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THESIS TITLE

"STUDY OF ARCHEOLOGICAL METALLIC ARTEFACTS, RAW MATERIALS AND CONSERVATION WITH BIOLOGICAL AGENTS"

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XXXV cycle

To the supporting pillars of my life:
my parents and my grandmother

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ENGLISH ABSTRACT

This research project, funded by the Basilicata Region and supported by the "Industrial Doctorate 4.0", had the objective of characterizing from a physicochemical and biological point of view seven metal artefacts found during archaeological excavations, in the Siris area of Policoro (Matera, Southern Italy), and preserved at the National Archaeological Museum of Siritide, and subsequently to seek green cleaning protocols. The PhD project was divided into four phases: 1) six-month practical activity at the National Archaeological Museum of Siritide (Policoro, Matera); 2) a three-month study period with Prof. Sariel Shalev concerning the different variables that can contribute to initiate corrosion processes; 3) five weeks collaboration at theArc'Antique Institute in Nantes for the application of a traditional cleaning method. This conservation-restoration and research laboratory was chosen because it specializes in terrestrial and underwater archaeological assets. The Arc'Antique team comprises people with complementary skills, which allows its real versatility in its intervention skills; 4) six weeks period at the University of Neuchâtel to evaluate the application of a green cleaning protocol using the *Beauveria Bassiana* Vuill. fungi as Prof. had already performed studies about green protocols by using biological agents to conserve/restore archaeological metal artefacts.

The seven archaeological metallic artefacts conserved at the Siritide National Archaeological Museum (Policoro, Matera) were identified by using a multi-analytical approach, with non-destructive analytical techniques, including optical microscopy (OM), X-ray fluorescence (XRF), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), followed by a microbiological investigation. Macroscopically, the

metal findings appeared to deteriorate due to severe corrosion and some specimens including arrowhead and lancet were respectively subjected to XPS and XRD analysis . The oxidation state, observed with the optical microscope (OM) on the surface of a lead stick and an arrowhead, did not allow the recognition of the constituent materials. The raw materials were qualitatively analysed by XRF, carried out on each artefact, and in the case of an arrowhead by XPS. Nevertheless, the XRD technique has been decisive in the characterization of a hand.

The chemical-physical characterization phase was followed by the evaluation phase of the variables that can initiate and initiate the corrosive processes including pH, T, climate, and type of excavation.

The information acquired from the chemical-physical analyzes is crucial for evaluating a suitable green or traditional conservation/restoration protocol, based on the raw materials, for cleaning the artefact or for trying to stabilize the corrosive processes in progress, in such a way that these processes do not become irreversible and the archaeological asset is no longer recoverable. Finally, the performance of a traditional protocol and a green one with the use of a biological agent, *Beauveria bassiana* Vuill. fungi, were evaluated.

The traditional protocol, in use at the Arc'Antique Institute (Nantes-France), first of all provides for the elimination of corrosion products on the metal artefact. This type of cleaning can be mechanical when the corrosion products present are highly compact and electrochemical, instead, when there are corrosion products of the porous type. After the corrosion products have been removed, the manufactured articles are subjected to a "microsablage" finishing technique which allows the residual corrosion products to be eliminated. Finally, the artefact is covered with a layer of Paraloid 72 andthen covered with wax.

At the University of Neuchatel (Switzerland) the fungi *Beauveria bassiana* Vuill. was used as a biological agent. This fungus produces oxalic acid as a secondary metabolite and for this reason, it has been evaluated exclusively on copper plates since oxalic acid in contact with copper forms copper oxalates which allow the creation of biopatina. In addition to the fungi, its filtrate was also used both in gel and in liquid medium and a comparison with commercial oxalic acid was also made to evaluate its efficacy. The results showed that the presence of the fungus is necessary for 100% efficacy to be achieved; as regards the samples treated with the filtrate of *Beauveria bassiana* Vuill., the formation of copper oxalates is present in 66% of the cases, however, with the use of commercial oxalic acid, efficacy is found only in 33% of the cases. As we can see, the obtained results show that the presence of *Beauveria bassiana* Vuill. is necessary. to achieve greater effectiveness.

Comparing the two types of protocols it is clear that the strong point of the application of the traditional protocol is that it can be used on all metals even if with small variations within the protocol depends on the type of corrosive process present; the main disadvantage concerns very long times. However, if the times are a disadvantage for the application of the traditional protocol, the application ofthe green protocol represents a strength as it involves times of about seven days, but the disadvantage of this type of protocol is that it can only be applied on metallic copper artefacts. In light of this, it is possible to think of studying other biological agents to draw up green protocols for other metals, always taking into consideration the type of metal dossier to be treated and also the costs.

ITALIAN ABSTRACT

Questo progetto di ricerca, finanziato dalla Regione Basilicata e supportato dal “Dottorato Industriale 4.0”, ha avuto l’obiettivo di caratterizzare dal punto di vista fisico- chimico e biologico sette manufatti metallici rinvenuti durante gli scavi archeologici, nell'area Siris di Policoro (Matera, Italia meridionale), e conservati presso il Museo Archeologico Nazionale della Siritide, e successivamente di cercare protocolli di pulitura green. Il progetto di dottorato si è articolato in quattro fasi: 1) attività pratica di sei mesi presso il Museo Archeologico Nazionale della Siritide (Policoro, Matera); 2) periodo di tre mesi di studio con il Prof. Sariel Shalev inerente alle diverse variabili che possono contribuiread iniziare o accelerare i processi di corrosione; 3)collaborazione con periodo di cinque settimane presso l’Istituto Arc’Antique di Nantes per l’applicazione di un metodo di pulitura tradizionale. Questo laboratorio di conservazione-restauro e ricerca è stato scelto perché specializzato in beni archeologici terrestri e subacquei. Il team di Arc'Antique è composto da persone con competenze complementari, che gli consentono una reale versatilità nelle sue capacità di intervento.;

4) periodo di sei settimane presso l’Università di Neuchatel per valutare l’applicazione di un protocollo di pulitura green utilizzando il fungo *Beauveria bassiana* Vuill. in quanto la Prof.ssa aveva già eseguito studi su protocolli green adoperando agenti biologici per conservare/restaurare manufatti metallici archeologici.

I sette manufatti metallici archeologici conservati al Museo Aercheologico Nazionale della Siritide (Policoro, Matera) sono stati caratterizzati utilizzando un approccio multianalitico, con tecniche analitiche non- distruttive, tra cui la microscopia ottica

(OM), la fluorescenza a raggi X (XRF), la diffrazione dei raggi X (XRD), la spettroscopia fotoelettronica a raggi X (XPS), a cui è seguita un'indagine microbiologica. Macroscopicamente, i reperti metallici sembravano deteriorarsi a causa di una grave corrosione ed alcuni campioni tra cui punta di freccia e lancetta sono stati sottoposti ad analisi rispettivamente XPS e XRD. Lo stato di ossidazione, osservato con il microscopio ottico (OM) sulla superficie di un bastoncino di piombo e di una punta difreccia, non ha consentito il riconoscimento dei materiali costituenti. L'analisi delle materie prime è stata svolta qualitativamente da XRF, effettuata su ogni manufatto, enel caso di una punta di freccia da XPS. D'altra parte, la tecnica XRD è stata decisiva nella caratterizzazione di una lancetta.

Alla fase di caratterizzazione chimico-fisica è seguita la fase di valutazione delle variabili che possono far iniziare e accelerare i processi corrosivi tra cui pH, T, clima, tipo di scavo. Le informazioni acquisite dalle analisi chimico-fisiche sono determinanti per valutare un idoneo protocollo di conservazione/restauro green o tradizionale, in base alle materie prime, per la pulitura del manufatto o per cercare di stabilizzare i processi corrosivi in atto, in modo tale che tali processi non diventino irreversibili e il bene archeologico non sia più recuperabile. Infine, sono state valutate le applicazioni di un protocollo tradizionale e di un protocollo green con l'uso di un agente biologico, fungo *Beauveria bassiana* Vuill.

Il protocollo tradizionale, in uso presso l'Institute Arc'Antique (Nantes-Francia), prevede dapprima l'eliminazione dei prodotti di corrosione sul manufatto metallico, quando presenti. Questo tipo di pulitura può essere meccanico quando i prodotti di corrosione presenti sono fortemente compatti ed elettrochimico, invece, quando sono presenti prodotti di corrosione di tipo poroso. Dopo che i prodotti di corrosione vengono rimossi, i manufatti vengono sottoposti ad una tecnica di finitura “microsablage” che consente di eliminare i prodotti di corrosione residui. Infine, il manufatto viene coperto con uno strato di Paraloid 72 e poi ricoperto con la cera.

All’Università di Neuchatel (Svizzera) è stato usato come agente biologico il fungo *Beauveria bassiana* Vuill. Questo fungo produce come metabolita secondario l’acido ossalico e per questo motivo è stato valutato il suo esclusivamente su placche di rame poiché poiché l’acido ossalico a contatto con il rame forma gli ossalati di rame che consentono di creare la biopatina. Oltre al fungo, è stato utilizzato anche il suo filtrato sia in mezzo gel che in mezzo liquido ed inoltre, è stato fatto anche un confronto con l’acido ossalico commerciale per valutarne l’efficacia. I risultati hanno evidenziato che è necessaria la presenza del fungo affinchè si abbia il 100% dell’efficacia; per quanto riguarda i campioni trattati con il filtrato di *Beauveria bassiana* Vuill., la formazione degli ossalati di rame è presente nel 66% dei casi, invece, con l’utilizzo dell’acido ossalico commerciale si riscontra un’efficacia solo nel 33% dei casi. Si può desumere, dunque, dai risultati ottenuti che è necessaria la presenza di *Beauveria bassiana* Vuill. per ottenere una maggiore efficacia.

Comparando i due tipi di protocolli si evince che il punto di forza dell’applicazione del protocollo tradizionale è che può essere adoperato su tutti i metalli anche se con con piccole variazioni all’interno del protocollo a seconda del tipo di processo corrosivo presente; lo svantaggio principale riguarda invece le tempistiche molto lunghe. Se le tempistiche sono però uno svantaggio per l’applicazione del protocollo tradizionale, per l’applicazione del protocollo green esso rappresenta un punto di forza in quanto si tratta di tempistiche di circa sette giorni, ma lo svantaggio di questo tipo di protocollo è che esso può essere esclusivamente applicato su manufatti metallici in rame. Alla luce di ciò, si può pensare di studiare altri agenti biologici per stilare protocolli green per gli altri metalli, sempre tenendo in considerazione il tipo di dossier metallico da dover trattare ed anche i costi.

1. INTRODUCTION

If many years ago people asked themselves the question of what was the limit between science and restoration because they thought they were between two parallel universes with nothing in common, today things are different because they realized that close collaboration between disciplines is necessary, but it is also necessary to apply a common language.

Art lovers, humanists, were considered thinkers of the arts extraneous to the reality of things while science lovers, positivists, were accused to perceive nothing beyond physicality and proven objective evidence (Avilia, 1980).

According to Thucydides, archeology had a lot of importance because it had the precise purpose of "demonstrating" and compatible with the demonstration function, it also had the value of "history", of reconstruction of the past and therefore it was intended as "research" in the full sense of the term as it served, precisely, to reconstruct history, demonstrating it "scientifically" through the archaeological finds found (Parmeggiani, 2003).

The history of human civilization is linked to the use of metals by man. Ancient civilizations knew eight elements: copper, tin, lead, zinc, iron, gold, silver, and mercury; there were elements of lesser use but probably the most famous were antimony and platinum. Hardly any aspect of the modern world could function without an intimate knowledge of the mining, purification, and use of the metals that have played such an important part in mankind's past and it is no surprise that objects fabricated from metals form a significant part of the world's cultural heritage. The metal history and that of metallurgy can be considered to be directly interconnected (Walker, 2008).

Metals, in fact, directly influence their evolution and it is linked to the production of weapons, agricultural tools, objects of workship, and everyday life (Artioli, 2010; Robert, 2013).

The first metal objects, according to the archaeological record, are part of grave goods, jewels, weapons, and precious objects that were buried in the ground for safekeeping or as part of ritual offerings.

The use of metal, therefore, implies that the ancients knew where and how to extract it, and how to treat it to obtain a metallic state. It is important to study methods for slowing down or inhibiting corrosive processes about cultural heritage because they are the basis of the history of civilization (Aceto, 2005).

Metal artefacts, over time, have the disadvantage of undergoing corrosion phenomena. There are two orientations about leaving or not the corrosive phenomena on the metal product. An orientation tends to preserve some corrosion types to give importance to the historical value acquired over time. The other tendency tends to restore the original surface layer. Proceeding with a correct restoration intervention, it is first of all necessary to carry out an accurate investigation of the current corrosion layer of the artefact along with nature alterations present through the identification and characterization of the corrosive phenomena that can allow to identify the type of cleaning that can be carried out according to the type of corrosion; outline guidelines for identifying the right restoration-conservation protocol must be applied. As a result, complementary non-destructive analytical techniques allow the identification of the corrosion products, that can reveal the conditions of burial processes and provides indications about restoration and conservation (Fabrizi, 2019).

1.1 Metal's properties

Studying metals means looking at the physical, chemical, and mechanical characteristics and how these characteristics have influenced the way the metals have been used. There are many metals in the periodic table, but only gold, silver, and copper occur naturally on Earth in pure form. Metals are a class of chemical elements with specific chemical properties that depend on the electronic structure: they are tough but can be bent or shaped by hammering, they are good conductors of electricity and heat, and polished metals often have shiny and light-reflecting surfaces.

As preciously only silver, iron, gold, platinum, and copper exist in nature in their native state, or as pure elements, while the others, combined with other elements exist in the form of metal ores, especially as oxides (Me_mO_n) and sulfides (Me_mS_n).

Metallic materials have a crystalline solid structure that is at the level of microscopic in the form of so-called grains whose geometry depends both on the type of metal.

The moment of transition from liquid to solid state begins around the nuclei of crystallization the growth of each crystal in various directions (Artioli, 2010).

A further aspect that interests the conservation of artistic artefacts consists both in the microscopic observation and the chemical and structural analysis of the alterationphases observed.

In particular, these analyses make it possible to study and evaluate the effects and the dislocation of the corrosion on the surface of the metal; i.e. if a uniform alteration has occurred without affecting the innermost parts, if the corrosion has also involved intergranular areas or if cyclical pitting corrosion is present. Archaeometry research uses all available techniques and methods developed within specialized disciplines (i.e. physics, chemistry, geology, biology) (Artioli, 2010).

Since there are so many different metals that can be alloyed in different types of compositions and since they can be subsequently modified by mechanical and thermal treatments, it is possible to produce metal objects with different physical and chemical characteristics. As a general rule, pure metals are softer and more easily deformed than alloys. The ductility of metals, i.e. the extent to which they can be deformed without fracturing, can be attributed to metallic bonding and the ability of metal atoms to slide past each other along the planes of the crystal lattice.

Even a pure metal will become harder when hammered at room temperature because "cold working" introduces defects in the crystal lattices that inhibit atomic planes from sliding past each other. This can be overcomed by simply heating the metal above a certain temperature which depends on the metal and the amount of deformation it has received. This introduces enough thermal vibration to allow the crystal lattices to relax and rearrange themselves into a more orderly arrangement. This process is called annealing and it was discovered very early in the history of metallurgy.

- Copper

Copper and its alloys were and are widely used in many environments for theirexcellent corrosion resistance characteristics, high thermal and electrical conductivity, ease of processing, welding, and a wide range of mechanical properties. Copper-based alloys are divided in two according to the type of binders: brass (Cu-Zn alloys) and bronze (Cu-Sn alloys). The properties of alloys are strongly influenced by the tin content and the presence of other binders. By increasing the amount of tin, the material becomes more fluid in the liquid state, and an increase in hardness and brittleness is also achieved (MacLeod, 1982).

- Bronze

Archaeologists have given the name "Bronze Age" to the European and Asian prehistoric periods in which men developed the metallurgy of copper and its alloys. During this historical period, bronze was used to make tools, weapons, and instruments stronger and lighter than those made of stone or copper; to the component, metals were added, mostly as impurities, even arsenic, which contributed to making the alloy even harder (Gordon, 2008)

The first bronze castings date back to the third millennium B.C. and simple stone or terracotta molds were used. In Roman times zinc and lead alloy was introduced, which made the spindle more fluid. From the beginning of the 20th century, silicon was introduced as the main binder of copper and today most bronze for industrial and artistic uses is a copper-silicon alloy (Gordon, 2008).

- Iron artefacts

Carbon is easily soluble in liquid iron and at high temperatures will diffuse into solid iron to form steel. Above the austenitization temperature of 912°C, however, the metal can accommodate much higher concentrations of carbon in its structure (up to 2.04% by weight at 1146°C).

Steel is an alloy made of iron and carbon, with a carbon content by weight ranging from 0.20 to 2.04% by weight. Carbon acts as a hardening agent, preventing atomic planes and dislocations in the iron atom's crystal lattice from sliding past each other. The hardness of steels can be enormously increased by correct heat treatment which consists of rapid cooling. The heat treatment process for most steels involves heating the alloy to the formation of austenite with subsequent and immediate cooling so that the transformation into ferrite or pearlite does not take place (Dillmann, 2004).

Stainless steels represent an immensely important class of ferrous metals. Despite the strength, low cost, and wide-ranging applications of iron and steel, they are very prone to rapid corrosion when exposed to moist air (Gordon, 2008).

- Silver

Silver alloys can have a silver content of over 90% and are used in coins, tableware, candlesticks, and jewelry; while fine or pure silver is too soft to be made into large or delicate objects and therefore it is limited to coinage and electronic components where its low electrical resistance is put to use (Butcher, 2005).

- Gold and platinum

Gold is the easiest native metal to find as it does not corrode and appears as a bright yellow nugget on the ground, is very soft when pure and easily shapes into simple objects, and can also be hammered into thin sheets. Alloys of precious metals such as gold and platinum have a variety of uses and are used to make jewelry but also in situations where extreme resistance to chemical corrosion is required. (Artioli, 2010).

- Aluminum alloys

Over the last century, aluminum and its alloys have become one of the most important metals in mass production. Aluminum is considered to be the most widely used non-ferrous metal. To this time it is used to make items as varied as aircraft, where it is used for its high strength-to-weight ratio, and aluminum baking sheets (Georgantzia, 2021).

1.2 Type of degradation

The definition of corrosion given by the International Organization for Standardization (ISO) is: "Physicochemical interaction between a metal and its environment which results in changes in the properties of the metal and which may often lead to impairmentof the function of the metal, the environment, or the technical system of which these form a part". ISO and the International Union of Pure and Applied Chemistry (IUPAC) give a wider definition of corrosion, also involving the degradation of the non-metals as well as metallic materials: "Corrosion is an irreversible interfacial reaction of a material (metal, ceramic, polymer) with its environment which results in consumption of the material or dissolution into the material of a component of the environment. Often, but not necessarily, corrosion results in effects detrimental to the usage of the material considered. Exclusively physical or mechanical processes such as melting or evaporation, abrasion or mechanical fracture are not included in the term corrosion" (IUPAC, 2014).
The study of the degradation of metal shows that can be influenced by several parameters including the surrounding environment, the increase in O_2 and the change in pH. This study is critical because it is possible to trace a series of information such as the raw materials used in the forging of weapons and tools and their origin to understand the trade routes.

The deterioration can also be of a biological type and can manifest itself for a long period, even in non-extreme environmental conditions with the presence, for example, of molds and parasites that cause rapid, serious, and irreversible degradation (MacLeod, 1991; Videla, 2002; Bouchard, 2003; Frost, 2003; Giumlia-Mair, 2005; Ingo, 2006; Figueiredo, 2007; Bernard, 2009; Mata, 2009; Campanella, 2009; Corsi, 2016;

Balassone, 2018).

Therefore, according to this definition, the products deriving from corrosion and those deriving from the phenomenon of abrasion must be distinguished. The primary products of the corrosion phenomenon are always solid or dissolved non-metallic chemicalspecies.

- Chemical corrosion

The identification of corrosion products continues to be studied because it can be originated from the aggression of chemical agents. Recently numerous chemical and /or physical methodologies have been applied for the identification of the chemical and structural composition of the patina formed in an archaeological context (MacLeod, 1991; Bouchard, 2003; Frost, 2003; Frost, 2004; Giumlia-Mair, 2005; Ingo, 2006; Figueiredo, 2007; Bernard, 2009; Campanella, 2009; Mata, 2009; Corsi, 2016; Balassone, 2018;). The corrosion processes are influenced by a vast series of factors depending both on the nature of the product (composition of the alloy, type of processing) and the environmental conditions (humidity, presence of pollutants, salts, climatic factors, etc.). The heterogeneous nature of alloy artfacts can cause the origin of local streams giving rise to phenomena of selective corrosion at the expense of thephase constituted by the less noble element (dezincification of brasses).

- Uniform corrosion

It is also called general (common) corrosion. General thinning occurs until the metal surface weakens. Metals corrode because they tend to return to their lowest energy levels, which is usually a state of oxidation. This type of corrosion is easily observable, and therefore protection by coating or painting is easy. From a technical point of view, this corrosion is preferred because it is predictable and therefore accepted in design (Jones, 1992).

- Galvanic corrosion

Galvanic corrosion, resulting from a metal connecting another conducting material in a corrosive medium, is one of the most common types of corrosion. When two different conducting materials in electrical contact with each other are exposed to an electrolyte, a current, called the galvanic current, flows from one to the other by Faraday’s law. A galvanic current generally causes a reduction in the total corrosion rate of cathodic member of the couple (Zhang, 2011).

- Pitting corrosion

A regional corrosion causes the formation of small pits in the metal. This type of corrosion is considered one of the harmful forms, as it causes perforation in the equipment. Pit corrosion occurs on the surface of metals with a metal oxide layer, such as aluminum and stainless steel (Frankel, 1998; Akpanyung, 2019).

- Crack corrosion

It is a localized type of corrosion that can be found in crevices in stagnant solutions (Hatch, 1984). Crack corrosion occurs in the gaps between two metal surfaces or between metals and non-metal surfaces.

- Intergranular corrosion

This corrosion is the chosen deterioration of the granular boundary region (Hatch, 1984) even though the mass granule of the metal or alloy is not attacked (Hatch, 1984). That is the corrosion occurs along the boundary line of the grains in the crystal structure of a metal.

- Subshell corrosion

Corrosion that occurs under the shell formed by corrosion products on the surface of the metal or for another reason is called sub crust corrosion. This type of corrosion occurs when the shell gold is damp and the shell gold does not get enough oxygen. Since there is no liquid movement under the shell, an environment similar to crack corrosion is created. The bottom of the shell corrodes as anodes, while the crust circumference is preserved as a cathode (Alp, 2014).

- Filiform corrosion

It is a corrosion phenomenon that walks under the paint or coating layer on the surface of the metal. This corrosion can be considered a type of crack corrosion and is also called sub-crust corrosion. The corrosion event acts under the shell in a similar way to the movement of a worm. This corrosion mechanism is like the mechanism of formationof crack corrosion. Corrosion starts from a weak point of the coating (Senoz, 2011).

- Selective corrosion

It is a corrosion event that occurs as a result of the corrosion of one of the elements in an alloy and its departure. The best example of this type of corrosion is brass alloy. Zinc is oxidized before copper, corroded and moved away. Brass is an alloy consisting of approximately 70% copper and 30% zinc. This alloy, which was initially yellow in color, gradually acquires the color of copper red after the corrosion of zinc. A similar phenomenon is observed in other alloys. Aluminum, iron, cobalt, chromium or other elements are selectively removed from the alloys (Cabrini, 2019).

- Erosive corrosion

The phenomenon of corrosion of the metal due to the relative movement between a metal and the corrosive medium is called erosion corrosion. When corrosive solutions flow rapidly through the surface of the metal, erosion occurs as well as corrosion. This leads to an increase in the corrosion rate. The reason for this is that the corrosion products formed are dragged away by the fluid. In the direction of the flow, visible cavities and rounded grooves in the form of a waveform are formed. Erosive corrosionis seen in many metals. The most sensitive metal is copper and copper alloys. In erosioncorrosion,the surface is clean and no corrosion product is visible (Alp, 2014).

- Stress corrosion (stress corrosion cracking)

If a metal in a corrosive environment is also under a static stress, the fracture of the metal by cracking becomes faster. Any pits or cracks on the surface of the metal become sensitive to stress, creating a favorable environment for the onset of corrosion. Although corrosion products normally form a protective shell on the surface of the metal, they cannot form a shell when under stress. As a result, corrosion continues rapidly, causing the metal to crack in that area. Alloys are more susceptible to this type of corrosion than pure metals (Di Fazio, 2022).

- Fatigue corrosion

A metal under a dynamic stress, which periodically acts in the form of loading-unloading, gets tired over time. The metal, which is in a tired state, can crack under the influence of smaller stresses than normal. The combined effect of fatigue and corrosion causes the metal to crack in a short time (Yaohan, 2022).

- Hydrogen fragility

As a result of a corrosion reaction or in the application of cathodic protection, hydrogen atoms are formed on the surface of the metal. They are adsorbed on the surface of the metal. Some of the hydrogen atoms, enter the metal and settle in the cavities in the metal. These hydrogen atoms then form a hydrogen molecule, causing a large increase in volume. Since the hydrogen molecule does not have the opportunity to diffuse through the metal, it causes the metal to crack by making a great pressure in the metal cavities (Alp, 2014).

- Elettrochemical corrosion

An electrochemical corrosion cell has four basic components: an electrical connection between the anode and the cathode for the circulation of the anode, a cathode, an electrolyte solution, and the electronic current. In case any of the specified components are lost or deteriorated, the electrochemical corrosion process will stop. Therefore, these elements are very important for corrosion control (Trethewey, 1995).

- Patina

About the corrosion phenomenon of bronze, there is a formation of "patina": theexternal layer that is formed spontaneously in contact with air, carbon dioxide (CO_2), andhumidity. (Chiavari, 2007). The term "patina" is broadly associated with the presence of a corrosion layer that marks the original surface. Robbiola et al. (2008) characterized thecorrosion structure of patina based on the preservation of the marker of the original surfaces of bronze artefacts as Type 1, also known as "noble patina", and Type II, "vile" patina". Type 1 structure is characterized by the presence of two layers: athin layer that isprotective grown in contact with the metal overlapped by a second thicker porous layer, thanks to its structure, this patina is stable and plays a protective role. Type 2 is patina with a thicker and more complex structure and it is characterized by three layers: in presence of Cl^- ions (aggressive environment), the outer porous corrosion layer, can let penetrate the Cl ions through the layer creating a high concentration of chloride ions, together with tin and copper compounds, at the metal corrosion layer interface (inner layer). The outer layer is characterized by copper compounds (II) such as hydrochloride (malachite), hydroxylates, and hydoxyphosphates. Between the outer layer and the inner layer, there is the intermediatelayer. When the type 2 patina is present, the surface of themetallic artefact is destroyed or deformed as a result of a severe attack by the environment, there is the aggressive action of chlorides that are metastable and can react with atmospheric humidity and there is the breaking of the outer layer, a phenomenon better known as a bronze disease (Robbiola, 2008).

- Microbiological corrosion

Microbiological corrosion, known as microbially influenced corrosion (MIC) is a type of corrosion in which the deterioration of metallic as well as non-metallic material occurs due to the presence and activities of microorganisms (Zarasvand, 2013). The process requires the presence of three elements: the metal, the electrochemical solution, and the microorganisms. The peculiar characteristic of MIC, for corrosion not influenced by microorganisms, lies in the fact that the latter can initiate, facilitate or accelerate the electrochemical corrosion reactions that take place on the metal surface, leading to degradation of the material found in aqueous environments. Microbiological corrosion can occur either locally, in the form of pitting, or in a general way, and this depends on the metal substrate that is attacked and the environment in which it is located (Videla, 2002).

Microbiological corrosion is no different from normal corrosion phenomena. It manifests itself in the form of some microorganisms by increasing the reaction rate of the corrosion. Microbiological corrosion events also participate in corrosion events that occur for other reasons and have an effect on increasing the corrosion rate.

Microbiological corrosion is difficult to detect by simple visual inspection. Verifying the presence of microbiological corrosion, a series of observation information is reported below:

1. Formation of pit corrosion rather than surface corrosion
2. Formation of microbiological secretory masses
3. Release of hydrogen sulfide in anaerobic systems
4. Formation of iron hydroxide in aerobic systems
5. Large population of bacteria or fungi
6. In aqueous systems or waterless systems, accumulation of water in some areas
7. pH between 4.0 and 9.0 (MIC usually occurs in this pH range)

It should be borne in mind that if one or more of the above symptoms are observed, there is a possibility of microbiological corrosion. Microorganisms never form a new corrosion reaction that has never been seen before.

The microorganisms involved are different and among these are bacteria, fungi, and diatom algae. Bacteria are however the main culprits of MIC and therefore deserve further investigation. Major bacteria involved in MIC are sulfate-reducing bacteria (Enning, 2012), manganese/iron-oxidizing bacteria (Rajasekar, 2005), iron-reducing bacteria (Herrera, 2009), sulfate-oxidizing bacteria (Monachon, 2019).

- Sulphate-reducing bacteria

These bacteria which reduce sulfates to sulfides: are considered the most active in the degradation of metals by microbiological corrosion and are the most studied ones. These are anaerobic bacteria, which can grow in environments such as water and soil where their nutrients can be found. Practically they are found in all environments without oxygen and containing sulfates even in small quantities. The two most important kinds of sulfate-reducing bacteria are Desulfovibrio and Desulfomaculum. Although they are anaerobic, the sulfate-reducing bacteria can also grow in the presence of oxygen, if present in extremely low quantities. The corrosion mechanism of sulfate-reducing bacteria is quite complex. the MIC phenomenon, promoted by sulfate-reducing bacteria, can occur according to the following reactions:

$Fe \rightarrow Fe^{2+} + 2e^{-}$ (anode)

$2H^{+} + 2e^{-} \rightarrow H_2$ (cathode)

$SO4^{2-} + 4H_2 \rightarrow S^{2-} + 4H_2O$ (bacterial action and reduction of sulphate)

$Fe^{2+} + S^{2-} \rightarrow FeS$ (anode)

$3Fe^{2+} + 6OH^{-} \rightarrow 3Fe(OH)_2$ (anode). (Enning, 2012)

- Manganese/Iron-oxiding bacteria

These bacteria which oxidize iron and manganese: Iron-oxidant bacteria can oxidize iron from the ferrous to the ferritic state. The most important reaction mechanism is the following:

$4FeCO_3 + O_2 + 6H_2O \rightarrow 4Fe(OH)_3 + 4CO_2$

From this reaction it can be seen that the action of the iron-oxidizing bacteria has led to the precipitation of ferritic hydroxide which covers the metal surface with a slime layer. They are aerobic bacteria, but which require a low concentration of oxygen (<0.5 ppm) (Rajasekar, 2005).

- Iron-reducing bacteria

These bacteria are usually facultative anaerobes. They can live with or without oxygen (Kennet, 2017). They use oxygen aerobically and switch to ferric ion reduction used as a terminal electron acceptor under anaerobiosis. Some ferries on reducers can utilize this type of pathway to grow quite rapidly under anaerobic conditions. An iron-reducing bactewria is Shewanella putrefaciens (Herrera, 2009).

- Sulphate-oxidizing bacteria

They oxidize sulfides to sulphates, iron, and sulfur: they can be both aerobic and anaerobic. Among the different species of these bacteria, the most important is that of Thiobacillus, which survives at particularly low pH values (the optimum is at pH = 2.5) (Monachon, 2019).

1.3 Protocols for the restoration and conservation of metallic archaeological artefacts

After excavation, archaeological artefacts tend to suffer from changes of their state in order to find equilibrium with the new environment. These modifications can lead to the activation of dormant corrosion mechanisms. Moreover, thestorage and display conditions inside museums are unfortunately uncontrolled, with frequent fluctuation of temperature and relative humidity that enhance corrosion processes. Furthermore, the presence of pollutants in indoor environment can cause the development of further corrosion. So, corrosion can lead to serious damages of metallic artefacts. In order to combat corrosion phenomena again, various strategies have been developed to increase the strength of alloys. The research for appropriate protective systems, began in the ancient world, when natural waxes, oils and resins were used to protect copper alloys from corrosion. Nowadays, different strategies are applied in orderto avoid or, at least, to decrease corrosion mechanisms.

It is possible to intervene on the product by coating it with a layer that guarantees adequate protection conservation.

There are different ways of protection such as chromating, the use of paints and varnishes or the use of corrosion inhibitors and ceramic coatings. (Brandi, 1981; Appelbaum, 2007; Chiavari, 2007). Because of their toxicity, traditional corrosion inhibitors are no longer allowed due to an increase in environmental awareness and a change in the laws. Environmentally friendly corrosion inhibitors are becoming more popular (Abdel-Karim, 2022). Therefore, there are many treatments against biological corrosion including the use of biocide substances, the use of non- biodegradable synthetic polymer coatings, the control of environmental and chemical- physical conditions in order to inhibit the growth of microorganisms. Two types of protocols are used in the field of restoration and conservation of archaeological metal artefacts: traditional (painting and chromation); green (use of biological agents, fungi and bacteria). For copper metal artefacts, were applied protective chemical treatments such as: protective coating such as Incralac©, Paraloid B72 or microcrystalline waxes, inhibitors as benzotriazole or a combination of the two are commonly used by conservator- restorers on archaeological objects (Argyropoulos, 2007). Nevertheless, these treatments interact with the artefact changing its aspect, particularly its colour and brightness(Faltermeier, 1998). Moreover, they are not completely efficient in the protection of the object and most of them are toxic for the operators and the environment (Cano, 2010). For this reason, conservator-restorers are looking for novel efficient and harmless alternatives to such treatments.

- Traditional protocols

- Painting and varnishes

One of methods to protect a metal surface from the external environment is painting. It is a heterogeneous system consisting of pigments in a matrix, called film-forming, which allows its application and physical continuity.

One of the most important ways to protect against corrosion is the selection of corrosion-resistant material. When choosing the material, the environment where the metal has to be used should be taken into consideration. Corrosion-resistant metals and alloys should be selected according to the chemical properties of the environment in which the metal will be used. It should be taken into account that the selected metal or alloy is also resistant to microbiological corrosion. The surface of the material to be used can also be protected from corrosion by applying protective coatings such as epoxypaint, metal coating or plastic. However, the point to be considered here is that when the coating material is decomposed, corrosive compounds are not released into the environment and this coating material is not used as a nutrient by bacteria. (Videla, 1996). The liquid part of the paint, the vehicle, is made up of several elements such as binders, plasticizers, solvents, adducts and auxiliaries. The binder is the constituent of the paint capable of forming the film. The solvent is present in the mixture, necessary to give the right viscosity for distribution on the surface. Plasticizer additives may be present to modify the properties of the coating by increasing its deformation capacity without breaking and are used for films based on thermoplastic polymers. The solid part is made up of pigments which are synthetic substances which can block the corrosive process or increase the impermeability and adhesion to the support of the coating. The film is formed either by physical means, i.e. by simple evaporation of the solvent and thinner,or by cross-linking of the polymer chains by reaction with atmospheric oxygen or by the presence of a hardener.

The protective action of the paints is based on the barrier effect against the diffusion of oxygen and water through the layer, added to the protective effect of the active pigments. Several coats of paint are used: a primer, a possible intermediate and a finishing coat.

An effective paint for bronze is Methacrylate or Incralac, based on acrylic resin and benzotriazole. This gives a transparent, brilliant layer with elastic properties and is thermally and hygrometrically stable.

Paraloid B72 is another possible solution, it is a medium hardness copolymer based on ethyl methacrylate and methyl methacrylate (70/30). Its use reduces microporosity, making the object more compact, less brittle, reducing the absorption of water both on the surface and in depth and does not alter the natural colors being the resin transparent and resistant over time (Videla, 1996).

Its advantages are:

flexibility, not attract dust, no plastic deformations products,very stable.

The application can be carried out by brush, spray or immersion; in the industrial field, spraying is clearly preferable (Pedeferri, 1993; Periolatto, 2017).

- Chromatation

Another technique to secure the surface against foreign chemicals is the use of conversion layers. These are chemical or electrochemical coatings to increase the corrosion resistance of the base metal. The conversion layers are very thin (0.5 µ or less thick) and have the property of providing corrosion protection. The conversion layersare obtained by spraying and, above all, by immersion (Bianchi, 1989)

With the chromating process there is a surface of chromium oxides with a protective and decorative function. The process involves treating the metal with an acid solution with or without external current.

The main components of the chromating bath are two:

hexavalent chromium compounds both in the form of chromic acid and chromates

organic compounds that are called catalysts or activators.

The protection mechanism is two fold since the presence of Cr^{6+} in the film mass gives a cathodic inhibiting action and the non-porous nature of the film excludes the penetration of moisture that could remove Cr^{6+} (Bianchi, 1989).

- Benzotriazole (BTA)

Since the most concerning problem of archaeological objects is the reactivation of corrosionmechanisms, it was decided to compare the biopatina treatment with BTA, a commonly used corrosion inhibitor. BTA does not remove copper chlorides from the corrosion layers but forms a layer of Cu-BTA polymeric complexes. Some authors have thought it advisable to carry out a preliminary mechanical cleaning, in particular on pustules and chloride craters. After this step, the artchaeological metal artefact were impregnated under vacuum with a 1% w/w benzotriazole solution in deionized water (pH 6) for 3 hours (Golfomitsou, 2004) using a vacuum chamber Salvis© (50 mbar pressure) and then immersed for 28 days under agitation in the same solution. It should be noticed here that there is no agreed protocol in the literature for the application of benzotriazole solutions. Many authors (Dugdale, 1963; Cotton, 1967; Brostoff, 2003; Watkinson, 2010) reported that BTA forms a polymeric complex with copper on top of the corrosionproducts actin as a barrier for corrosion and that this film is very thin and chemically resistant. Recently, it has also proposed that BTA protects copper in both aqueous and gaseous environments polluted with sulphur dioxide, hydrogen sulphide and salt mist (Watkinson, 2010). Also, the chosen solvent for the preparation of BTA solution seems to be arbitrary and subject to individual practices.

- Green protocols

In the last few years, there is a pressing need to develop novel conservation methods compatible with the metallic artefact’s patina and there is an interest for the synthesis of inorganic materials by biological agent because they are environmental friendly processes. The nature of corrosion products present on the surface of archaeological artefact, is related with the environmental context. For this reason, research studies have been carried out on the use of biological agents and their potential inhibitory effect on other corrosion phenomena (Joseph, 2013). The use of fungi, such as *Beauveria bassiana* Vuill., like corrosion inhibitor could represent an innovative treatment for archaeological metal artefact. This biological use is in contrast with traditional protocol (painting) that has only the role to create a barrier against aggressive environments (Joseph, 2012).

Beauveria bassiana Vuill. is a cosmopolitan anamorphic ascomycete fungus. It is an entomopathogenic fungus that attaches itself as a spore to the insect's cuticle and germinates by colonizing the host with its hyphae. It is capable to colonize more than 700 host species and has been isolated in both fallow and cultivated environments (Inglis, 2001; Meyling, 2006). For these properties, *Beauveria bassiana* Vuill. has always been considered an excellent biological control agent for pests in organic agriculture and has been used in the development of numerous commercial products (Inglis, 2001).

Despite its primary pathogenic behavior, this fungus can also live in soil as a saprophyte, but extensive proliferation and dispersal are limited (Keller, 1989; Hajek, 1997). In this environment, the population's survival depends on the production of infectious conidia released from the host's cadaver over time after sporulation (Gottwald, 1982). Furthermore, some evidence has shown that B. bassiana could be included in the range of endophytic fungi. Its presence in plant tissues of corn, cocoa and poppy (Quesada-Moraga, 2006) could protect plants from herbivorous insects (Elliot, 2000; White, 2002). *Beauveria bassiana* Vuill. has also been isolated in air samples (Shimazu, 2002; Ulevicius, 2004) and air transport and deposition could explain its occurrence on plant phyllopians (Meyling, 2006). Some abiotic factors can affect the development and survival of *Beauveria bassiana* Vuill., like temperature, humidity, and solar radiation. Non-optimal temperature can inactivate or activate spores' germination and reduce or accelerates mycelial growth. In *Beauveria bassiana* Vuill., the optimum temperature is between 23 and 28°C, the minimum between 5 and 10°C, and the maximum at about 30-38°C, depending on the strain (Zimmermann, 2007). The synergic effect of high temperature and high or low humidity affects spores' viability. The optimal range of relative humidity is between 100 and 92% (Hallsworth, 1999). The UV component of solar radiation, in particular, UV-A and B is the most dangerous factor for fungal survival and in simulated sunlight, irradiation experiments just half of *Beauveria bassiana* Vuill. conidia were still viable. Numerous studies have been done on the toxicity and risks related to human health regarding the use of this fungus as a biological control agent. Hazards from human exposure are extremely rare. This fungus is the ideal candidate to be used in green protocols for the restoration and conservation of archaeological metal artefacts composed of copper and copper alloys (Langle, 2006; Zimmerman, 2007; Shahid, 2012).

Entomopathogenic fungi can produce different metabolites to perform specific activities and functions. Secondary metabolites of the genus Beauveria are bassianin, bassiacridin, beauvericin, bassianolide, beauverolides, tenellin and oosporein (Strasser, 2000; Glare, 2004). *Beauveria bassiana* Vuill. also produces an important extracellular organic acid, oxalic acid (Roberts, 1981), which can solubilize specific cuticular proteins (Vey,2001). Oxalic acid is one of the strongest organic acids and it is produced by many fungi. Its production is often associated with metal solubilization, increasing its bioavailability. Indeed, fungal growth needs the availability of essential elements, such as sodium, potassium, copper, zinc, cobalt, calcium, magnesium, manganese, and iron. Among these elements, all metals, particularly heavy metals, can become toxic if present in high concentrations. The production of oxalic acid is also related to this issue providing metal immobilization, and environmental detoxification (Gadd, 1993; Burford, 2003; Jarosz-Wilkolazka, 2010). Indeed, free ions of toxic metals can be complexed by oxalic acid forming highly insoluble biogenic crystals of metal oxalates and thus reducing the toxicity of the medium (Gadd, 1993; Gadd, 2010). During fungal growth, the production of organic acids is influenced by the pH and the buffering capacity of the environment, the carbon sources and the presence of certain metals. In the presence of copper metals, though, the production of oxalic acid may increase. During my research project in Neuchâtel, I conducted experiments with her and her staff to construct an artificial biopatina to restore or preserve copper artefacts using *Beauveria bassiana* Vuill.

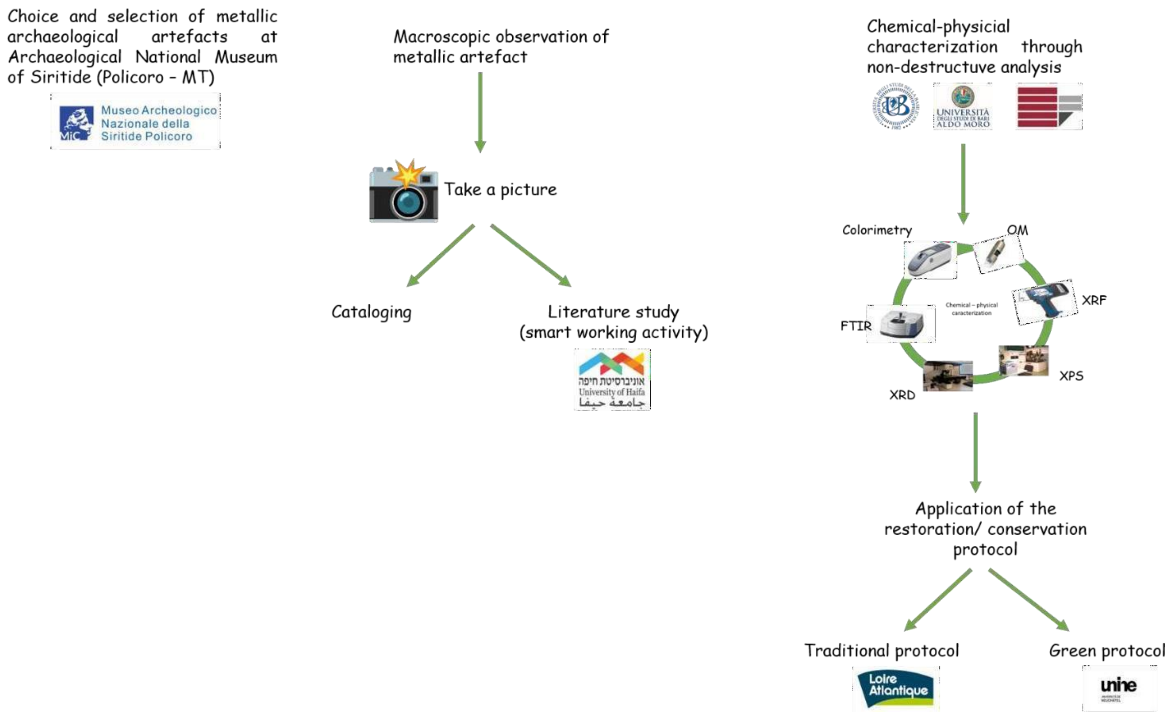
1.4 Aims

The study of archaeological metal artefacts is very important for reconstructing history, learning about civilizations and trade routes.

The phases of this research project were:

- Choice and selection of archaeological metal artefacts preserved in the National Archaeological Museum of Siritide - Policoro (Matera, Italy).
- Mapping of main raw materials used for the artefacts and indicate thresold of production techniques, aimed to identify the supply and manifacturing areas.
- Identification of the main forms of alterations and degradation and definition of danger indexes to indicate threshold values beyond which restoration is necessary. This result allowed us to evaluate the right planning of restoration interventions in a perspective of maintenance and enhancement of a given collection.
- Definition of adequate cleaning protocols about the type of product and the type of degradation identified. This result allows choosing the best type of protocol to remove the surface patina without compromising the metal substrate and evaluating the possible use of biological agents for cleaning and conservation protocols.

1.5 Flowchart



2. EXPERIENCE AT SIRITDE NATIONAL ARCHAEOLOGICAL MUSEUM – POLICORO (MT)

2.1 Selection of archaeological metal artefacts

A set of seven metal artefacts found during archaeological excavations in the Policoro area (Basilicata) and housed in the National Archaeological Museum of the Siritide, have been subjected to non-destructive chemical, physical and biological characterization (Table 1).

These samples (Figure 1, Table 1) were chosen because they represent a metal set and also because they had different intended uses and different places of discovery during excavations in the Policoro area (Siris-Herakleia archeological site, Figure 2).

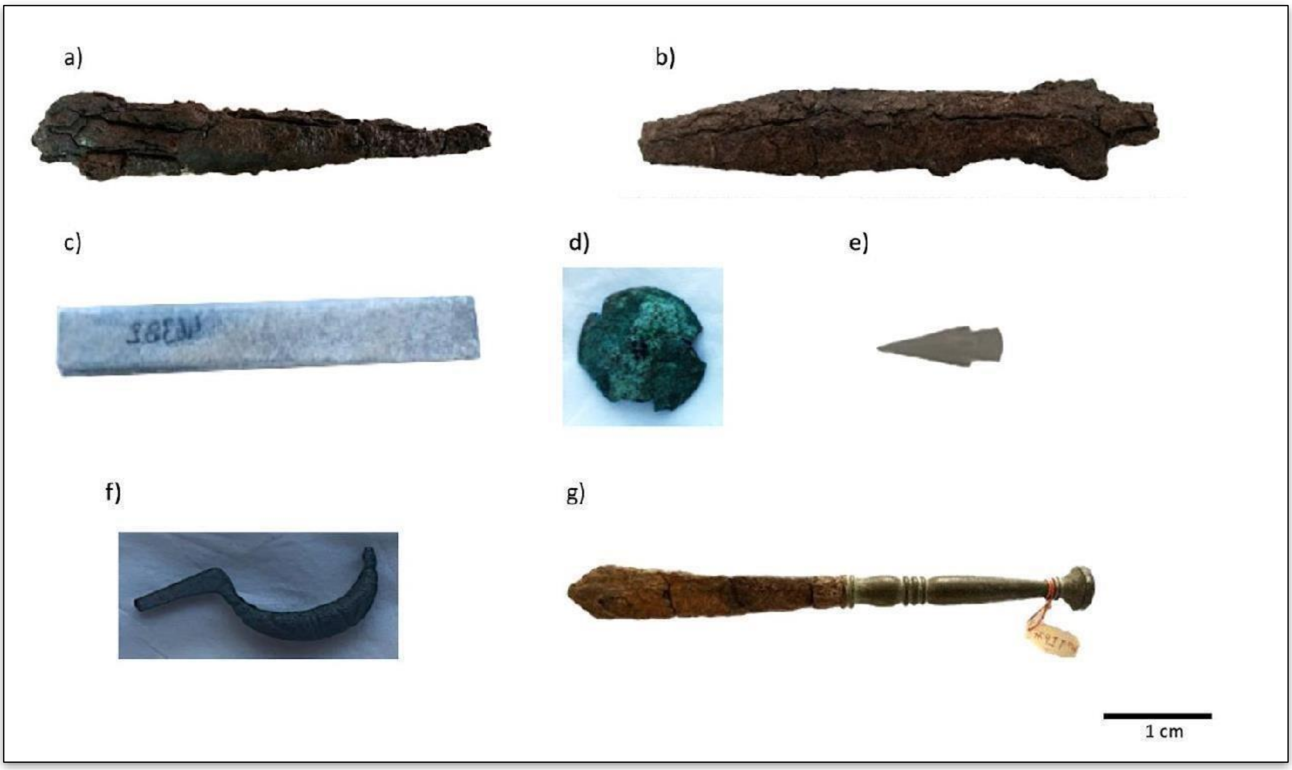


Figure 1. Studied archaeological metal finds: a) javelin tip; b) iron weapon; c) lead stick; d) flask cap; e) arrowhead; f) fibula; g) lancet.

Table 1. Archaeometric identification of metal artefacts collected from the Policoro area.

Sequence	**Identification code**	**Artefact**	**Archeological context**	**Excavations places**	**Size (cm)**
A	207559	Javelin tip	necropolis context	St. Arcangelo	length: 14 thickness: 0.6 – 2.5
B	216346	Iron weapon	sacral context	Demetra sanctuary	length: 14 thickness: 0.3
C	46382	Lead stick	votive offer	Siris acropolis	length: 5
D	46995	Flask cap	funerary context	Siris, western necropolis	diameter: 2.4
E	35207	Arrowhead	housing context	Herakleia acropolis	length: 2.5
F	38747	Fibula	votive offer	Siris archaic temple	length: 5 thickness: 0.5 – 1.0
G	35514	Lancet	housing context	Herakleia acropolis	length: 12.9

Figure 2. Aerial view of ancient Siris and Herakleia in Policoro (Google Maps Satellite on 31 March 2023).

2.2 Geological framework

Policoro is located in the Metapontum plain, on the Gulf of Taranto. It lies between the mouth of the Cavone River and the settlement of Marina di Ginosa in the South Eastern territory of the Basilicata region along the Ionian coast. It includes the final stretch and the mouth of the Sinni River. The area of Policoro falls within a sector of the Fossa Bradanica where "terraced marine deposits" or "deposits of terraced coastal prisms" of the Middle-Upper Pleistocene age (1.5-0.12 Ma) emerge, resting on sub-Apennineclays, sandy, sandy-gravelly and sandy-silty deposits of the alluvial and coastal plains of the Holocene age (Pescatore, 2009; Sabato, 2011) (Figure 3).

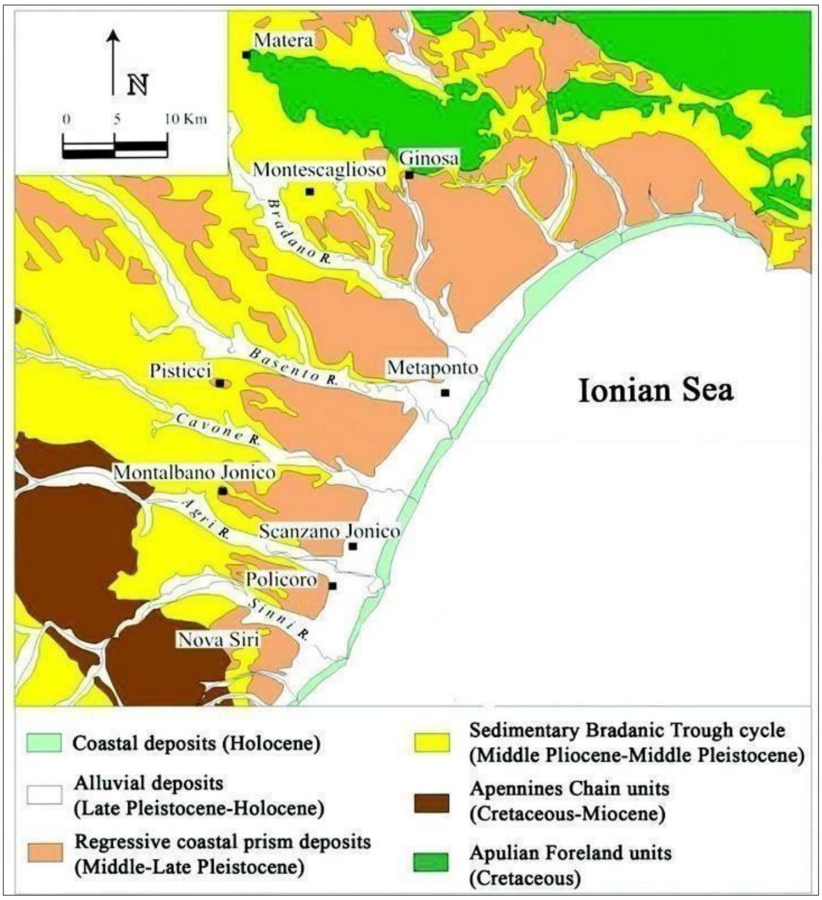


Figure 3. Schematic geological map of the Bradanica trench sector. Sheet N° 508 "Policoro" (modified after Pescatore et al., 2009; Sabato et al., 2011).

2.2.1 Siris and Herakleia

Siris owes its name to the river on which it lays. The surrounding area, which today retains the name of Siritide, was exalted for its beauty by the poet Archilochus (8^{th} century BC). The inhabitants of the city of Colophon had set out to colonize the town of Siritide. In the second half of the 6^{th} century BC. Siris was eliminated by the Sybarites who were allied with the Achaean colonies of Crotone and Metaponto. This warfare perfected an Achaean hegemony that had already been initiated with the foundation anti-Tarantine of Metapontum at the end of the 7th century.

The subsequent fate of the Syrian settlement remains obscure to us both before and after destruction of Sybaris in 510. After Siris was destroyed, the colony of Thuroi was founded at the behest of Pericles and among the first inhabitants was Herodotus. This new colony was founded to have the Doric colony of Taranto as a rival, with which Thuroi soon came into conflict. Eventually, however, the two rivals allied and decided to found a new colony "Herakleia" (Seibert, 1963). Herakleia, in the second half of the 4th century BC, passed under the dominion of Alexander Milosso. In 212 BC, Hannibal used Herakleia as a supply base during his campaigns in Southern Italy. Only in 90 BC, Herakleia assumed the name of the municipality. The grandeur of Herakleia is due tothe bronze plates, found in 1732, which constituted a document of great importance for linguistic, constitutional, social, and economic history. The presence of spring waters and canals, present in the area together with the rural landscape, was the ideal place for the construction of sanctuaries such as those of Dionysus, Demeter, and Ascelpio. The sanctuary of Demeter is located on the southern slope of the valley and is located preciselybetween the city of Heraklea and the Castle of the Baron (Osanna, 2011). This sanctuary was the most frequented sacral pole. During the archaeological excavations carried out, iron weapon (Figure 1b, Table 1b) was found.

2.3 Analytical techniques

The most widely used investigation techniques for the analysis of materials of archaeological artistic interest, in addition to the purely analytical parameters of accuracy, precision and sensitivity, they must have the appropriate characteristics to enable them to be applied to the survey scientific of the works of art; among these the most important is the non-destructiveness. The non-destructive techniques are that preserve the integrity of the sample submitted for analysis: it follows that are considered non-destructive techniques that can be used directly on the find, and those techniques that provide for the analysis of a portion of the object, provided that this portion does not change during the analysis itself (non-destructive analysis). From the point of view of the archaeologist, the art scholar, the conservative, and the restorer, therefore, a technique is considered non-destructive because it does not require sampling. The archaemotric research uses all available techniques and methods developed within specialized scientific disciplines (i.e. chemistry, physics, geology, biology) to mainly serve conservation science and archaeology (Balassone, 2018). The characterization of archaeological materials plays a big role in determining the production technology of that period. Physical and chemical analysis techniques, can be used in the characterization of archaeometric manufacts. These analytical techniques play an important role in determining the properties of metal materials such as composition, element distribution/regional chemistry, surface/interface chemistry, grain size, distribution, morphology and texture, and phase identification/molecular structure. The goal of these techniques is to aid archaeology to unravel the human past and use the information derived from material to understand past societies (Artioli, 2010).

These analytical techniques used on the samples studied were carried out at University of Basilicata (Potenza), University of Bari and University of Calabria.

- Optical microscope

This technique gave us indications on the physical structure of the metal or alloy examined, such as the distribution of the phases, the shape and size of the grains, the state of the sliding planes, the presence of inclusions and micro-activities. This analysis also makes it possible to obtain very important information on the state of conservation and on the history of the building, production, but not on its composition.

Differential interference contrast: interference and polarization effects are exploited. The interference allows to analyze transparent substances but with refractive index different from the Medium in which the sample is placed. There are two polarizers, one of which for the light beam exiting the sample, thanks to which the irregular areas will be differently colored.

The Dinolite AM4113TFVW Portable Digital Optical Microscope was used for the investigation. The analysis were performed using visible light and UV LEDs with magnifications of 50 units of measurement (real size of about 7 mm) and 200 units of measurement (real size of about 2 mm).

Technical features: Resolution: 1.3 Mpixel, Magnification: 10 × ÷ 50 ×; 200 ×, Illumination: 4 UV LEDs with 400nm + emission; 4 built-in white LEDs, Frame rate: more than 30 fps, Interface: USB 2.0, Sensor: Color CMOS, Manual calibration and measurement with accuracy of approximately ± 3μm. This instrument was used to analyze the arrowhead and the lead stick at the University of Calabria (Arcavacata di Rende, Cosenza, Italy).

- X-Ray Fluorescence (XRF)

This investigation allows the non-invasive analysis of the chemical elements, majority and trace, providing information on the original materials and those of degradation and / or restoration, on the executive technique and on the state of conservation, as well as on the probable origin and dating of the cultural property. However, this technique allows the analysis of chemical elements with an atomic number greater than that of sodium ($Z > 11$), forming the sample within a maximum thickness of approximately 100 μm fromthe surface. In fact, it is a non desctructive technique that allows to perform both compositional and / or screening analysis without destroying the sample. Among its characteristics, it has also the advantage of being highly sensitive because its detection levels up to a few ppm (mg/Kg). The investigated volume depends, in fact, from the elements present both in the material and chemical surroundings, determining the so-called *matrix effect*. This technique can be used on artefacts on which it is not possible to carry out micro- reliefs or which are in such a state of conservation that do not make possible the sampling (Ingo, 2006). The analysis were conducted using a portable instrumentation assembled mainly composed of three units in addition to the software for the management of the instrument and for the acquisition of data and precisely:

- X-ray source (Mini-X Amptek with power up to 50 kV);
- Detector (X-123 SDD Amptek with a resolution of 0.135 keV on the Kα line of the Mn);
- Self-built support necessary to keep the source-sample-detector geometry constant;
- Acquisition interval range: from 1 to 27 KeV.

With the system used, the fluorescence radiation of the sample was recorded by a solid state SDD (Silicon Drift Detector) which allowed to identify in a single measure all the detectable elements in the sample. This instrument was used to analyze javellin point, iron weapon, flask cap, fibula and lancet at the University of Bari, Italy.

Instead, to analyze the arrowhead and lead stick was used the portable XRF instrument with following chacteristics:

- X-ray source (Mini-X - Amptek with power up to 40 kV);
- Detector (X- 123SDD – Amptek with resolution of 125 - 140 eV);
- Acquisition interval range: from 1 to 40 keV;

This instrument was used at the University of Calabria (Arcavacata di Rende, Cosenza, Italy).

X-Ray Diffractometry (XRD)

This technique has more advantages like high sensitility, non-destructive nature, easy sample preparation, fast speed. It has been used to do both qualitative and quantitative analysis. It is used to study the crystalline content. It can be used to determine the crystallinity by comparing the integrated intensity of the background pattern to that of the sharp peaks (Singha, 2006; Chauhan, 2011). Under certain conditions, such as 0°C for water at 1 atm, a new arrangement of atoms or molecules may become stable, leading to a phase transition. At this point, new diffraction peaks will appear or old ones disappear according to the symmetry of the new phase (Skakle, 2005). Every mineral is defined by a characteristic crystal structure that gives a unique X-ray diffraction pattern,allowing rapid identification of minerals present within a rock and soil sample (Skoogh, 2007). The Empireneal Diffractometer (Panalitycal) was used in this study. Technical characteristics of this instrument are the use of Bragg-Brentano geometry, an X-ray tubewith copper anode used as a source, the mono-chromatizer used is a nickel filter and the detector is a 3D PixCell. This instrument was used to analyze the probe at the University of Bari, Italy.

X-Ray Photoelectric Spectroscopy (XPS)

This technique called ESCA (Electron Spectroscopy for Chemical Analysis), provides a total elemental analysis, except for hydrogen and helium, of the first 10–200 Å (depending on the sample and instrumental conditions) of any solid surface stable under vacuum or can be made vacuum stable by cooling. Information on chemical bonding is also provided. Of all the instrumental techniques currently available for surface analysis, XPS is generally considered to be the most quantitative, the most easilyinterpretable, and the most informative with regards to chemical information. For these reasons it was highly recommended (Pintori, 2022).

The XPS analysis were conducted with the use of the double anode XPS PHOIBOS 100-MCD5 spectrometer (Mg Kα and Al Kα).

The spectrometer is equipped with a multi channel detector that allows to work at high lateral resolution (and in energy), maintaining high sensitivity in the electronic counting. The spectra were acquired with the Mg Kα (1253.6 eV) source operating at 10kV and 100 mA, in 'medium area' mode with a lateral spot of approx. 2 mm. This instrument was used to analyze arrowhead at the University of Basilicata (Potenza, Italy).

- Biological analysis

Sampling and isolation of the bacterial strains collected on the surfaces of the metal finds were carried out according to the Recommendation of the Ministry of Cultural Heritage n.3/1980, by using both sterile and dry swabs. Subsequently, biological samples were isolated, dissolving in 500 µL of sterile Ringer's solution, vortexing for 10 minutes, and pipetting a 100 µL aliquot into 5 mL of Plate Count Broth (PCB); after 24 hours of incubation at 30°C, the culture was used for inoculation on Plate Count Agar (PCA) plates, which were further incubated at 30°C for seven days. Then the plates were observed by using a standard light microscope to check for the presence of biological colonizers on the metallic material.

The biological analysis did not reveal the presence of microorganisms. Since then, the Museum has usually been exhibited the metal finds to the public and they had probably been subjected to cleaning by using disinfectants. Therefore, we could not state if their visible corrosion derives from a microbiological deterioration or is due to other environmental issues that arose in the archaeological site of origin. For this reason, the biological data are not discussed in the following sections.

2.4 Results

Table 2 shows the non-destructive analyses performed for each artefact. Only XRF was applied to the whole metal finds selected by the Museum because this techniqueallowed us to know the raw materials and the probable alloys of the object under examination, given a future application of a green cleaning protocol. The other techniques helped to understand the main corrosion products that appeared on the macroscopic observation.

Table 2. Diagnostic analysis performed on the artefacts.

Sequence	**Artefact**	**OM**	**XRD**	**XRF**	**XPS**
a	Javelin tip			X	
b	Iron weapon			X	
c	Lead stick	X		X	
d	Flask cap			X	
e	Arrowhead	X		X	X
f	Fibula			X	
g	Lancet		X	X	

Legend: OM Optical microscopy; XRD X-Ray diffractometry; XRF X-Ray fluorescence; XPS X -Ray photoelectric spectroscopy.

- Optical Microscopy (OM) study

This technique provided information about the physical structure of the metal finds (Moropoulou, 2013).

Figures 4 and 5 show the lead stick and arrowhead surface obtained by OM characterization.

From the analysis done with the optical microscope, it can be seen that the composition of the patinas/concretions present on the surface of the lead stick (Figure 4) are characterized by a high inhomogeneity of thicknesses

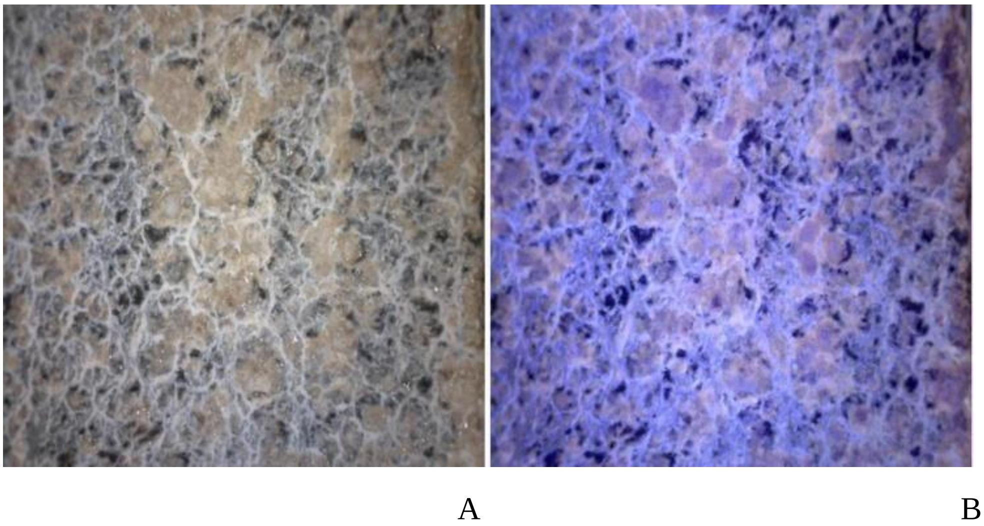


Figure 4. Optical microscopy analysis of the lead stick (50 units magnification; [A] vis. and [B] UV pictures).

The analysis carried out on the arrowhead (Figure 5) under an optical microscope reveals that the artefact shows different thicknesses and compositional inhomogeneities of the patinas formed as a result of the typical corrosion processes of bronzes (formationof copper oxides, and decuprification).

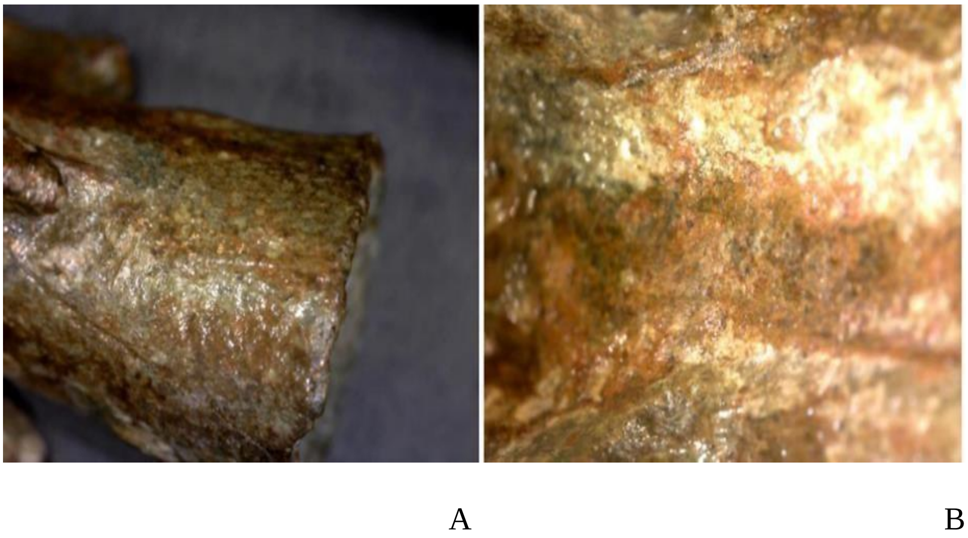


Figure 5. Optical microscopy analysis of the arrowhead ([A] 50 and [B] 200 units vis. magnifications)

X-Ray Fluorescence (XRF) analyses

The corrosion and oxidation phenomena did not allow for visually recognizing of the component materials. While with the help of the XRF application, it was possible to detect all the elements of the alloy used for each studied sample and to determine the main component. Table 3 shows the results of the XRF analyses.

Table 3. Elements and materials of the artefacts

Sequence	**Artefact**	**Identification code**	**Ca**	**Ti**	**Mn**	**Fe**	**Cu**	**Zn**	**Sn**	**Pb**	**Material**
a	Javelin tip	207559	X	-	tr	X	tr	tr	-	-	Iron
b	Iron weapon	216346	tr	-	-	X	tr	-	-	-	Iron
c	Lead stick	46382	tr	-	-	-	-	-	-	X	Lead
d	Flask cap	46995	tr	-	-	tr	X	tr	X	tr	Bronze
e	Arrowhead	35207	tr	-	-	tr	X	-	X	X	Copper
f	Fibula	38747	tr	tr	-	tr	X	tr	X	tr	Bronze

g	Lancet blade	35514	tr	-	-	X	tr	tr	-	-	Iron
	Lancet handle		tr	-	-	tr	X	X	X	tr	Brass

Legend. X: main element; tr: traces

- Javelin tip

The XRF results (Figure 6) obtained for the javelin tip (Table 1, Figure 2a) highlighted Fe as the main constituent and Ca that probably belongs to some exogenous alteration materials. Traces of other elements were also detected (Table 3).

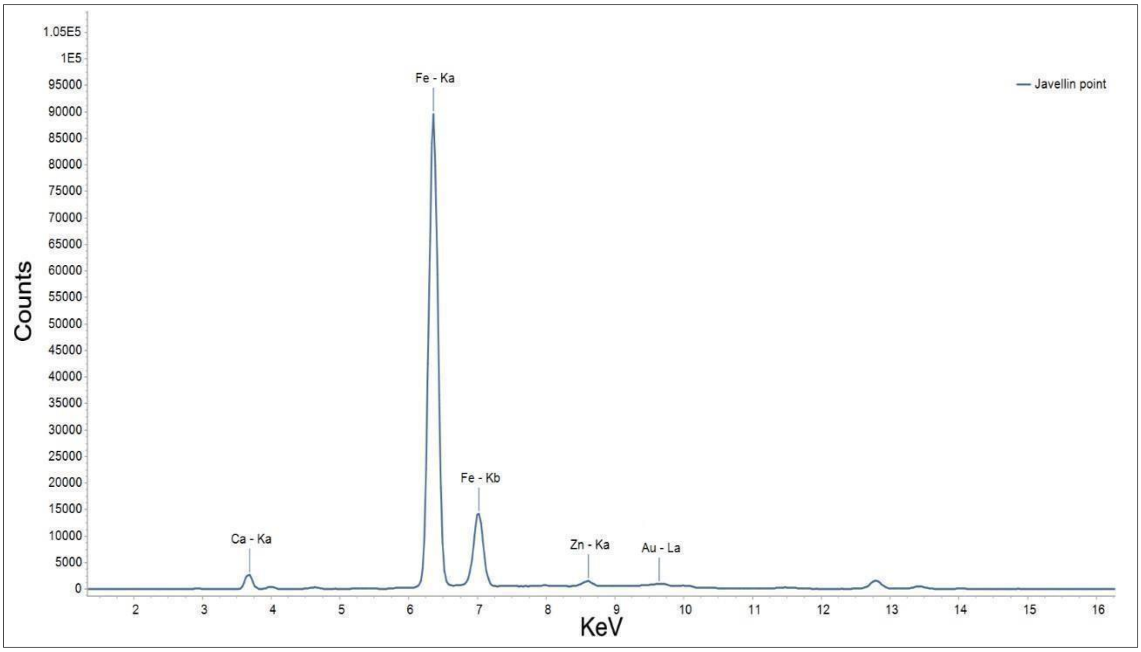


Figure 6. XRF analysis of the javelin tip carried out at University of Bari (Bari).

- Iron weapon

The iron weapon (Table 1, Figure 2b) evidenced a somewhat deteriorated appearance. The XRF analysis showed Fe as its main component (Figure 7). Traces of Ca and Cu were also found (Table 3).

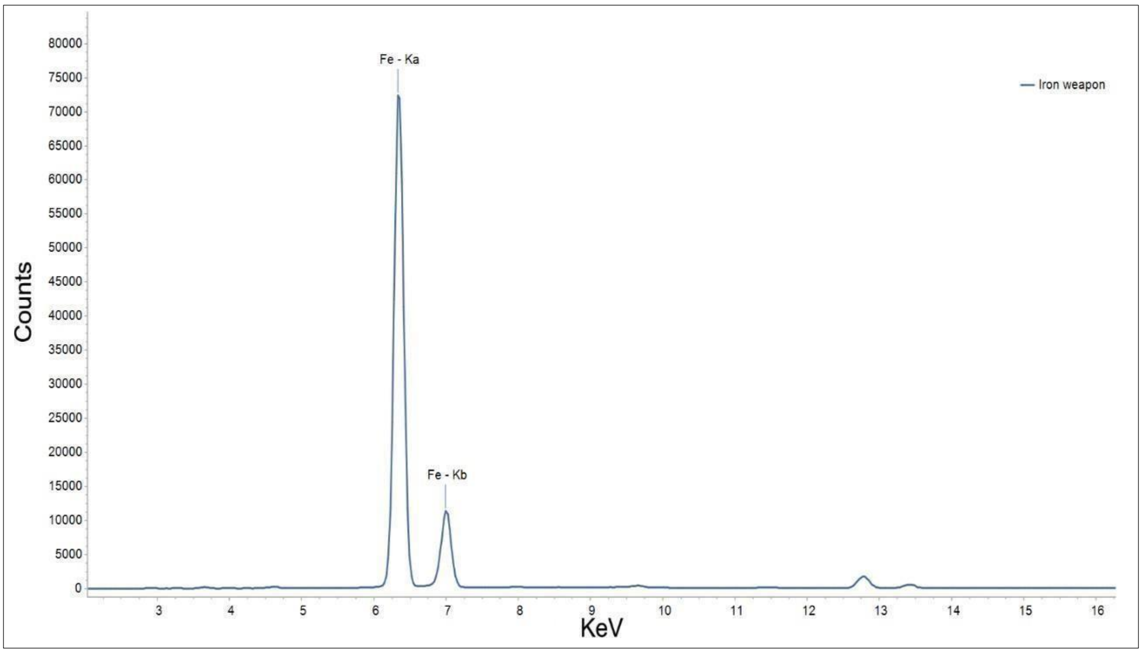


Figure 7. XRF analysis of the iron weapon carried out at University of Bari (Bari).

- Lead stick

The lead stick (Table 1, Figure 2c) is made of Pb, showing severe degradation even with macroscopic observation.

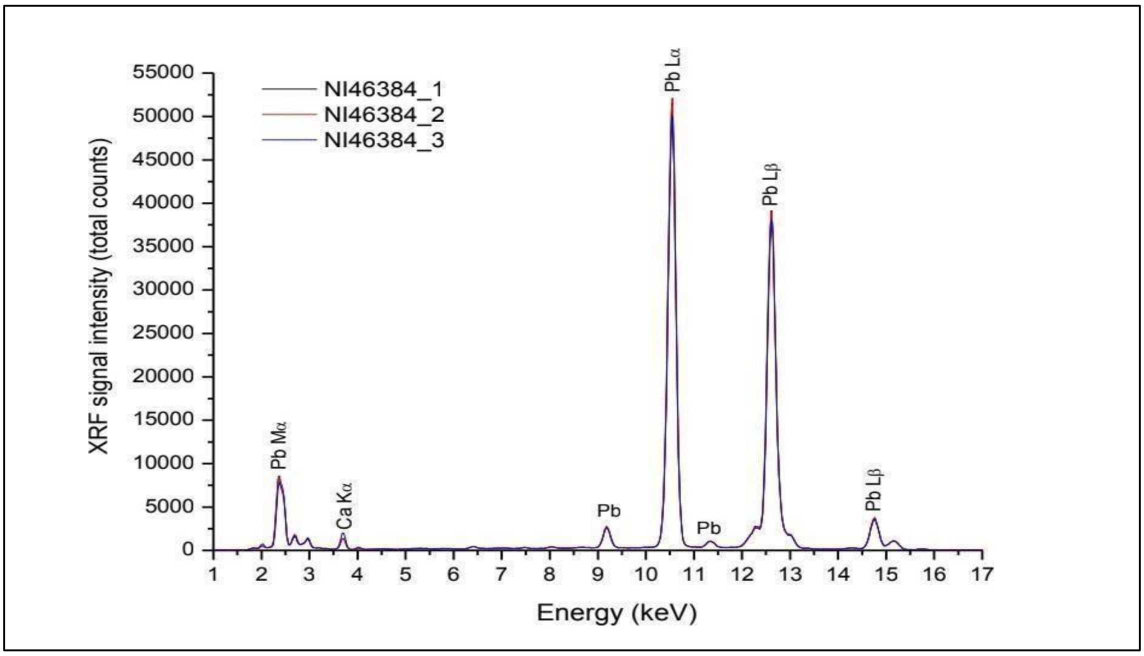


Figure 8. XRF analysis of the lead stick carried out at University of Calabria (Avarcata di Rende- Cosenza).

On this find, it was possible to carry out analyses with the portable OM (Figure 4) and XRF (Figure 8). Also, in this case, traces of Ca due to natural contamination were evidenced by XRF (Table 3).

- Flask cap

The flask cap (Table 1, Figure 2d) already appeared with macroscopic observation deteriorated significantly. XRF analysis (Figure 9) highlighted the presence of Cu and Fe as elemental components of the alloy and Ca as a contaminant (Table 3).

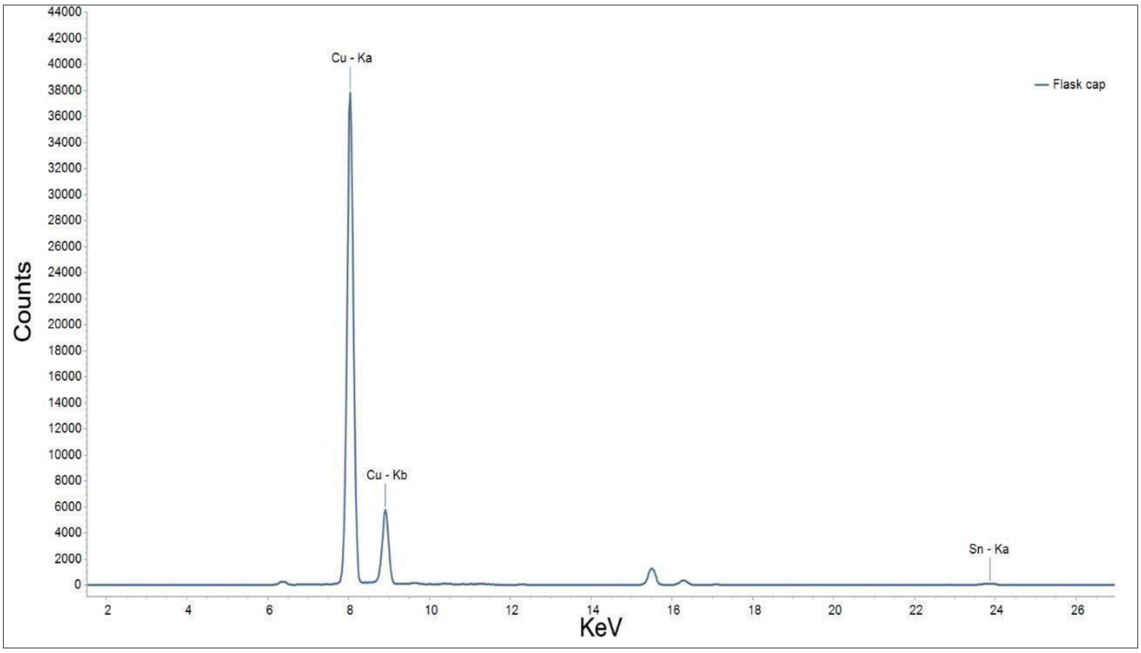


Figure 9. XRF analysis of the flask cap carried out at University of Bari (Bari).

- Arrowhead

XRF analysis of the arrowhead (Table1, Figure 2e) showed that Cu (prevalent), Ni, Pb (high), Sn, Zn , and Fe constitute the elemental composition of the alloy, and Ca is in the trace as a contaminant (Table 3, Figure 10).

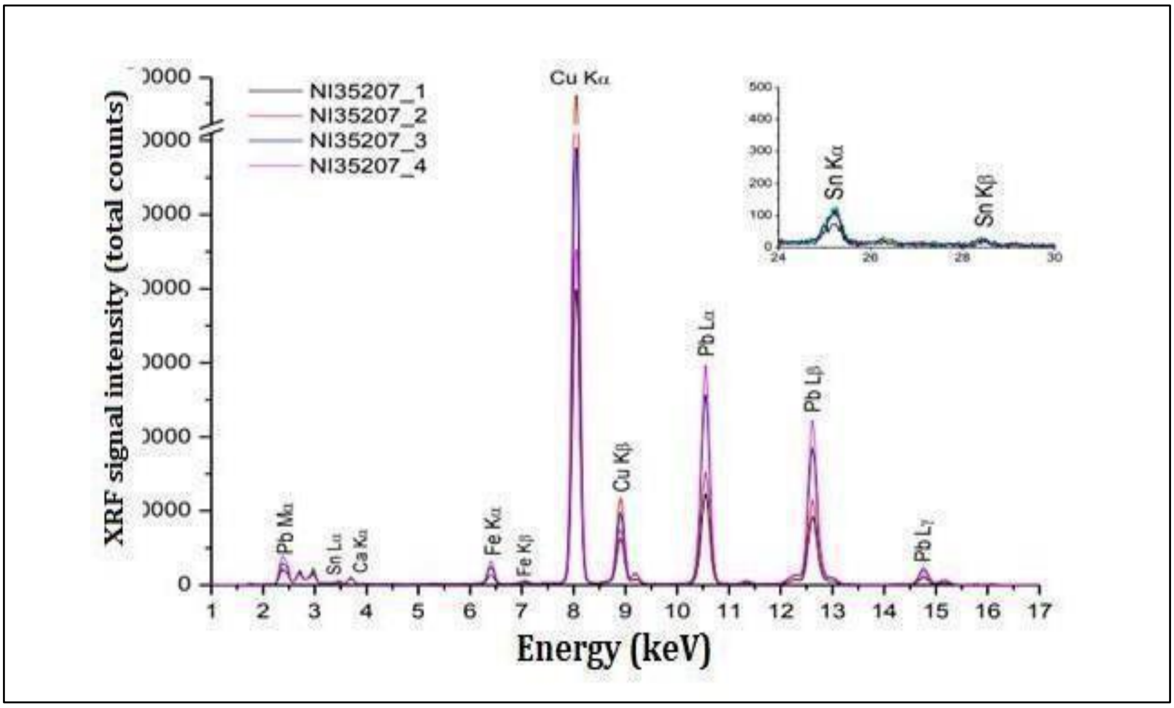


Figure 10. XRF analysis of the arrowhead carried out to University of Calabria (Avarcata di Rende - Cosenza).

- Fibula

The XRF analysis of the fibula (Table 1, Figure 2f), showed that the main components were Cu, Sn, Pb, Fe, and Ca (Table 3, Figure 11). The object appeared oxidized but still intact.

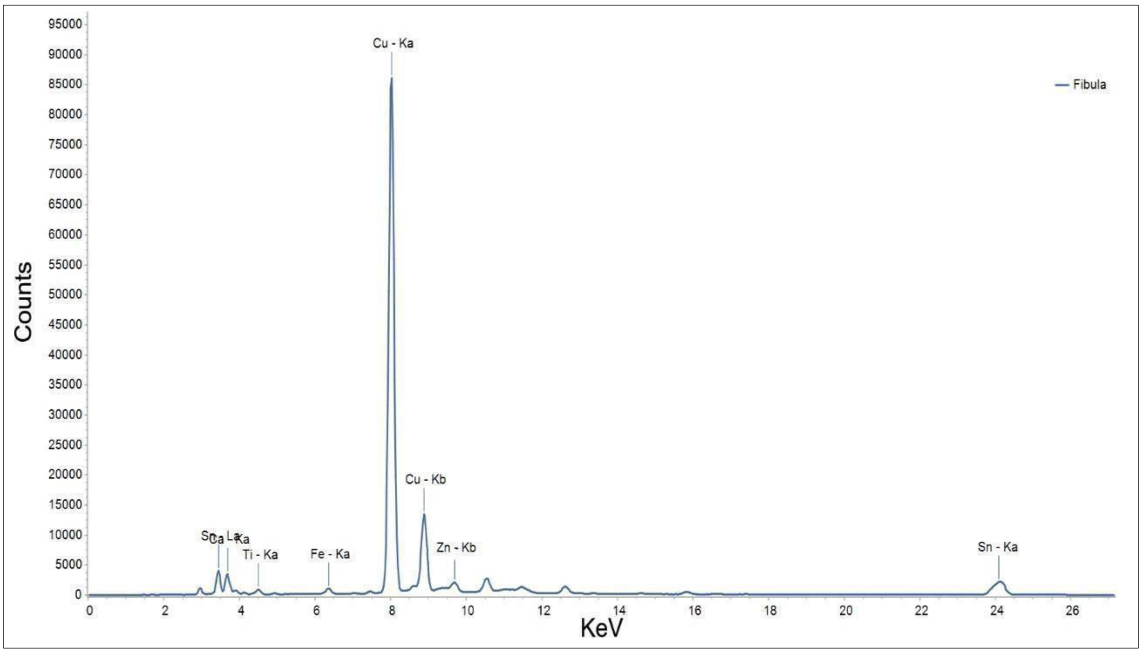


Figure 11. XRF analysis of the fibula carried out at University of Bari (Bari).

- Lancet

Two XRF analyses had to be carried out on the lancet (Table 1, Figure 2g) since the lancet blade and the handle appeared to be produced with different materials to macroscopic observation. The XRF analysis of the lancet blade (Table 3, Figure 12) cleared that it was created with only iron as a raw material. In contrast, the XRF analysis of the lancet handle (Figure 13) showed that it was produced with an alloy of Cu, Zn, and Fe (Table 3).

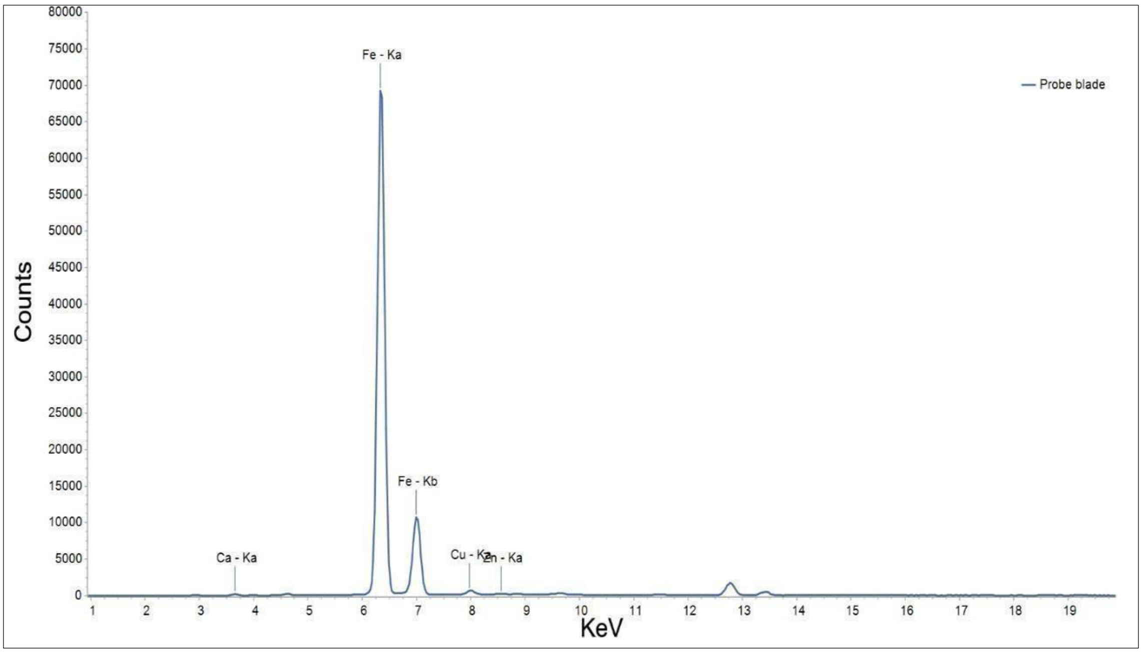


Figure 12. XRF analysis of the lancet blade carried out at University of Bari (Bari)

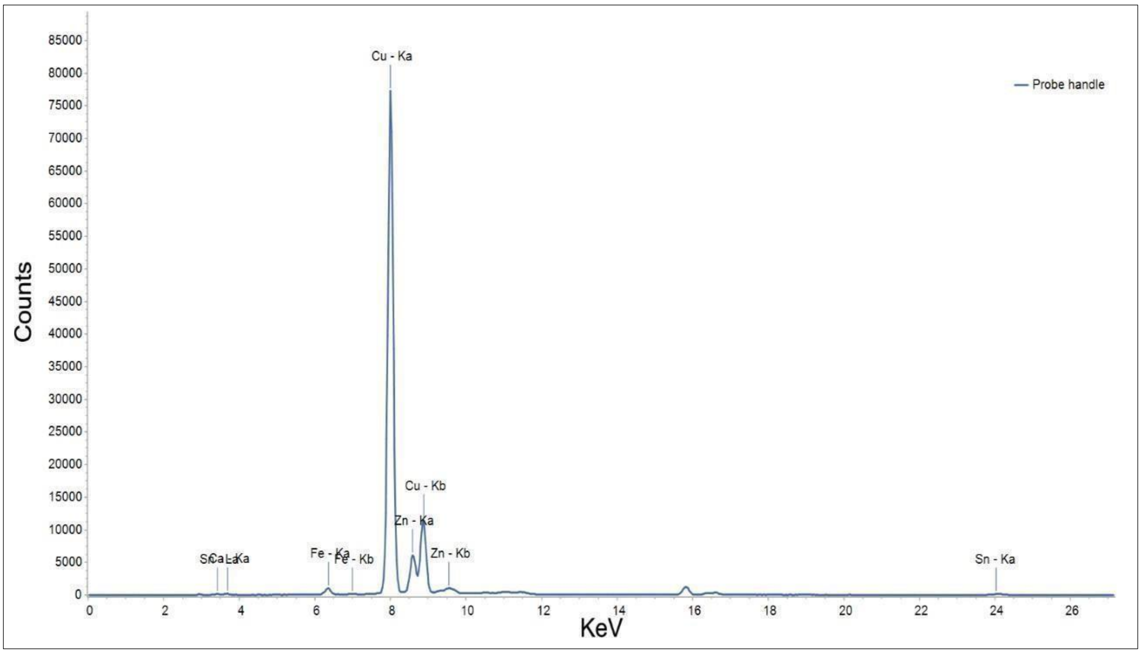


Figure 13. XRF analysis of the lancet handle carried out at University of Bari (Bari)

- X-Ray Diffractometry (XRD) analyses

XRD analysis was performed on both the blade and the handle confirming the results obtained by XRF. The diffraction pattern generated by the blade (Figure 15) revealed the occurrence of iron, aluminum, quartz, calcite, and analcime, while the X-ray pattern of the handle (Figure 16) showed the presence of metallic iron and hydroxide, metallic and oxides copper, lead oxide, and quartz. The presence of two broad peaks centered at 2θ ~ 18° and 29°, respectively, indicates the presence of micro-crystalline graphite and an amorphous component on the blade.

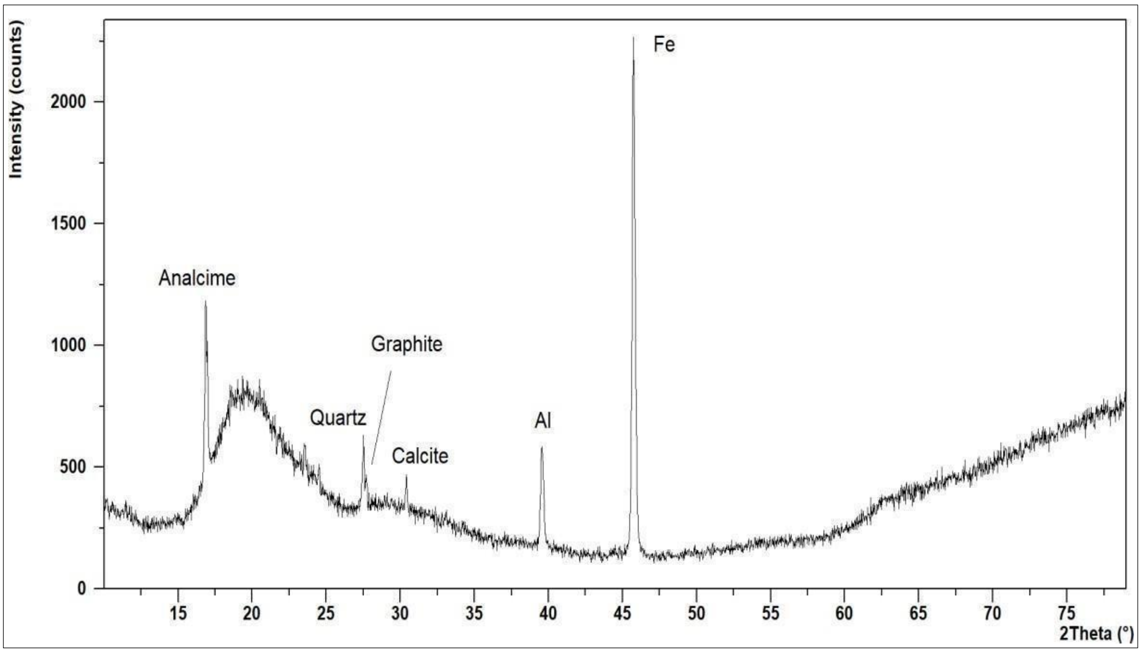


Figure 14. XRD analysis of the lancet blade.

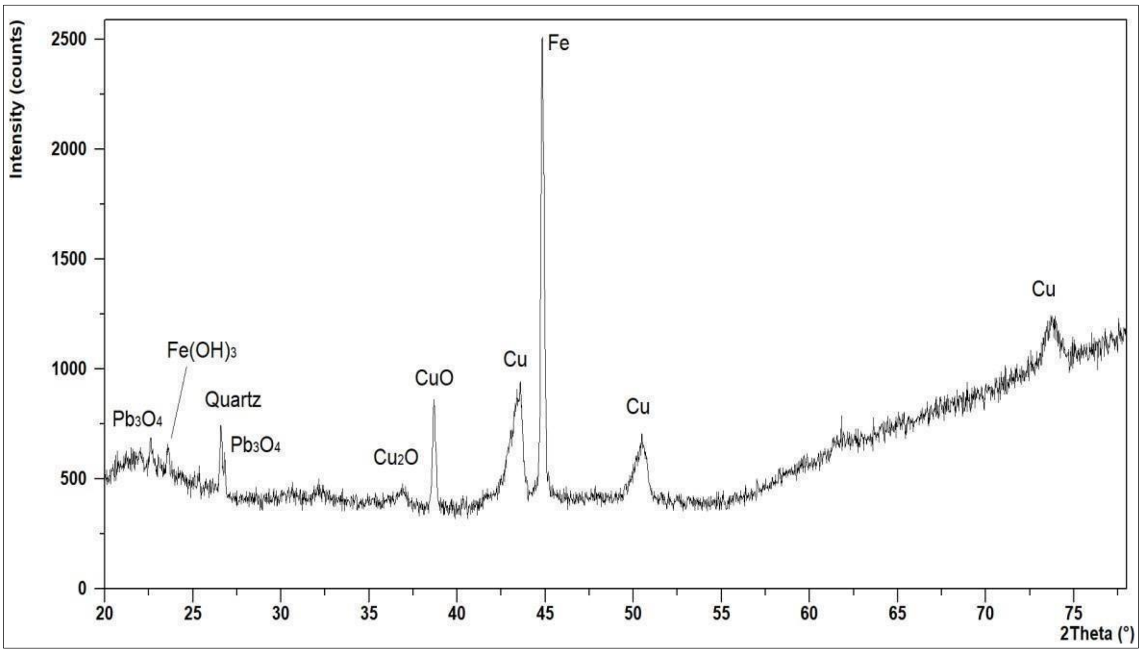


Figure 15. XRD analysis of the lancet handle.

X-Ray Photoelectric Spectroscopy X (XPS) analyses

The XPS analysis (Figure 14) carried out on the arrowhead (Figure 1e) showed the presence of the following elements: carbon (C1s); oxygen (O1s); copper (Cu2p); nitrogen (N1s); calcium (Ca2p); lead (Pb4f); sulphur (S2p); silicon (Si2p); sodium(Na1s). The presence of chlorine (Cl2p) was only probable.

Aluminium (Al2p), if present, is covered by other secondary signals, while Sn, ifpresent, is covered in negligible quantities. Sn is a component of the bronze alloy together with Cu.

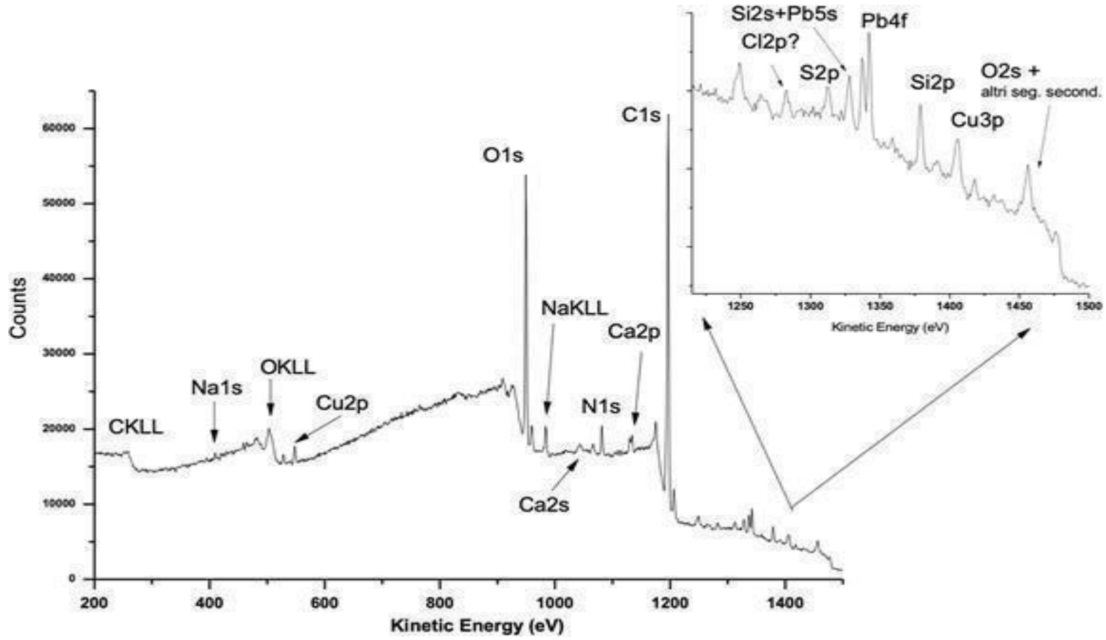


Figure 16. XPS analysis of arrowhead.

3. EXPERIENCE ABROAD

3.1 Smartworking activity at the "The Leon Recanati Institute of Haifa" (Haifa–Israel)

The study of metallurgy has changed over the centuries and shows how temperature, pH, humidity and climate can influence the artefacts with the consequence of creating a corrosive phenomena that can initiate or even to accelerate a corrosive process. In the 6th century BC, the southern Levant was rich in copper artefacts (Berna, 2007). In this period, the local production of metal products began.

Subsequently, during the Chalcolithic period (IV-III century BC) there was the presence of two distinct metallurgical industries; one for the production of tools and the other one for the manufacture of prestige/cult metal objects (Levi, 1989). Metallurgy appears to be the most sophisticated of the Late Chalcolithic technologies, exemplifying the remarkable achievements of Late Chalcolithic communities, including a high investment in raw material acquisition and technological know-how. Furthermore, it appears that at least some of the copper objects were cult paraphernalia integrated into late Chalcolithic cult practices with little or no utilitarian function. Late Chalcolithic metallurgy comprised two discrete production techniques (Caspi, 2010) that refer to the later stages of production, namely the open cast technique, usually by using pure copper, probably originating from Fay-nan, and the considerably more sophisticated 'lost wax' technique, frequently by using " non-local copper-based alloys " with significant arsenic, nickel, and antimony content (Rosemberg, 2020).

It is important to consider, also, the place where the archaeological metal finds are found.

In the case of underwater locations, it is necessary to consider whether the water is fresh or salty, the percentage of elements present, the bacteria that can live in this type of water and evaluate whether these bacteria can give rise to corrosive processes (Hamilton, 1976). Today it is preferred to leave the artefacts in an underwater environment as these artefacts when recovered from the water, a place where they have found their balance, can undergo even irreversible corrosive processes because theclimatic conditions change.Therefore, it is preferred to leave the covered objects where they are and thus increase marine tourism.

3.2 Experience to Institute of Arc’ Antique (Nantes- France) to evaluate the traditional protocol

I spent a period abroad at the Arc'Antique Institute in Nantes (Nantes-France) toobserve the traditional cleaning protocol of archaeological metal artefacts seeing that this conservation-restoration and research laboratory is specialized in underwater and terrestrial archeological assets. When the archeological metal artefact arrived at the Insitute, a chemical-physical and also tomographic characterization of the metal artefacts was experimented.

Figure 17. Artefacts after tomographic analysis

If the metal artefact is covered with concretions, the first step will be to eliminate it through mechanical or electrolytic cleaning. Mechanical cleaning is recommended when the artefact is covered by hard and compact concretions that cannot be removed electrolytically. The electrolytic treatment, nevertheless, is more suitable for porous concretions. This type of cleaning requires the use of a power generator and the cleaning is conducted at a constant potential. This procedure requires the use of a reference electrode and a multimeter (V). The solution in which the artefact is immersed is potassium hydroxide (KOH) (Figure 18) (Siano, 2004).

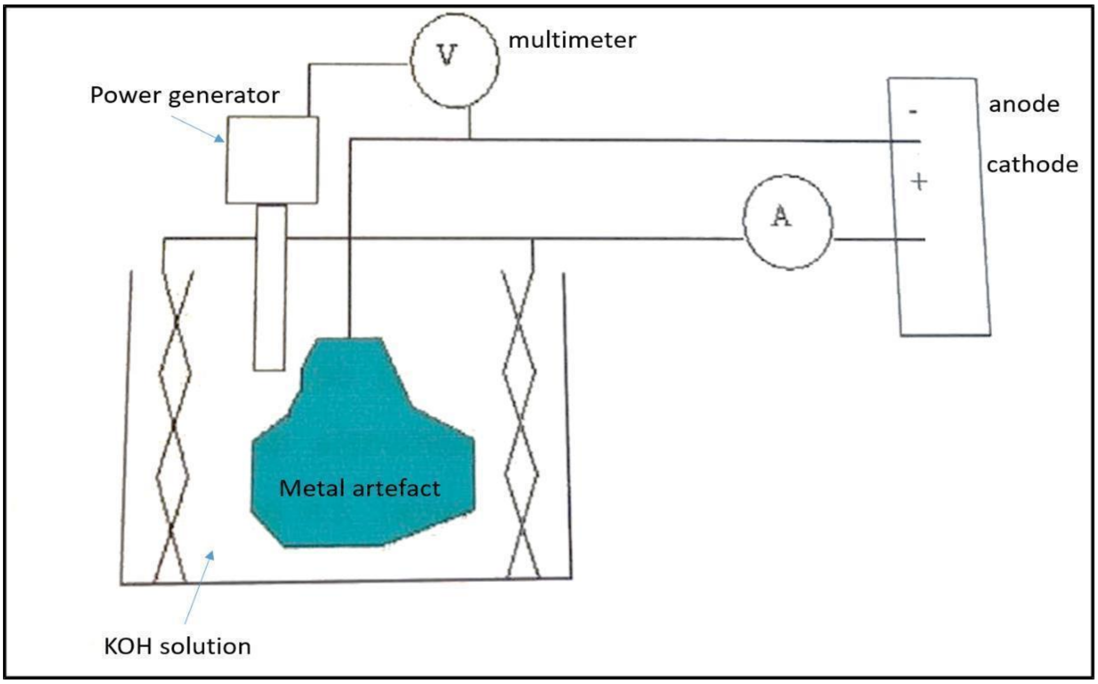


Figure 18. Electrolytic cleaning (Siano, 2004).

Electrolytic cleaning allows a homogeneous and respectful cleaning of the original surface. This type of cleaning is suitable for lead which, due to its low hardness, can be seriously damaged during mechanical cleaning. This type of treatment is also used when there are inscriptions on the artefact. Electrolytic cleaning of metal artefacts is not always recommended it is forbidden when there are natural patinas on the artefacts which must be preserved because they too have cultural value. Electrolytic cleaning cannot be applied to iron materials because the original surface of the product could be lost. After all the concretions have been eliminated, it is also necessary to eliminate any type of trace of corrosion defined as active through the stabilization process (Siano, 2004).

After electrolytic cleaning, the artefacts must be stabilized and then rinsed in deionized water. After removing the concretions and once the artefact has stabilized, you can move on to the next step. The stabilization process is followed by rinsing with deionizedwater. Cathodic protection is used when there are cast iron parts; while immersion is sufficient for bronze artefacts. The next step is that of the finishing which consists in using the microsablage technique (Fig 19) which allows the elimination of any type of residual concretion (Siano, 2004).

Figure 19. Microsablage technique

If the artefact is in bronze, then you need to treat them with benzotriazole, a corrosion inhibitor, and then you need to cover them with a layer of Paraloid 72 (Figure 20) which is a varnish and finally add the wax, which, however, decreases the shine of the artefact.

Figure 20. Paraloid 72. Varnish that is added to the artefact and then removed once it has dried

Whether it is electrochemical cleaning or stabilization, it is necessary to establish in advance the various types of treatment to which the product must be subjected, therefore a physical characterization is always necessary, moreover it can be useful, in addition to the experience in the sector, also the use of a potentiostat and the use of artificial samples that simulate the approach of ancient materials. The potentiostat is a piece of equipment that allows you to trace voltammetric curves (potential of the polarized objectas a function of the intensity that passes through it) revealing the different electrochemical reactions that occur during the application of a cathodic and anodic potential. This type of graph determines the electrolytic trend of cast iron parts (-0.85 V/ENH, ENH: normal hydrogen electrode), that of stabilization (-0.8 V/ENH) and that of stabilization of copper-based alloys. The monitoring of both cathodic or anodic treatmentsconsists in measuring the evolution of the intensity over time at these particular potentials.This tool is rarely used on archaeological metal artefacts, but man-made artefacts that mirror the characteristics of the archaeological artefacts are used in such a way as to avoid side effects on the archaeological artefacts.

3.3 Experience to Universitè de Neuchatel (Swtitzerland) to do experiments with *Beauveria bassiana* Vuill. of copper plates.

The period abroad carried out at the University of Neuchâtel (Switzerland) allowed me to acquire the green protocol with the use of *Beauveria bassiana* Vuill.

3.3.1 Copper samples and patina preparation

Eighteen copper samples (3 x 3 cm) (Figure 22) were cut from a naturally aged copper roof from Universitè de Neuchâtel, Switzerland. The surface of the samples was washed with 95% ethanol and then the samples were air dried.

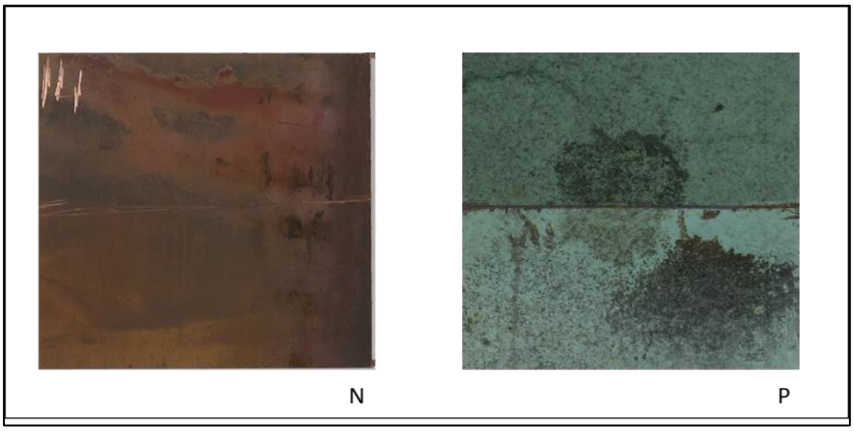


Figure 22. Copper sample (3 x 3 cm). *Legend. N: nude, P: opaque*

These samples were marked with a line to differentiate between the part that should be treated (below the line) and the part that should not be treated (above the line).

In this part of research project, *Beauveria bassiana* Vuill., fungi filtrate and commercial oxalic acid in liquid medium and gel medium were used to do an artificial patina on the copper samples for seven days. Before the treatment, the samples were analyzed with Fourier transform infrared spectroscopy (FTIR) and colorimetry analysis. After the the treatment (7 days), the samples were washed with 95% ethanol to remove the substance and then they were analyzed another time with FTIR and colorimetry to see the differences.

3.3.2 *Beauveria bassiana* Vuill. and chemical oxalic acid

- Fungal strain

The S6 strain of *Beauveria bassiana* Vuill. belongs to the fungal culture collection of the Laboratory of Microbiology, Department of Biology, Faculty of Science, Universitè de Neuchâtel. The culture was maintained on plates and malt slants of 12 g/L.

- Liquid culture

The fungus was grown in 100 mL Pyrex bottles (Figure 23) with 57.6 mL of malt medium (12 g·L^{-1}). A piece of copper was also inserted into the bottles to obtain high concentration of oxalic acid. Obtaining the filtrate (without spore), was the final result.

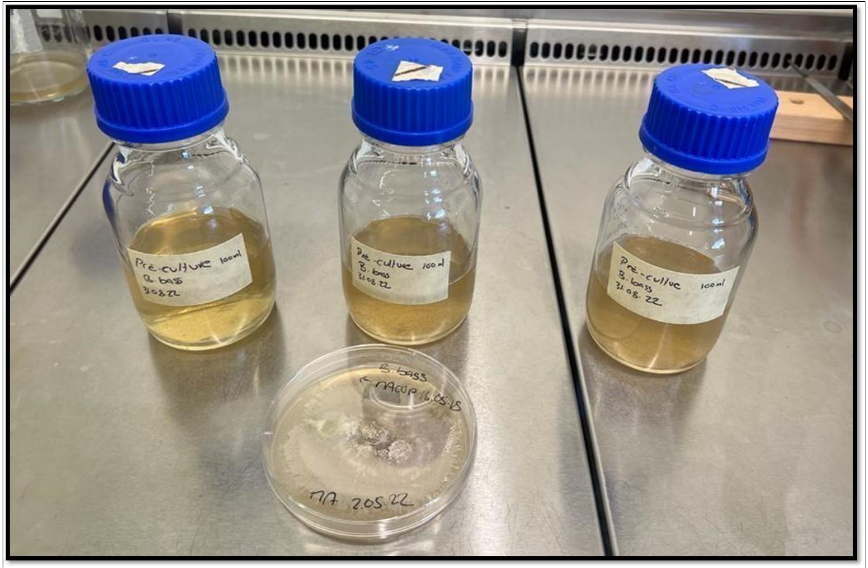


Figure 23. Pyrex bottles with malt medium and Petri plate with *Beauveria bassiana* Vuill.

- Chemical oxalic acid

Studying the oxalic acid’s effectiveness produced by the fungi ICP-MS, was necessary to analyze its concentration.

- Gel

The gel was prepared by adding a gelling substance to various solution (fungi, filtrate, commercial oxalic acid).

The copper plates were subjected to two different treatments by using both the liquid medium and the gel one to see if there were different types of results.

3.3.3 Medium

- Liquid medium

Three samples with *Beauveria bassiana* Vuill. (Figure 24), three with the fungi filtrate (Figure 25) and three with commercial oxalic acid (Figure 26) were analyzed in liquid medium.

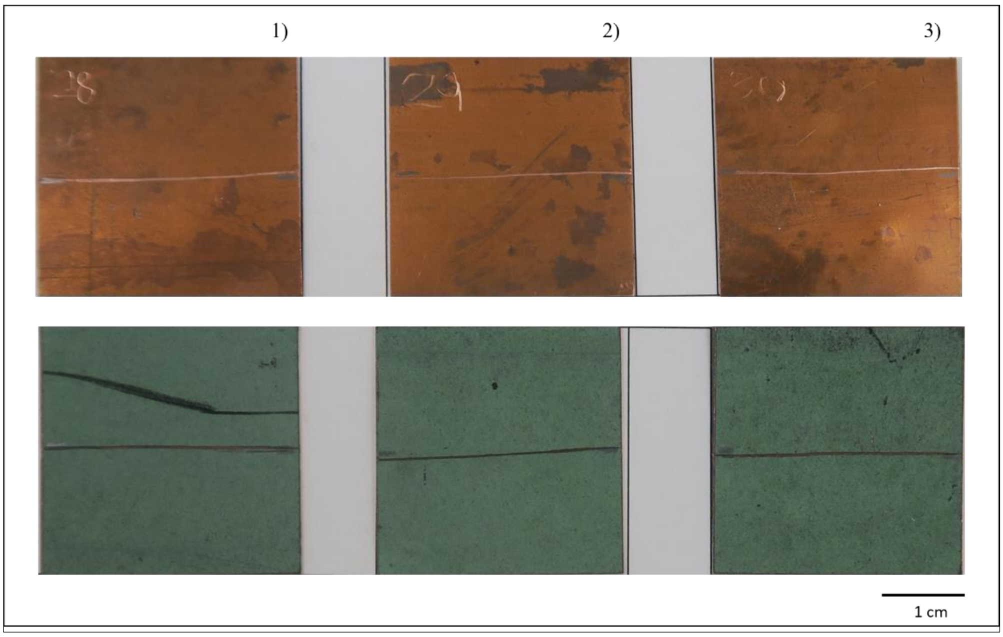


Figure 24. Samples pre-treatment with *Beauveria bassiana* Vuill. (liquid medium)

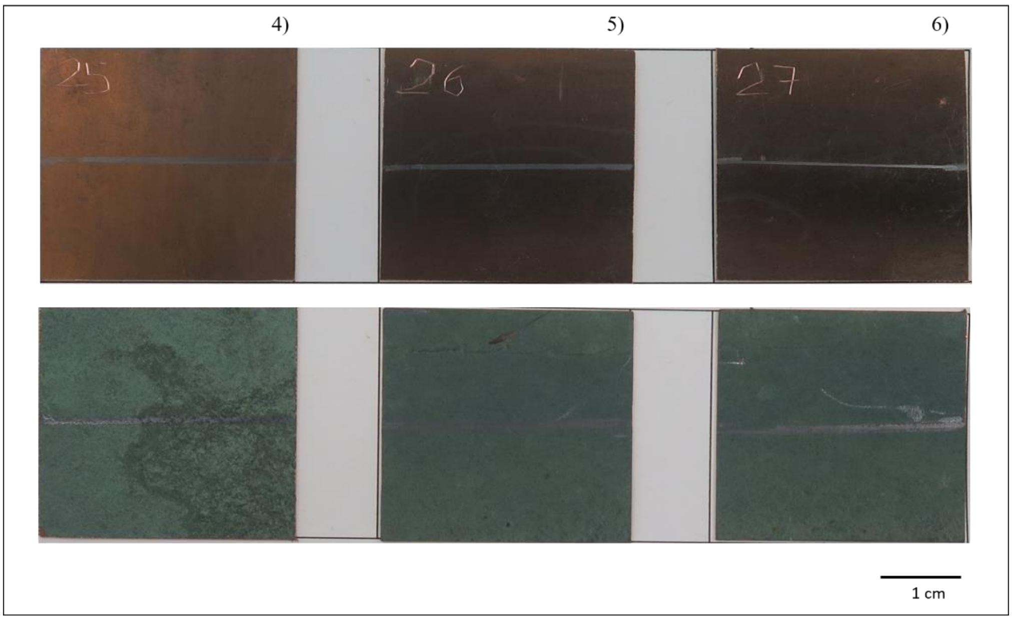


Figure 25. Samples pre-treatment with fungi filtrate (liquid medium)

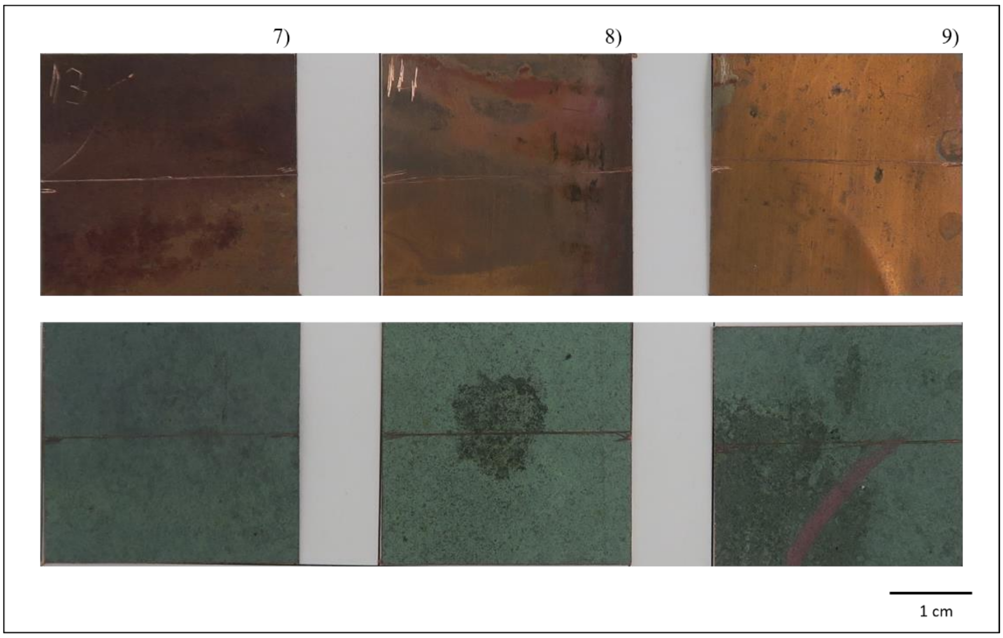


Figure 26. Samples pre-treatment with commercial oxalic acid (liquid medium)

- Gel medium

Three samples with *Beauveria bassiana* Vuill. (Figure 27), three with the fungi filtrate (Figure 28) and three with commercial oxalic acid (Figure 29) were analyzed in gel medium.

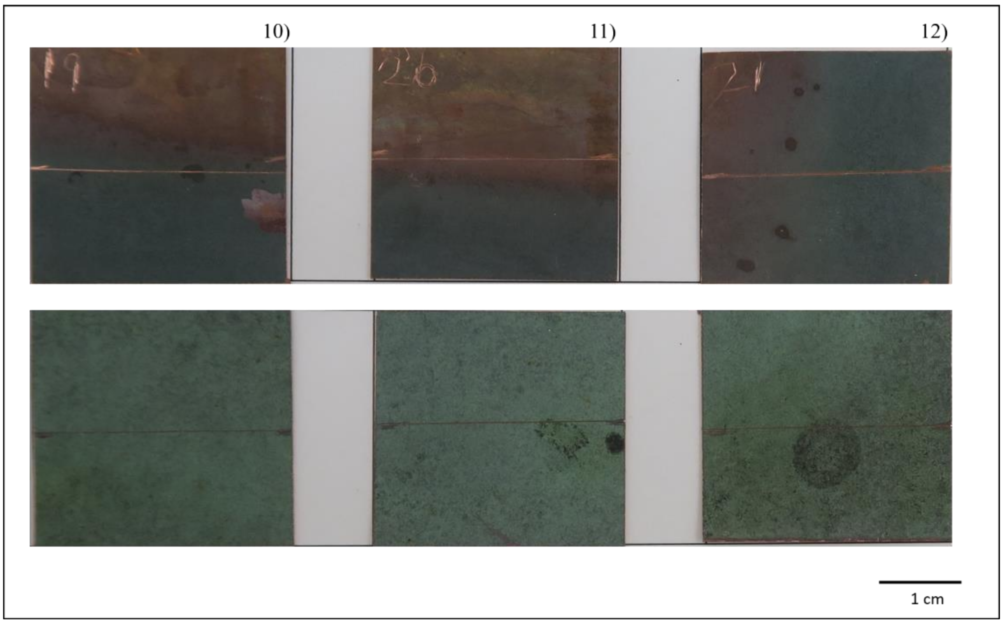


Figure 27. Samples pre treatment with *Beauveria bassiana* Vuill. (gel medium)

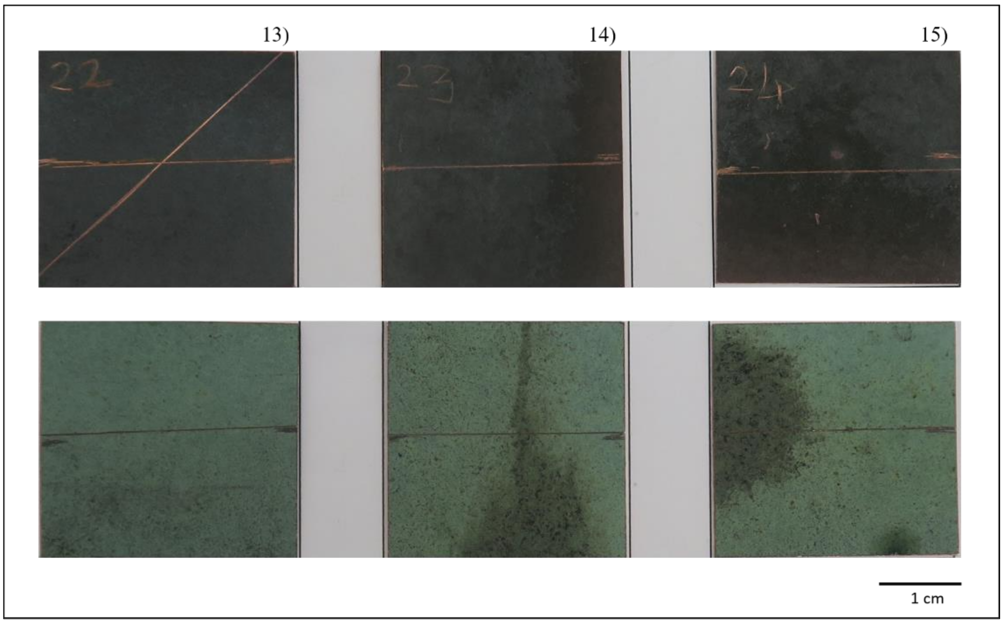


Figure 28. Samples pre-treatment with fungi filtrate (gel medium)

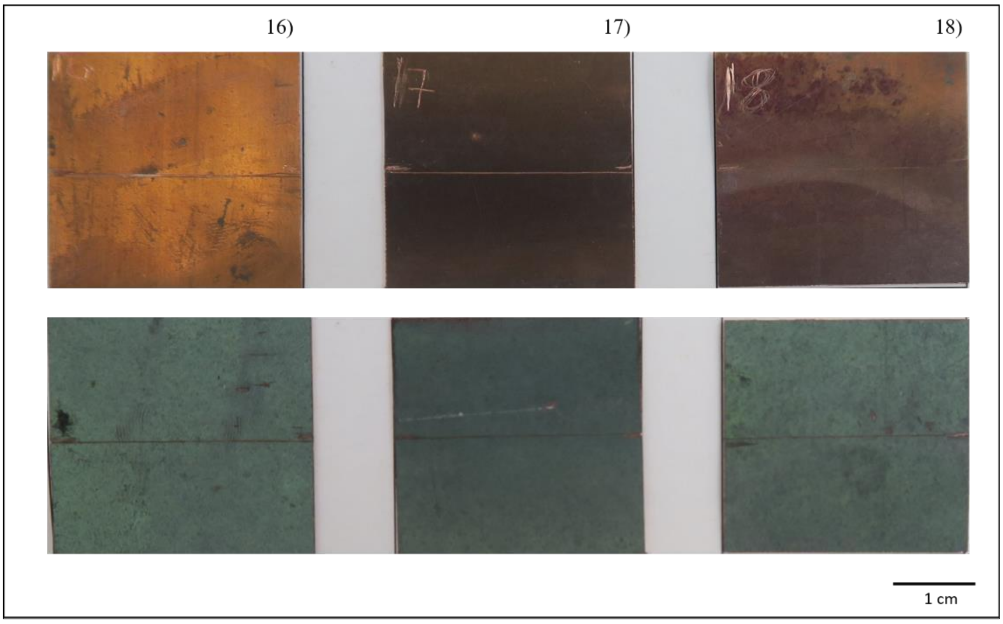


Figure 29. Samples pre treatment with commercial oxalic acid (gel medium)

3.3.4 Analytical techniques

The analytical techniques that have been used on the copper plates are:

- Fourier transform infrared spectroscopy (FTIR)

It is considered a very effective technique for studying and understanding the chemistry and surface chemistry in various types of materials.

It is a method for determining the structure of molecules that collects a molecular vibrational spectrum. When exposed to infrared radiation, the sample molecules selectively absorb radiation of specific wavelengths that causes the pole momentum of the sample molecules to change (Liu, 2022).

As a result, the vibrational energy levels of the sample molecules, transfer from the ground state to the excited one. The frequency of the absorption peak is determined by the vibrational energy gap. The number of absorption peaks is related to the vibrational freedom of the molecule. The intensity of the absorption peaks is related to the change of the pole moment and the possibility of a transition of the energy levels. Therefore, by analyzing the infrared spectrum, information about the structure of a molecule can easily be obtained (Ismail, 1997).

FTIR was performed without any sample preparation by using an iS5 Thermo Scientific spectrometer with a diamond Attenuated Total Reflectance (ATR) crystal plate (iD5™ ATR accessory). All spectra were acquired in the range 4000–650 cm^{-1}, at a spectral resolution of 4 cm^{-1}. Data collection and post-run processing were carried out using Omnic™ software. The FTIR mapping on samples sections was performed using a Thermo Scientific Nicolet iN10 MX FTIR microscope in ATR mode. All spectra were acquired in the range 4000–675 cm^{-1}, at a spectral resolution of 4 cm^{-1} and a step size of 20x20 mm.

FTIR analyses were performed on each sample from both sides (N and P) before and after treatment.

Colorimetry

In 1859 Maxwell published his Theory of *Color Vision*, which is the origin of the development of the quantitative measurement of colors (Colorimetry), today internationally codified (CIE XYZ).

Colorimetry is the measurement of color. That is the determination of the concentration of a substance through the measurement of the absorption of the relative light to it. In colorimetry, natural or artificial white light is used as a source of the optical system, and the analysis are carried out with an instrument called ***colorimeter.***

The colorimetric analysis is based on the principle that many substances when reacting with a chemical compound, produces a color change which will be reduced to the concentration of the substance to be measured (Higueras, 2023). When a substance is exposed to a beam of light of intensity (I0) a part of the radiation is absorbed by the substance, emitting radiation of intensity (I). The difference in intensity is used for the colorimetric determination. The amount of energy absorbed is given by the Lambert- Beer law:

$A=\log Io/I$ (Noboru, 2006).

A Minolta CM-508D spectrophotometer was used with the following measurement conditions: Specular Component Included (SCI), Illuminant D65 (daylight containing UV component, colour T 6504K), d/8° geometry, 10° observer, measurement areadiameter 8 mm, illumination with Xe flash light source 100% UV containing all UV components or 0% UV containing no UV components, CIELab 1976 colour space. On each sample, three measurements were carried out at different spots and the mean value calculated. In order to describe the colour variation before and after treatment theparameter ΔE* (colour difference) was calculated for each treatment using the CIE1976 equation (1).

$$\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

Values lower than 3 are considered not perceptible to the human eye while ΔE* bigger than 5 are considered clearly perceptible (Culliver, 2022).

3.3.5 Results

The FTIR carried out before the treatment on all the samples showed Brochantite on the P side (Figure 30); while on the N side, a mineral was not present in the software data (Figure 31).

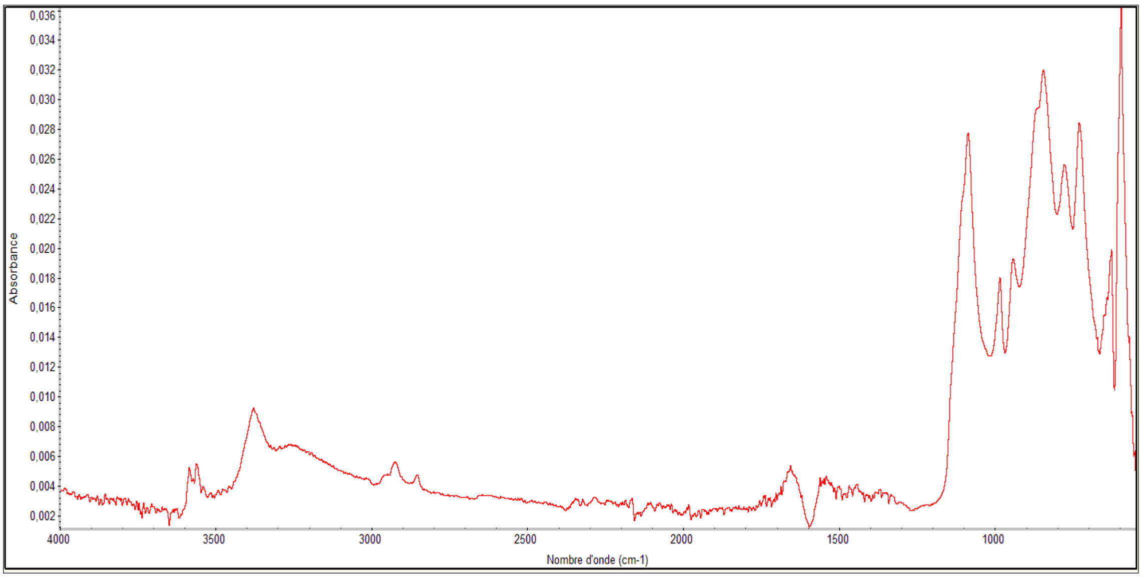


Figure 30. FTIR carried out on P side (pre-treatment)

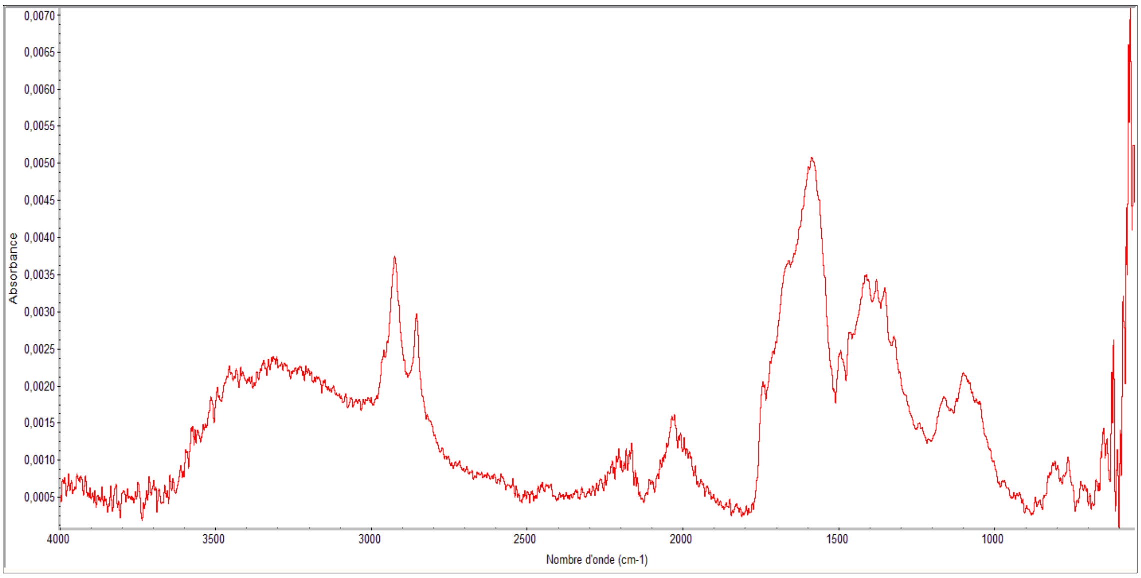


Figure 31. FTIR carried out on N side (pre-treatment)

After the treatment of seven days, The FTIR analysis and colorimetry were repeated to evaluate the presence of copper oxalates and if the colour of copper plates changed. These results obtained from the FTIR analysis on the samples confirm the obtained results in another study (Albini, 2016) in which there was the presence of *Brochantite* before the treatment on copper plates.

- Liquid medium

- *Beauveria bassiana* Vuill.

FTIR analysis, carried out on both sides, shows the formation of copper oxalates (Figure 32, Figure 33). The Figure 34 shows the difference between pre and after treatment with *Beauveria bassiana* Vuill. in liquid medium.

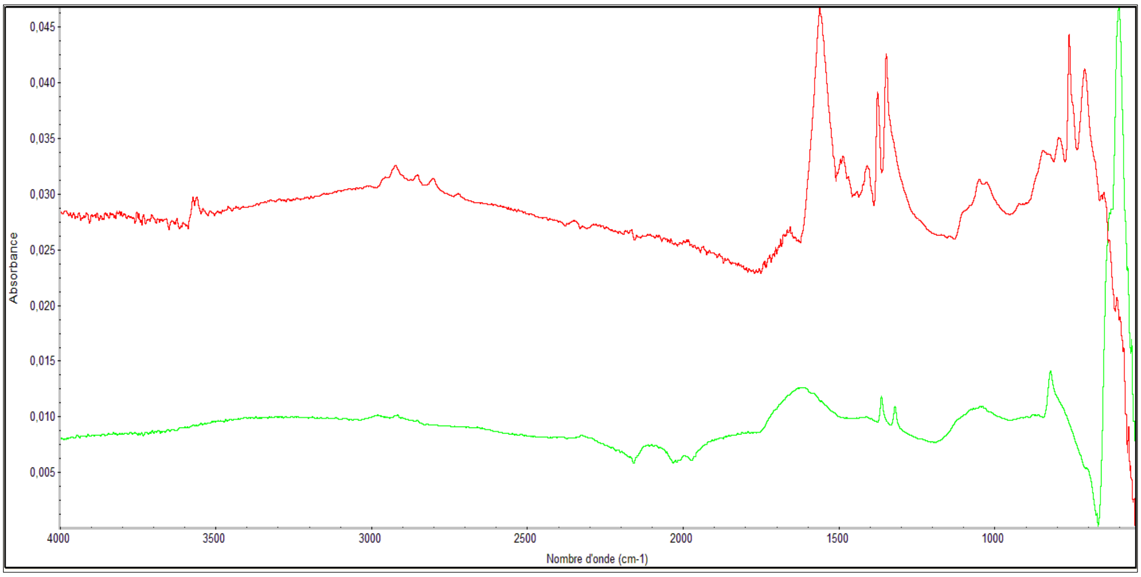


Figure 32. FTIR carried out post treatment on N side treated with *Beauveria bassiana* Vuill. in liquid medium

Legend: copper oxalates (red); sample (green)

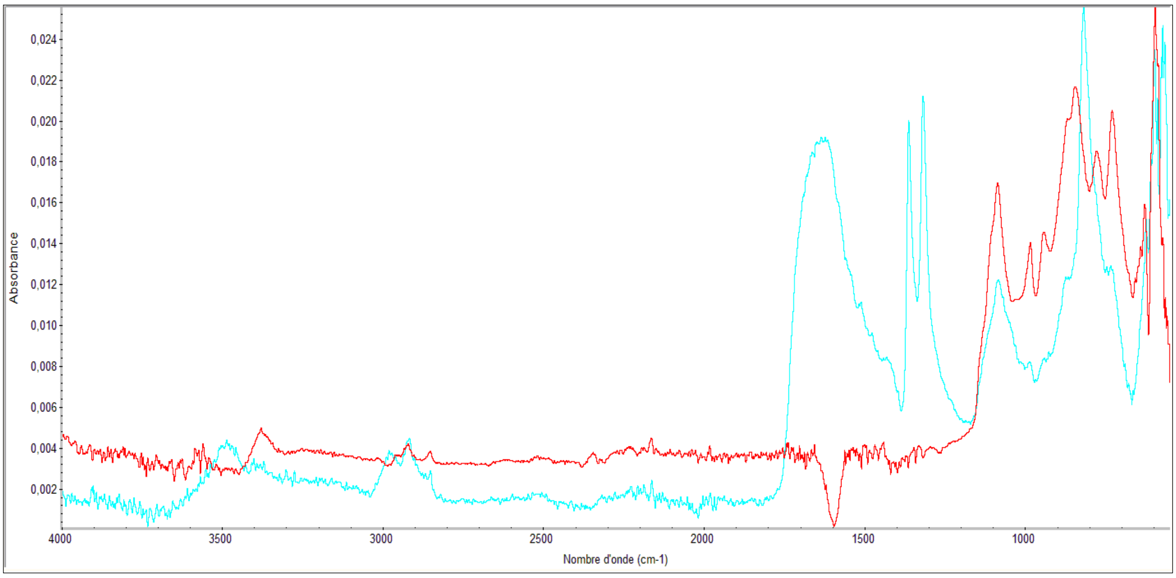


Figure 33. FTIR carried out post treatment on P side treated with *Beauveria bassiana* Vuill. in

liquid medium.
Legend. Copper oxalates (red), sample (blue)

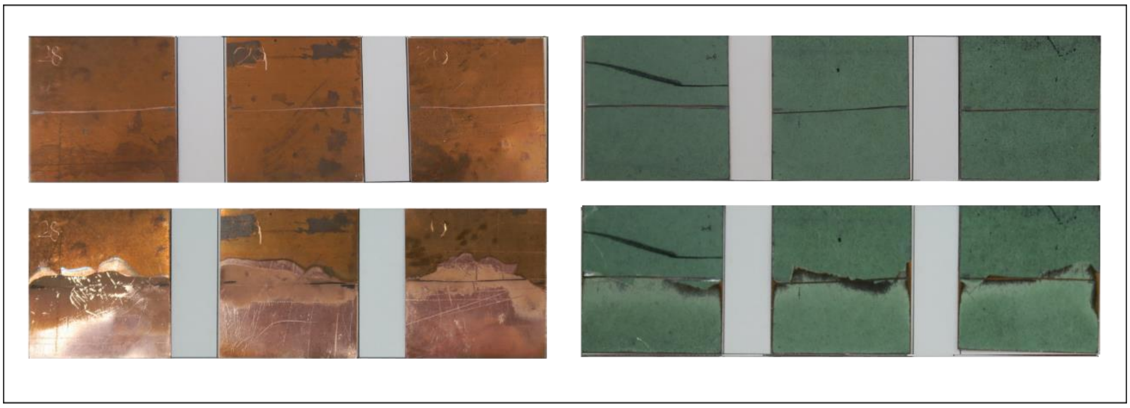


Figure 34. Difference between pre (above) and after treatment (below) with *Beauveria bassiana* Vuill. in liquid medium

Table 4 shows the colour difference ΔE* calculated before and after treatment with *Beauveria bassiana* Vuill.

Table 4. *Colour difference ΔE* calculated before and after treatment with Beauveria bassiana* Vuill.

Samples	**N**	**P**
1	25,4233	7,0748
2	20,3768	3,3865
3	22,2915	5,2683

The colorimetric analysis carried out after the treatment with *Beauveria Bassiana Vuill.* in liquid medium, reveals the colour change on the N part in all three samples; while the significative color changes on part P is found in 2 out of 3 samples (1 and 3 samples).

- Fungi filtrate

After the treatment with fungi filtrate, two of three samples, analyzed with FTIR, showed the formation of copper oxalates on both sides (N e P) (Figure 35 and Figure 36). The Figure 37 shows the difference between pre and after treatment with fungi filtrate in liquid medium.

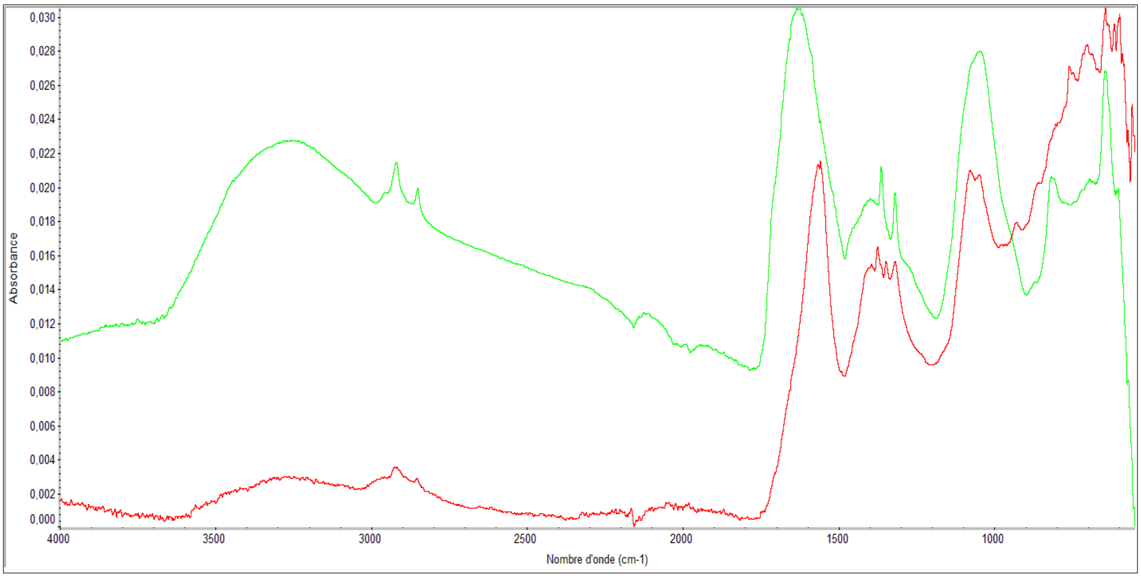


Figure 35. FTIR carried out on N side treated with fungi filtrate in liquid medium
Legend. Copper oxalates (red), sample (green)

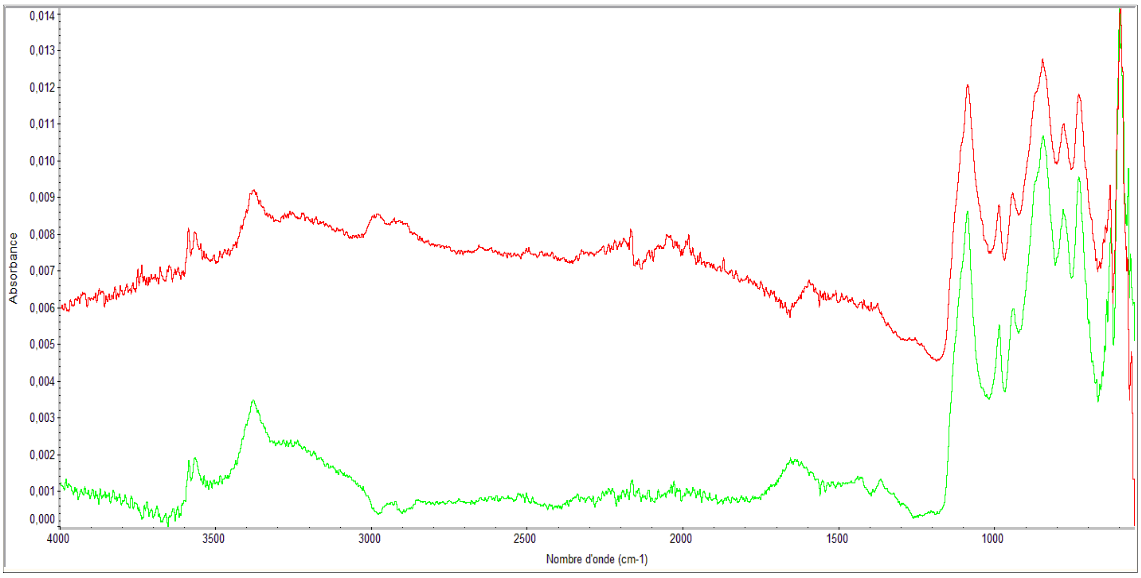


Figure 36. FTIR carried out on P side treated with fungi filtrate in liquid medium. *Legend. Copper oxalates (red), sample (green)*

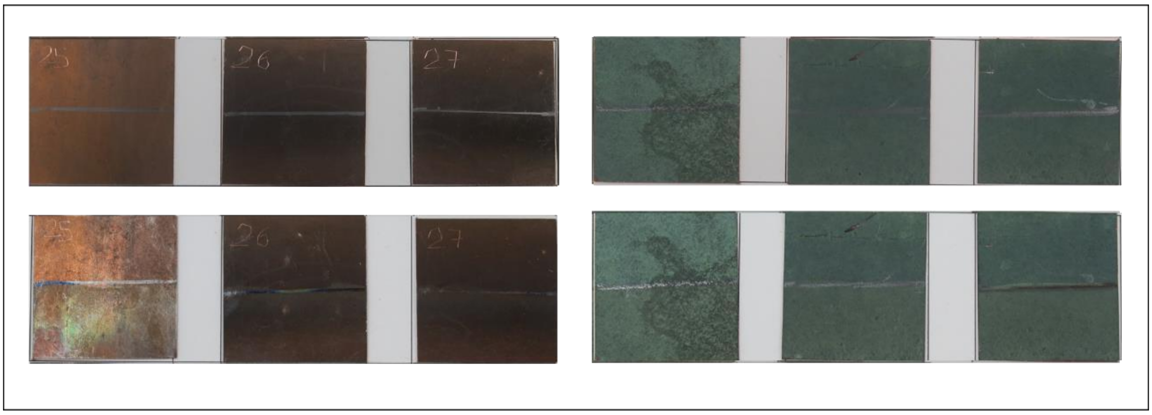


Figure 37. Difference between pre (above) and after treatment (below) with fungi filtrate in liquid medium

Table 5 shows the colour difference ΔE* calculated before and after treatment with fungi filtrate.

Table 5. *Colour difference ΔE* calculated before and after treatment with fungi filtrate*

Samples	**N**	**P**
4	6,7482	11,4584
5	3,6356	20,4994
6	5,1257	19,1119

The colorimetric analysis carried out after the treatment with fungi filtrate in liquid medium reveals the colour change on the N part in 2 out of 3 samples (4 and 6 samples); while the color change on part P is found in 3 out of 3 samples (4,5, and 6 samples).

- Commercial oxalic acid

The samples that were treated with commercial oxalic acid at the same concentration as the oxalic acid produced by fungi, showed by FTIR analysis that only one out of three samples formed copper oxalates on both sides (N and P). The Figure 38 shows the difference between pre and after treatment with commercial oxalic acid in liquid medium.

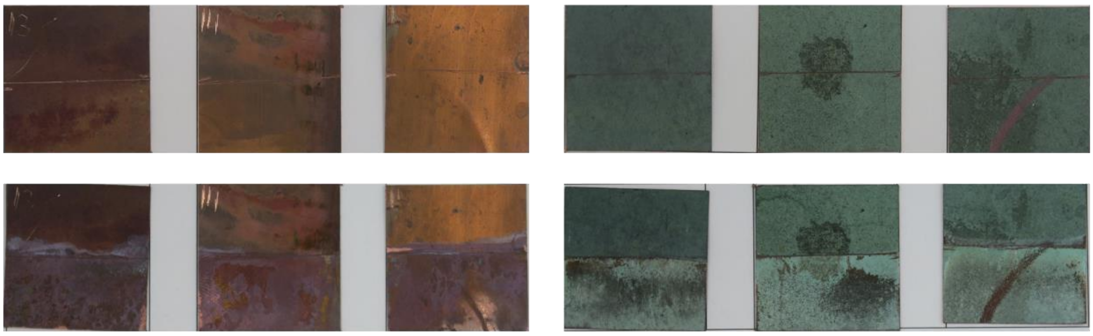


Figure 38. Difference between pre (above) and after treatment (below) with chemical oxalic acid in liquid medium

Table 6 shows the colour difference ΔE* calculated before and after treatment with commercial oxalic acid.

Table 6. *Colour difference ΔE* calculated before and after treatment with commercial oxalic acid*

Samples	N	P
7	25,8301	6,6486
8	16,8638	9,9456
9	38,1764	7,8443

The colorimetric analysis carried out after the treatment with commercial oxalic acid in liquid medium reveals the colour change on both sides (N and P).

- Gel medium

We analyzed the samples after treatment in gel medium with *Beauveria bassiana* Vuill., fungi filtrate and commercial oxalic acid with FTIR and colorimetry analysis.

- *Beauveria bassiana* Vuill.

All three samples analyzed with FTIR showed the formation of copper oxalates on both sides (Figure 39 and Figure 40). The Figure 41 shows the difference between pre and after treatment with *Beauveria bassiana* Vuill. in gel medium.

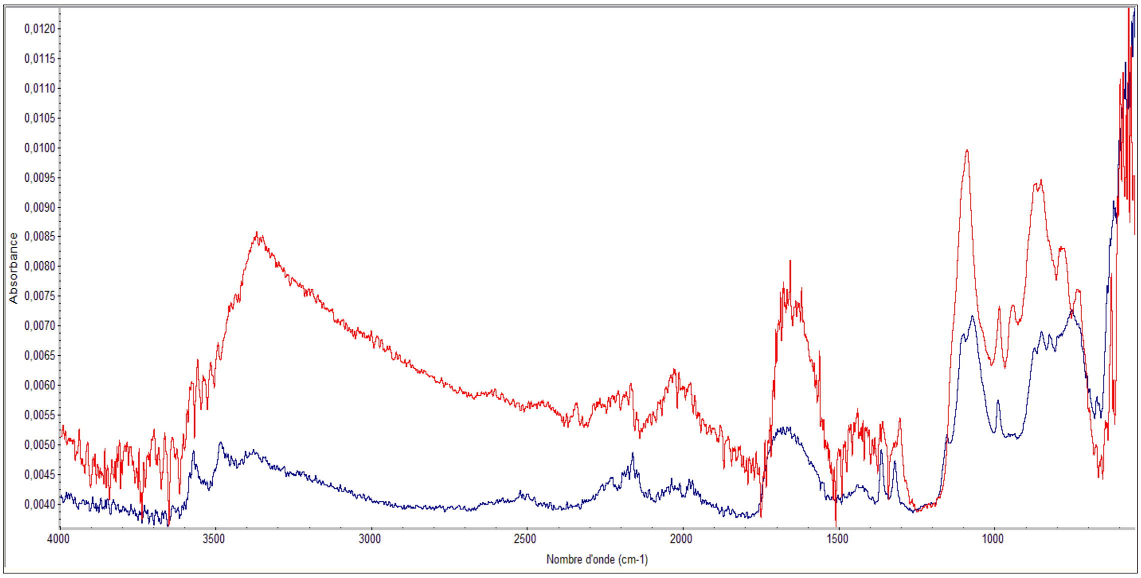


Figure 39. FTIR carried out on N side treated with *Beauveria Bassiana* Vuill. in gel medium
Legend. Copper oxalates (red), sample (blu)

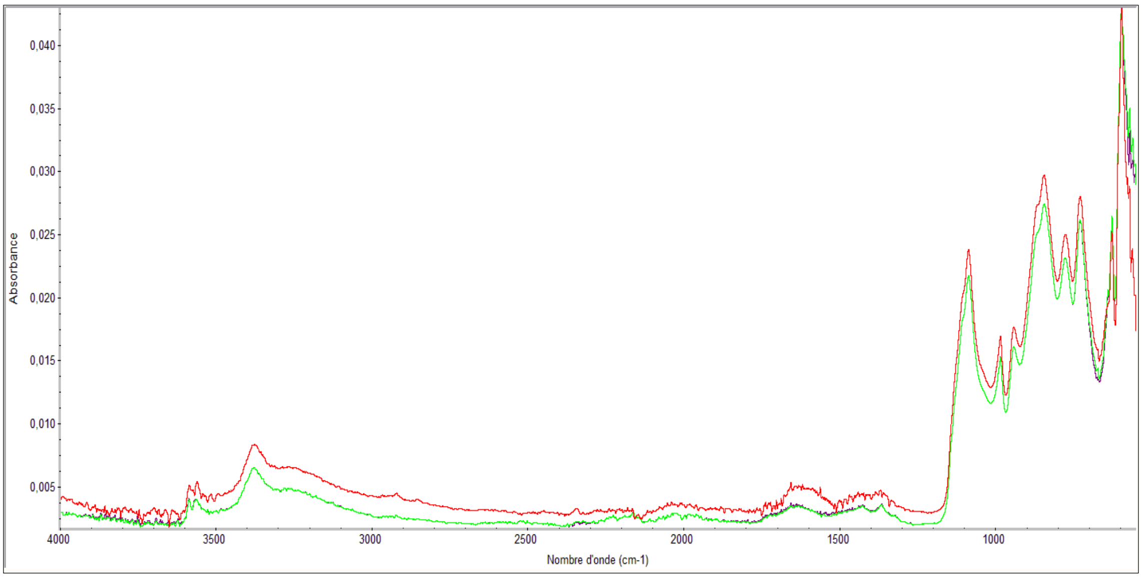


Figure 40. FTIR carried out on P side treated with *Beauveria bassiana* Vuill. in gel medium
Legend. Copper oxalates (red), sample (green)

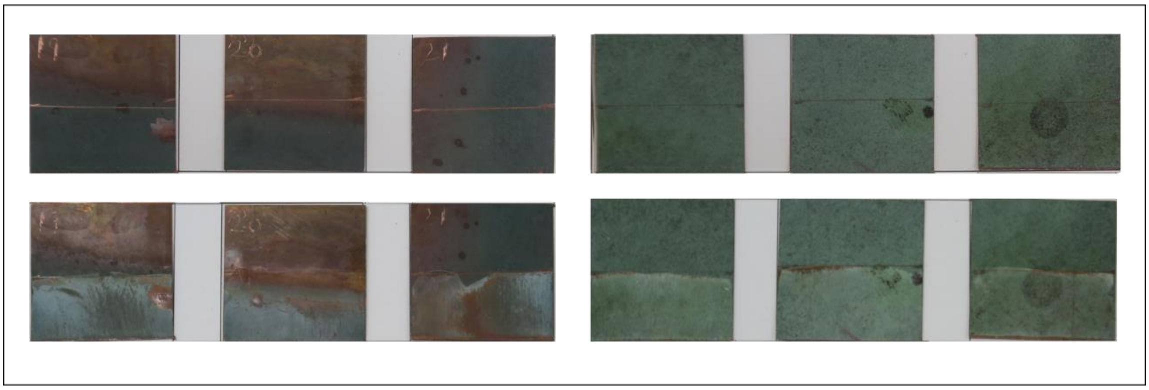


Figure 41. Difference between pre (above) and after treatment (below) with *Beauveria bassiana* Vuill. in gel medium

Table 7 shows the colour difference ΔE* calculated before and after treatment with *Beauveria bassiana* Vuill. in gel medium.

Table 7. *Colour difference ΔE* calculated before and after treatment with Beauveria bassiana* Vuill.

Samples	**N**	**P**

10	7,72	21,3724
11	11,9659	5,4626
12	6,7712	6,3718

The colorimetric analysis carried out after the treatment with *Beauveria bassiana* Vuill. in liquid medium reveals the colour change on both sides (N and P) in all 3 samples.

- Fungi filtrate

Only two samples of three analyzed with FTIR showed the formation of copper oxalates (Figure 42 and Figure 43). The Figure 44 shows the difference between pre and after treatment with fungi filtrate in gel medium.

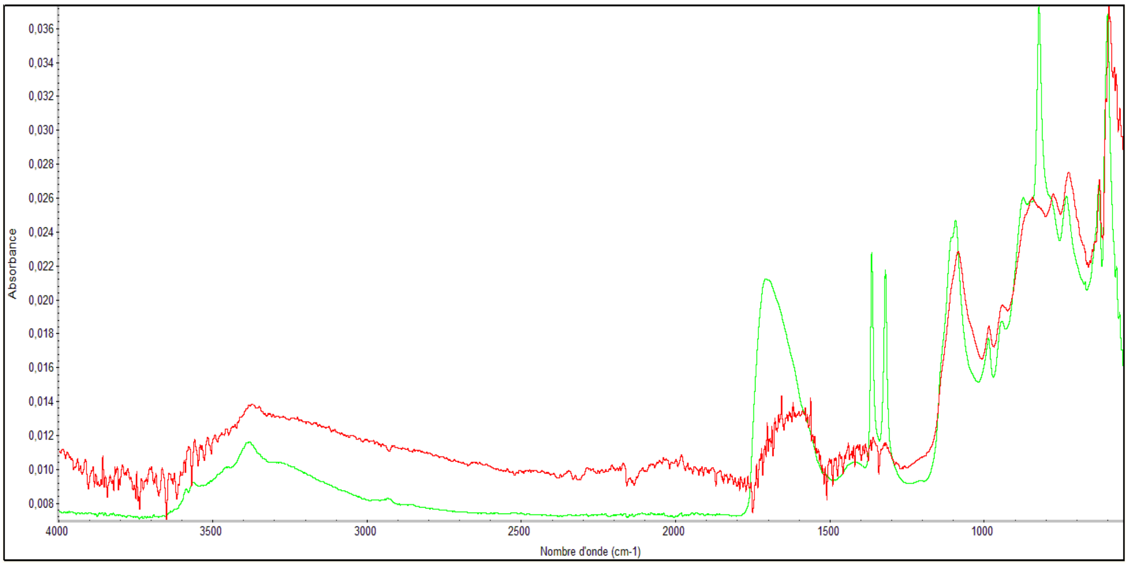


Figure 42. FTIR carried out on N side treated with fungi filtrate in gel medium

Legend. Copper oxalates (red), sample (green)

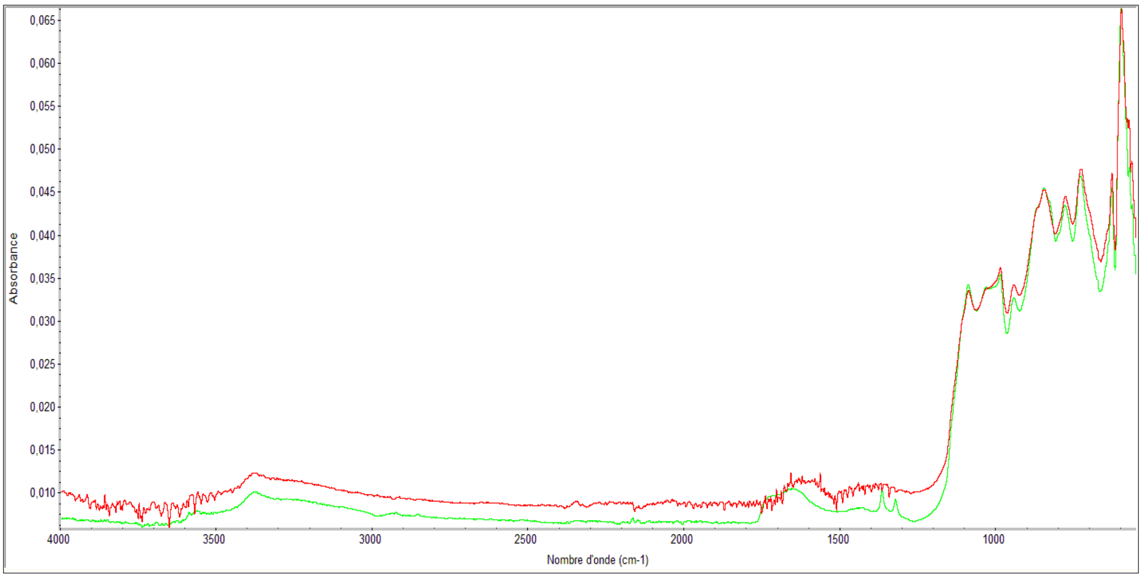


Figure 43. FTIR carried out on P side treated with fungi filtrate in gel medium
Legend. Copper oxalates (red), sample (green)

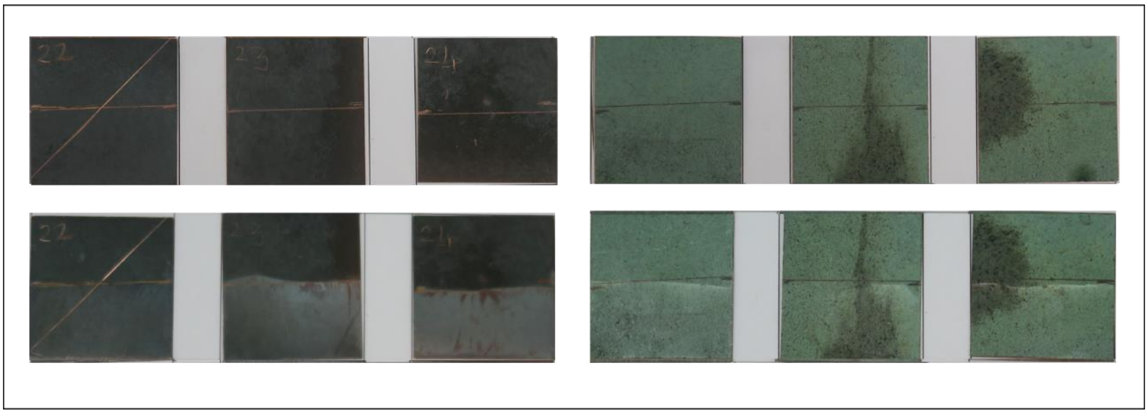


Figure 44. Difference between pre (above) and after treatment (below) with fungi filtrate in gel medium

Table 8 shows the colour difference ΔE* calculated before and after treatment with fungi filtrate in gel medium.

Table 8. *Colour difference ΔE* calculated before and after treatment with fungi filtrate in gel medium*

Samples	**N**	**P**
13	6,7482	4,2011

14	26,1666	13,2846
15	33,2458	7,7489

The colorimetric analysis carried out after the treatment with fungi filtrate in gelmedium reveals the significative colour change on the N part in 3 out of 3 samples (13, 14 and 15 samples); while the color changes on part P is found in 2 out of 3 samples (14 and 15 samples).

- Commercial oxalic acid

The samples that were treated with commercial oxalic acid showed by FTIR analysis that only one out of three samples formed copper oxalates on both sides. The Figure 45 shows the difference between pre and after treatment with commercial oxalic acid in gelmedium.

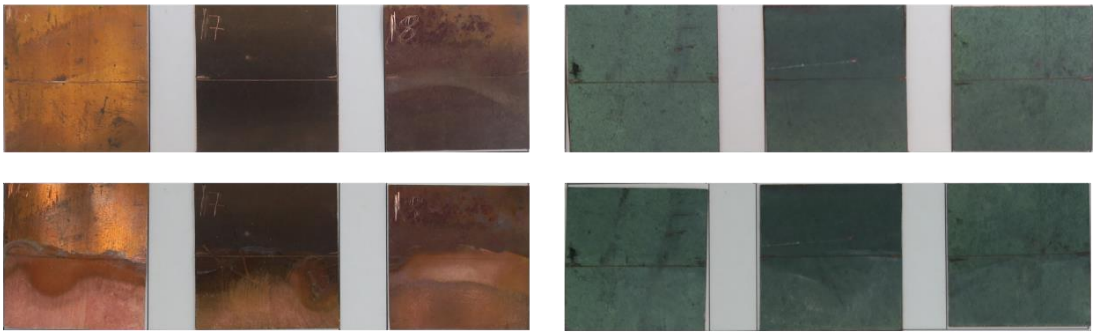


Figure 45. Difference between pre (above) and after treatment (below) with chemical oxalic acid in gel medium

Table 9 shows the colour difference ΔE* calculated before and after treatment with *commercial oxalic acid* in gel medium.

Table 9. *Colour difference ΔE* calculated before and after treatment with commercial oxalic acid in gel medium.*

Samples	**N**	**P**
16	26,697	12,8429
17	24,0501	8,513
18	24,4886	10,1502

The colorimetric analysis carried out after the treatment with commercial oxalic acid in gel medium reveals the colour change on both sides (N and P) in all 3 samples.

4. DISCUSSION

The study of the archaeological metal artefacts conserved in the National Archaeological Museum of Siritide (Policoro - Italy), analysed with Optical microscopy(OM) shows the presence of multiple layers made of both organic materials and oxidized metal. The artefacts appeared highly deteriorated, perhaps due to the environment in which they were found. The state of corrosion shows a granular appearance, but for the arrowhead, it is difficult to recognize the native surface state of the artefact.

FTIR analyses suggested that iron was the main constituent of the javelin tip (Figure 1a), iron weapon (Figure 1b), and lancet blade (Figure 1g). The alloy of the lancethandle (Figure 1g) was indeed brass for the presence of Cu, Zn, and Sn. The flask cap (Figure 1d) and fibula (Figure 1f) were bronze, with Cu and Sn as the main elemental constituent. Pb was the main constituent of the lead stick (Figure 1c) The lead stick examined is part of a set of 102 sticks contained inside an amphora (Figure 44) at the time of its discovery. They may have been used as weights for shipping nets (Verger, 2014). The stick is made of lead, showing severe degradation even with the naked eye.

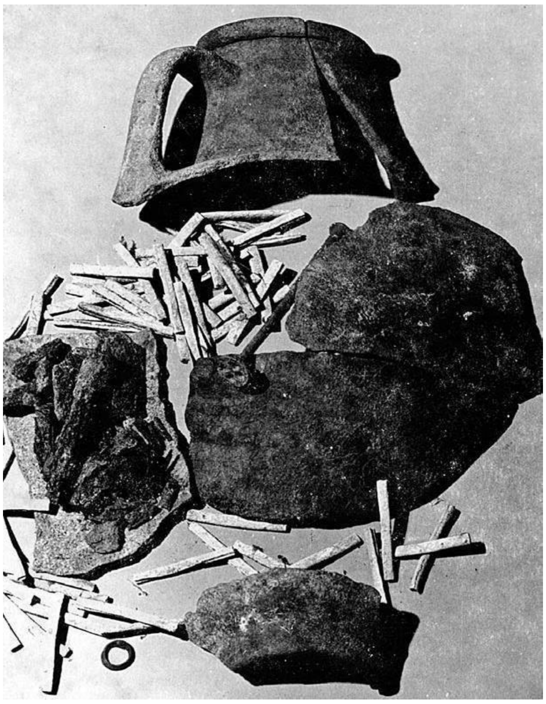


Figure 44. Amphora with 102 lead sticks (Verger, 2014).

Pb was also present in the arrowhead with Sn and Cu. This last element was the main substance for the arrowhead, revealing that the artefact, contrary to the information given by the Museum, was not made of Fe but of Cu. This information is essential because depending on the type of metal and the corrosion products, the protocol that canbe applied to restore or preserve the product also changes. Due to the need for more information on the chemical form in which lead is present, an XPS analysis wasperformed. The XPS analysis carried out on the arrowhead allowed the identification of the chemical species present which constituted the main corrosion products. Considering that the Pb signal studied falls at BE of 139.5 eV and comparing the values with the NIST XPS database, the presence of chemicals such as $PbSO_4$, $Pb(NO_3)_2$ was identified and widening the search range by ± 1 eV, the species found were PbS, $PbCl_2$, PbO_2 and $Pb(C_2H_3O_2)_2$.

As regards the presence of lead in the form of PbO_2, a constituent of the mineral known as plattnerite, the values reported by the NIST database for lead in the form of oxide are

PbO_2 = 136.8 – 138.2 eV; PbO = 137.6 - 138.2 eV; Pb_3O_4 = 137.8 eV.

As can be seen, the BE value of the various forms of lead oxide is more than 1 eV higher than the experimental value found. From this result, it can be assumed, with all the necessary precautions, which the lead found is probably not present in the form of oxide. About fibula (Figure 1f), it seems to be part of the collection of fibulas found in the tomb 11 of Madonnelle necropolis. It was contained in a Corinthian amphora in an infant burial. Using astragalus as a playing piece and depositing it in the tomb was a Greek practice in this period. So, the presence of the fibulas inside the tomb gives the certainty that it belongs to a child. The use of the fibula is a characteristic of the Greek period (Verger, 2014).

The correct chemical composition was also detected from the analyses carried out on the lancet (Figure 1g), an artefact that had the function of a surgical scalpel. According to the information given from Museum, it should be entirely made of iron, information which is partly true since the blade of the lancet is of iron (Fe). Still, XRF provided precise indications on the chemical composition of the lancet handle, reporting the presence of Cu, Zn, Fe, Pb, and Sn, and therefore revealing the presence of a binary alloy copper-zinc (Cu-Zn) which forms brass. This result was enjoyable as the lancet's presumed dating was considered the 4th-2nd century BC. Still, the period in which brass appeared in the Balkan area dates back to the end of the 2nd century BC (Bursak, 2022), in which the Western Roman Empire annexed the Balkan region. Therefore, it is possible to think that the artefact in question arrived at the Policoro (MT) area when began the trade routes between the two territories, now part of the same empire. After having subjected the metal artefacts found in the Museum to chemical-physical characterization, two types of cleaning/restoration protocols were evaluated.

About the use of traditional protocol when you want to stabilize the artefact you have to keep in mind the type of raw material from which it is made. If it is aluminum, the product must be protected with cathodic protection, if instead the artefact is made of copper or bronze (copper alloy), just immerse the objects but for a short time due to the high corrosiveness of deionized water against tin. The recent study about the last step ofthis protocol (Guilminot, 2023) have revealed that today, it is used the gel formed from xanthan gum and not wax. It adheres well to the surface of the object. However, xanthan gels are very difficult to remove, even after rinsing thoroughly. For archaeological finds, the use of gels is more problematic. Gels can penetrate the corrosion layers and cause flaking and/or leave residue after treatment. On a surface in good condition, as is generally the case with historic properties, even after thorough rinsing, a large amount of residual xanthan gel remains in the form of a thin layer spread over the surface. However, before proceeding with archaeological metal artefacts, it is advisable to use artificial artefacts that have the characteristics of archaeological artefacts, to fine-tune the protocol each time.

The application of a green protocol was evaluated at Universitè de Neuchatel (Switzerland).

Table 10 shows the different treatments carried out on the samples and the results obtained from the analysis carried out with FTIR and colorimetry

Table 10. *Comparison of the different treatments and relative results obtained from FTIR and colorimetry analyses.*

Liquid Medium	***FTIR***	***Colorimetry***	***Efficay***
Beauveria bassiana	3/3 Formation of	N side: 3/3	100%

Vuill.	copper oxalates	P side: 2/3	
Fungi Filtrate	2/3 Formation of copper oxalates	N side: 2/3 P side: 3/3	66%
Commercial oxalic acid	1/3 Formation of copper oxalates	N side: 3/3 P side: 3/3	33,3%
Gel medium			
Beauveria bassiana Vuill.	3/3 Formation of copper oxalates	N side: 3/3 P side: 2/3	100%
Fungi Filtrate	2/3 Formation of copper oxalates	N side: 3/3 P side: 2/3	66%
Commercial oxalic acid	1/3 Formation of copper oxalates	N side: 3/3 P side: 2/3	33,3%

From these results summarized in Table 10, it can be seen that the use of liquid medium or gel medium is indifferent as the same results were obtained in terms of the production of copper oxalates and colour change of the samples. Regarding the treatment carried out with *Beauveria bassiana* Vuill. and filtered fungi, a significant change in the colour of the samples is observed closely related to the formation of copper oxalates, effective at 100% and 66% respectively; while with the treatment carried out with commercial oxalic acid, there is a strong colour change, but with 1 sample out of 3 in which there is the formation of copper oxalates. From this result obtained, it can be seen that commercial oxalic acid does not create a patina to stabilize the products, but only a colour change.

5. CONCLUSIONS

This research project concerned the physical-chemical and biological characterization of some metal finds, generously entrusted to us by the National Archaeological Museum of Siritide - Policoro (MT).

About the information given by the museum regarding the metal artefacts and in the light of the results obtained from the chemical-physical characterization, it is clear that it is always necessary to analyze the samples with non-destructive analytical techniques so as to know the raw materials, type of production (local or foreign trade), probable traderoutes and precise dating as in the case of the lancet (Figure 1g). The presence of specificchemical elements within the alloy of the manufactured article can greatly influence the effectiveness of their cleaning or consolidation treatments. We, therefore, tried, as far as possible, to obtain a compositional analysis of their construction materials.

Optical microscope analysis and, even more profoundly, the spectra one obtained with non- destructive instrumental methods based on X-rays, have returned a reality that is sometimes different from the visual one. By applying these instrumental methods, it will be possible to make a more informed choice of the technologies to be used to preserve and conserve perishable archaeological resources. The metal finds granted by the Museum for this study were in a severe state of alteration due to corrosion. The inscriptions that described these objects in the Museum were not always accurate because they were based solely on visual observation.

It is necessary to carry out a chemical-physical characterization before displaying the artefacts in a museum.

In this research project, traditional and green restoration and/or conservation protocols were evaluated to analyze which had better efficacy on metal artefacts. Therefore, it was also necessary to evaluate the time taken, and the benefits that these cleaning/consolidation protocols can have when applied to archeological metal artefacts, but for both types of protocols, there are weaknesses and strengths.

The traditional protocol takes a long time (months) but can be applied to almost any kind of metal, but before applying the traditional protocol on the artefact, tests must firstbe carried out with artificial artefacts to avoid the occurrence of side effects directly on archaeological artefacts that are part of the cultural heritage. The disadvantage is that the last step of this protocol involved the use of wax. However, the wax caused the decrease in effectiveness.

As regards the use of *Beauveria bassiana* Vuill. as a green protocol, the strong point is time that this type of protocol requires only 7 days of application without any pre-treatment of the sample. The disadvantage is that the artefact must necessarily be made of copper, therefore a chemical-physical analysis is necessary to ascertain the raw materials of the artefact under examination.

In terms of health for the operator, some studies have highlighted the non-pathogenicity of *Beauveria bassiana* Vuill. towards humans even for long exposure times. This factor must be counted among the advantages of using green protocols as the oxalic acid produced by the fungi is non-toxic, on the contrary instead of commercial substances used in traditional protocols which after long exposure times can be harmful to health. Considering the advantages of green protocols, we are trying to draw up green ones for each type of metal which must have the following requirements: short application times, easy to use, non-pathogenic for the operator's health, excellent efficacy in orderto decrease future corrosive processes. Based on the results obtained with the green protocol using Beauveria bassiaa Vuill. as a biological agent on copper plates, we want to continue this project by studying other biological agents that can have the same efficacy for other types of metals.

Posterity is left free to choose which protocol type to use according to their needs.

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Figure 1. Studied archaeological metal finds: a) javelin tip; b) iron weapon; c) lead stick; d) flask cap; e) arrowhead; f) fibula; g) lancet.

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Abbreviations Index

- Ca: Calcium
- CO_2: Carbon dioxide
- Cu: Copper
- Fe: Iron
- Mn: Manganese
- MT: Matera
- N: Nude
- O_2: oxygen
- OM: Optical microscope
- P: Opaque
- Pb: Lead
- Sn: Tin
- Ti: Titanium
- XPS: X-ray photoelectric spectroscopy.
- XRD: X-ray Diffractometry
- XRF: X-ray fluorescence
- Zn: Zinc

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