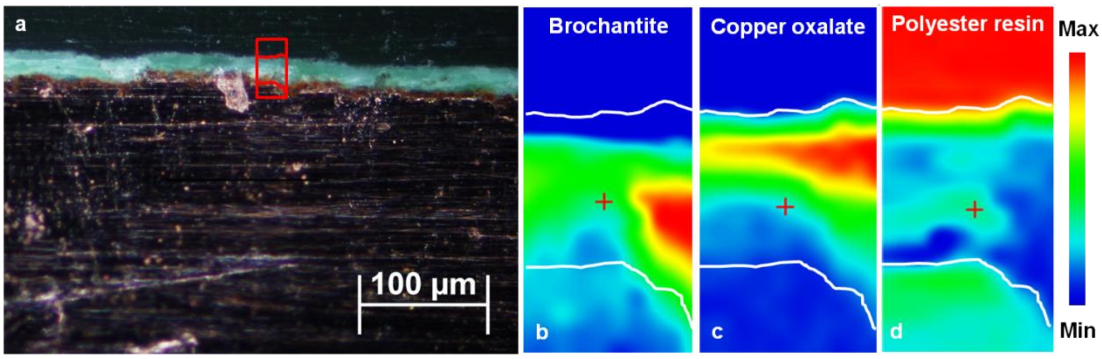

Figure 1 – ATR-FTIR raster scanning: a) visible light photomicrograph of a cross section of corroded coupon with an urban natural patina after treatment with *B. bassiana*, embedded in polyester resin: the box indicated the selected area for the ATR raster scanning. FTIR false colors representing b) the original patina composed of

brochantite (region of interest 1105 - 1085 cm^{-1}), c) the newly formed copper oxalates (peak area 1320 cm^{-1}) and d) the embedding resin (region of interest 1735 - 1715 cm^{-1}).

As enlightened several time, protective treatments efficacy is dependent on the underlying patina. Attention has thus been paid to test the same treatment on several kinds of coupons. Different copper and bronze coupons with either urban or marine patinas were indeed prepared and characterized with a complement of analytical techniques: XRD, SEM-EDS, optical, FTIR and Raman microscopies, colorimetry and electron Electrochemical Impedance Spectroscopy (EIS). The optimized fungal treatment was then applied on the coupons and the newly formed metal oxalates were investigated using the same analytical techniques as for the untreated patina. The different coupons treated with *B. bassiana* or reference materials (e.g. wax: Cosmoloid H80; silane: Dynasylan® F8263…) were exposed in a corrosion site (class 5, Genoa Harbor) in December 2011. Their long-term behavior and performance were compared and monitored over a six-months period. Different application procedures were also investigated on real artworks (archaeological finds, outdoor monuments). The procedure was adapted to better fit with the three-dimensionality of the objects.

In addition, the same approach was considered on other metal substrates, such as iron and silver, which are frequently found in cultural heritage artworks and also encounter several problems of active corrosion.

Liquid cultures of *B. bassiana* on iron objects gave very promising results: after a few weeks, hyphae seemed completely incrusted with red crystals (Joseph et al. 2012a). These latter were further analyzed by FTIR measurements and presented the characteristics absorbance bands at 1366(ν_sC–O), 1320(ν_sC–O), and 822 (δC–O) cm^{-1} of iron oxalates (Figure 2). The optimization of the incubation of *B. bassiana* over iron coupons is still an on-going work. In particular, further tests are currently being performed to lessen the amount of water used during application with a spray for the aspersion of the culture over the iron coupons.

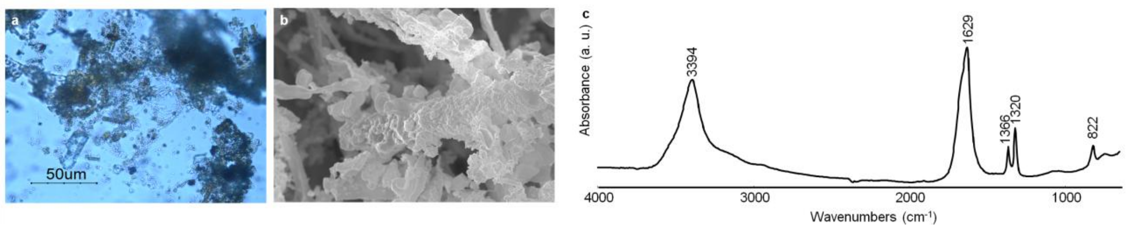

Figure 2 – *Beauveria bassiana* cultures on malt-agar medium with an iron washer. a) Optical microscopy observations. b) Secondary electron image. c) Transmittance FTIR spectrum (4000–650cm^{-1}) obtained from hyphae incrusted with red crystals.

On silver, a co-precipitation of copper and silver oxalates occurred. As this greenish patina would not be acceptable on silver objects, silver reduction was explored as a tarnishing remediation. First experiments showed the transformation of silver nitrate into nanoparticles of elemental silver by an unknown extracellular mechanism. The ability of *B. bassiana* to synthesize Ag^0 nanoparticles was tested either in liquid medium or within a cell filtrate (Figure 3). After three days of incubation, UV-VIS spectroscopy analysis showed a characteristic absorbance band at 420 nm, which was absent in the controls and can be attributed to the presence of Ag^0 nanoparticles due a Plasmon resonance phenomenon.

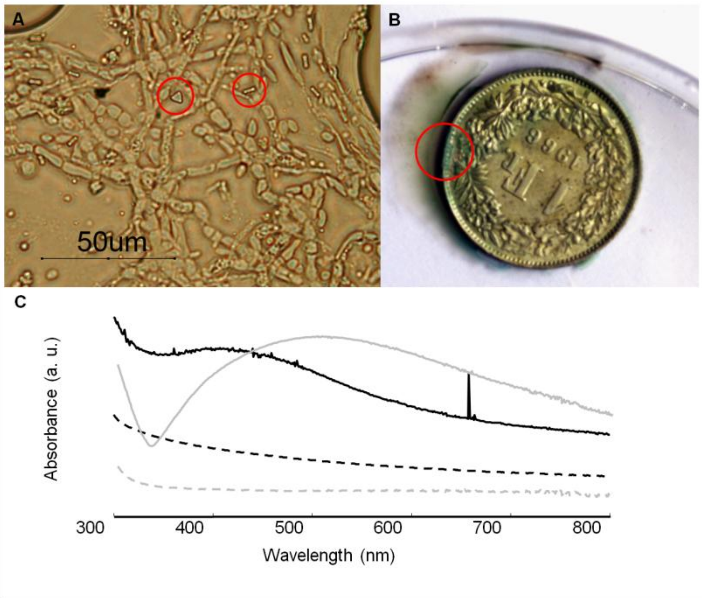

Figure 3 - Growth of B. bassiana on silver compounds. a) Optical microscopy observations of B. bassiana cultures on MAACAN medium with rectangular and bipyramidal crystals indicated by red circles. b) Sample C3 (copper-silver alloy) with copper oxalates formed around the area indicated by a red circle. c) UV-visible absorbance spectra of mycelium (black line) and cell filtrate (dash line) containing silver nanoparticles. As control the reference spectra for mycelium (grey line) and cell filtrate (grey dash line) were included.

- The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results (not exceeding 10 pages).

Biopatinas created are expected to be stable and insoluble, providing a very high protection and allowing the inhibition of the corrosion processes. They are an eco-friendly strategy with which maintenance costs may be reduced to minimum. The treatments may also have no side-effect on health and environment as non-toxic and naturally occurring microorganisms are used.

This project permitted also the development of partnerships with the French Corrosion Institute in Brest, the Institute of Marine Sciences in Genoa (CNR-ISMAR, Italy), in Neuchâtel or private conservator-restorers workshops such as Art Metal Conservation (AMC s.a.r.l). With the National Center for Metallurgical Research (CENIM) of the Spanish Council for Scientific Research (CSIC) in Madrid, a closed collaboration was established and allowed the successful submission to a JERICO grant (VII FP, Contract 262584) on the application of electrochemical techniques to the evaluation of coatings for cultural heritage. This grant, which will start during the last semester 2012, will allow the access to a natural ageing facility. The assessment of the protectiveness of selected coatings, among these those developed during the BAHAMAS project, will be hence possible.

As recognition of her work, the researcher was invited speaker to three conferences:

- the conference *Marie Curie Actions for an Innovative Europe* in 2010, as one of the fellows representing the 50.000th Marie Curie research fellow,
- the annual congress of the Swiss association of conservators-restorers *Innovation in conservation practice* in 2011,

- the *Bronze Conservation Colloquium* organized in Stuttgart in 2012.

Moreover, one of the outcomes of the BAHAMAS project is the development of an easy-to-use kit dedicated to conservators-restorers. In partnership with the industry and the school of conservation-restoration ARC (University of Applied Sciences Western Switzerland), an application was submitted to the Swiss Commission for Technology and Innovation (CTI) and is now under evaluation. In total, the researcher has published in 3 peer-reviewed journals: *Journal of Raman Spectroscopy* (accepted, under revision), *Frontiers in Microbiotechnology*, *Analytical and Bioanalytical Chemistry* (as forefront paper) and presented 7 posters:

- *IRUG10, 10th biennial conference of the Infrared and Raman Users Group, Barcelona, Spain, March 28-31 2012.*
- *RAA 2011, 6th International Congress on the Application of Raman Spectroscopy in Art and Archaeology, Parma, Italy, September 5-8 2011.*
- *Ecology of soil microorganisms, Prague, Czech Republic, April 27 – May 1 2011.*
- *Euro BioMat, European symposium on biomaterials and related areas, Jena, Germany, April 13-14 2011.*
- 4th *Swiss microbial ecology meeting, Endelberg, Switzerland, Februar 2-4 2011.*
- *9th International Mycological Congress, Edinburgh, UK, August 1-6 2010.*
- *1st International Congress Chemistry for Cultural Heritage (ChemCH), Ravenna, Italy, June 30 - July 3 2010.*