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# Tortoiseshell or Polymer? Spectroscopic Analysis to Redefine a Purported Tortoiseshell Box with Gold Decorations as a Plastic Box with Brass

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**Abstract**

The study and preservation of museum collections requires complete knowledge and understanding of constituent materials that can be natural, synthetic, or semi-synthetic polymers. In former times, objects were incorporated in museum collections and classified solely by their appearance. New studies, prompted by severe degradation processes or conservation-restoration actions, help shed light on the materiality of objects that can contradict the original information or assumptions. The selected case study presented here is of a box dating from the beginning of the 20th century that belongs to the Portuguese National Ancient Art Museum. Museum curators classified it as a tortoiseshell box decorated with gold applications solely on the basis of visual inspection and the information provided by the donor. This box has visible signs of degradation with white veils, initially assumed to be the result of biological degradation of a proteinaceous matrix. This paper presents the methodological rationale behind this study and proposes a totally non-invasive methodology for the identification of polymeric materials in museum artifacts. The analysis of surface leachates using $^{1}$H and $^{13}$C nuclear magnetic resonance (NMR) complemented by in situ attenuated total reflection infrared spectroscopy (ATR FT-IR) allowed for full characterization of the object s substratum. The NMR technique unequivocally identified a great number of additives and ATR FT-IR provided information about the polymer structure and while also confirming the presence of additives. The pressure applied during ATR FT-IR spectroscopy did not cause any physical change in the structure of the material at the level of the surface (e.g., color, texture, brightness, etc.). In this study, variable pressure scanning electron microscopy (VP-SEM-EDS) was also used to obtain the elemental composition of the metallic decorations. Additionally, microbiologic and enzymatic assays were performed in order to identify the possible biofilm composition and understand the role of microorganisms in the biodeterioration process. Using these methodologies, the box was correctly identified as being made of cellulose acetate plastic with brass decorations and the white film was identified as being composed mainly of polymer exudates, namely sulphonamides and triphenyl phosphate.

**Keywords**

Tortoiseshell, Cellulose acetate, Noninvasive methodology, Nuclear magnetic resonance (NMR) spectroscopy, In situ Fourier transform infrared (FT-IR) spectroscopy, Plastic objects, Biodegradation, Museum environment

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## Introduction

Tortoiseshell has been used by man since antiquity (found in pre-Egyptian tombs) as costume or art objects or ornamental gems. It is a natural proteinaceous biopolymer composed mainly by β-keratin obtained from the dorsal shells and plastron plates of sea turtles.[1] It became quite popular after the 17th century until the 20th century when it entered the CITES list of endangered species, terminating its legal market.

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**Figure 1.** Object under study. Details from front and back and sampling location in the box (△ microbial isolation and ◇ box micro-sampling; □ATR FT-IR, ☆NMR and ○SEM-EDS analysis).

This study was prompted by an urgent call from the Portuguese Ancient Art Museum regarding an early 20th century tortoiseshell box with gold applications that presented severe surface alteration with the formation of a white film and warping, allegedly due to microorganisms attack (Figure 1). Microorganisms can modify the surface aspect of the objects by producing biofilms, changing the color or making stains, leaving characteristic odor or even penetrate the materials with their own constituent structures, leading to the decomposition of the object.[2–5]

The study of this type of objects can be more complicated due to the fact that after the beginning of the 20th century several semi-synthetic plastics derived from cellulose began to be used to imitate tortoiseshell and other valuable natural materials such as amber, ivory, and coral.[6,7] Today, plastics (polymeric synthetic or semi-synthetic materials) are used to mimic all types of materials and are widely present in daily life objects, as well as an integral part of numerous modern art museum collections worldwide. The polymer additives include (non-exhaustively) plasticizers, antioxidants, UV stabilizers, dyes, fillers, pigments, flame retardants, and flavors.[8]

Within the field of cultural heritage, little is known about the condition of polymeric objects at early stages of degradation, before visible symptoms appear.[9] Even with knowledge transfer from other fields of research and with the recent advances in material characterization, our understanding of polymer stability remains rudimentary.[10]

Polymer degradation can be attributed to chemical, physical, and biological factors. Chemical factors can cause changes to the polymer matrix or their additives, which may result in physical changes or even complete degradation. Physical factors, such as the migration of additives or interaction with the environment, can also cause physical changes, such as warping or brittleness. Chemical degradation is mostly caused by the interaction of the polymers with oxygen, water, ozone, atmospheric pollutants, and radiation (light and heat).[11] Biodeterioration phenomena by microorganisms represent a combination of physical and chemical alteration processes in various materials, such as those constituting the objects that represent our cultural heritage. Storage objects inside structures intended for their preservation have created new man-made environments for microbial species such as fungi and bacteria to inhabit. These microorganisms are the most important agents of biodeterioration in museums, storage rooms, libraries, collections, and restoration studios.[4,5]

Characterization of the growing number of synthetic polymers like plastics in museums is thus essential for assessing the longevity of these materials, establishing proper storage conditions, and informing conservation practice.[12–15]

Various instrumental analytical techniques have been used to identify polymers and additives. The most useful of these unfortunately require sampling or are destructive techniques like pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS), vibrational spectroscopy (transmission Fourier transform infrared spectroscopy (FT-IR) or μ-FT-IR) and classic nuclear magnetic resonance (NMR).[6,16–19] Ideally, the characterization methods should be non-destructive to the artefact since many objects cannot be transported outside the museum collection for reasons of security or because of size.

In this research, non-invasive $^{1}H$ and $^{13}C$ NMR complemented by in situ ATR FT-IR was used to characterize the polymeric matrix (and additives) of the object under study. Other non-invasive methodologies such as variable pressure scanning electron microscopy with energy dispersive spectroscopy (VP-SEM-EDS) and microbiological assays were used to obtain the elemental composition of the metallic decorations and to access the microbial proliferation.

## Experimental

### Samples

The box under study belongs to the Portuguese National Ancient Art Museum and was registered as a tortoise box with gold incrustations (stars and cordon). The box presented severe alteration with formation of a white film on the surface. The sampling process followed the requirements for conservation purposes, minimizing the structural and aesthetical impact of the object (Figure 1).

First, six samples of the surface "biofilm" were collected in different areas to allow the microbiological assays, under aseptic conditions with sterile swabs, placed in suspension of transport MRD medium (Maximum Recovery Diluent, Merck)/NaCl 0.85% solution. Samples were conserved at 4°C until utilization.

For the material characterization of the box, a microsample was collected from a broken area and analyzed by micro-FT-IR. The elemental composition of the metallic decorations was done with VP-SEM-EDS.

Taking into consideration the preliminary results obtained in the first step of the analytical methodology, the box was further analyzed by a new non-invasive methodology that comprised NMR analysis of surface leachates combined with in situ ATR FT-IR, to allow the full chemical characterization of the object matrix composition. For the NMR analysis, solutions were obtained by the leaching technique. A small area of the object (1 $cm^2$) was percolated with approximately 1 mL of 99.96% deuterated methanol, $CD_3OD$ (Euriso-top, France). The percolate obtained was immediately placed in a NMR tube for further analysis.

### Microbiological Assays

Samples collected with cotton buds were diluted in 1 mL of sterile maximum recovery diluent (comp. NaCl 0.9%) and shaken mechanically for 1 h. Fungal isolation was performed using standard mycological medium (malt extract agar, potato dextrose agar, and Cook rose bengal). All cultures were grown for 7–20 days at 28°C: Identification of fungi was based on the macroscopic features of colonies grown on agar plates, and the micromorphology of the reproductive structures was identified by optical microscopy (OM). Fungal strains were identified following standard methods,[20] based on its macro- and micromorphological characteristics, such as colony diameter, texture, color, dimensions, and morphology of hyphae and reproductive structures (for sporulating isolates).

### SEM-EDS

In order to allow the visualization of the surface "biofilm" and the elemental composition of the golden applications, the box was examined without any surface treatment by variable pressure SEM-EDS using a Hitachi Scanning Electron Microscope S-3700N (Tokyo, Japan) in backscattering mode with the accelerating voltage of 18–20 kV and 40 Pa pressure. Elemental composition (point analysis and two-dimensional mapping) was obtained in an EDS BRUKER XFlash 5010 EDX spectrometer (Berlin, Germany) coupled to the SEM.

### Micro-FT-IR

The identification of the box matrix was firstly assessed by μ-FT-IR spectroscopy. Infrared analysis was carried out using a Nicolet Nexus spectrometer coupled with a Nicolet CONTINUM microscope with an mercury cadmium telluride (MCT-A) detector working in the range of 4000–650 $cm^{-1}$. The micro-sample was analyzed in transmission mode in micro-compression diamond cell. The area of analysis of the sample was defined by the double aperture contained in the microscope. For each spectrum, 256 scans were acquired with a spectral resolution of 4 $cm^{-1}$.

### ATR FT-IR

Infrared spectroscopy was performed directly on the object using an Alpha-R spectrometer from Bruker Optics, with an ATR module. Bruker OPUS 6.5 software was used for processing the spectra. The IR spectra were plotted in the region between 4000 and 350 $cm^{-1}$, with 128 scans and spectral resolution of 4 $cm^{-1}$. Contact between the ATR crystal and the object surface was controlled by multiple scan to ensure optimal analysis conditions and the applied pressure did not cause any visible physical change on the surface.

### NMR Spectroscopy

$^1H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III HD 400 spectrometer at 400 MHz. $^1H$ shifts are reported relative to the $^1H$ signal of $CH_3OH$ ($\delta = 3.31$ ppm) reference. $^{13}C$ shifts are reported relative to the $^{13}C$ signal of $CH_3OH$ ($\delta = 49.00$ ppm) reference.

## Results and Discussion

### Biofilm Analysis

Scanning electron microscopy micrographs and isolation procedures presented strong signs of microbiological contamination by bacteria and filamentous fungi. In the case of fungal contamination, it was possible to observe the proliferation of micellar structures and hyphae in the surface of the object (Figure 2). Taking into consideration the initial information that the box was made of tortoise, proteolysis activity assays were performed to evaluate the possible role of these microorganisms in its degradation. The results showed minimal proteolytic activity of these microorganisms and did not allow an adequate explanation on how

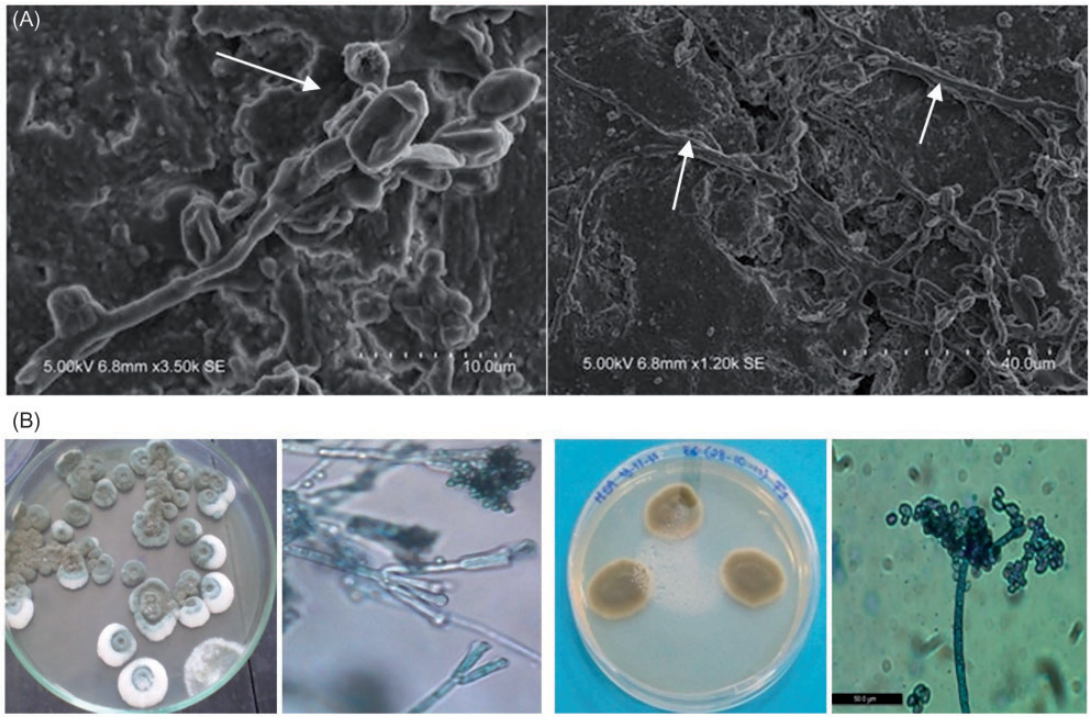


**Figure 2.** (A) SEM micrograph of the box. The arrows indicate the presence of filamentous fungi and spores (left) and hyphae proliferation by the surface (right). (B) Macroscopic and microscopic features of the isolated fungi of the genera *Penicillium* and *Cladoporium*, respectively.

these microorganisms were able to thrive in this substrata. Furthermore, *Penicillium* and *Cladosporium* were found to be the predominant filamentous fungi genera isolated. These fungi are known to have the ability to metabolize cellulose as has been shown in related studies that refer the cellulolytic activity of these genera of fungi.[21,22] These results prompted the team to further evaluate the chemical nature of the substrata and the possible involvement of the microorganisms on the degradation process of the piece.

## *Material Characterization*

The first approach to characterize the box substratum was the analysis of a micro-sample by micro-FT-IR. Surprisingly, the results showed that the box was made of cellulose acetate contradicting the initial information about its tortoiseshell nature and consistent with the presence of the identified cellulolytic filamentous fungi on the surface of the box.

Variable pressure scanning electron microscopy with energy dispersive spectroscopy was also performed on the box without any surface preparation to allow the characterization of the golden alloy. Again, the results contradicted the initial information showing that the metal applications are made of gilded brass instead of a gold alloy (Figure 3). The brass alloy has an average composition of 92% copper and 8% zinc while the gold layer is an alloy composed of 75% gold, 21% silver, and 4% copper.

To deepen the study on the chemical nature of the box substratum, a completely new non-invasive analytical methodology was envisaged by combining ATR FT-IR with $^{1}H$ and $^{13}C$ NMR. This approach was also intended to evaluate the feasibility of this methodology for the study of plastic artefacts without sampling.

Using non-destructive and non-invasive ATR FT-IR, it was possible to identify and classify the polymer type and also some additives. The ATR FT-IR spectra of the object (Figure 4) show three strong patterns of bands found in saturated molecules, like cellulose acetate, and typically referred to as "rule of three". The first band at 1734 $cm^{-1}$ is due to carbonyl stretch of the ester group, while the second band at 1221 $cm^{-1}$ is due to asymmetric stretching of C–C–O of the ester group. The last large band appearing at 1029 $cm^{-1}$ is the result of asymmetric O–C–C bond stretching attached to the carbonyl carbon. In addition, smaller bands located at 1368 $cm^{-1}$ are caused by methyl groups found in acetate esters. Other smaller bands present at 1590, 1488, and 961 $cm^{-1}$ suggest the presence of additives such as triphenylphosfate and benzenesulfonamide.

The presence of these polymer additives was further confirmed by analysis of the surface leachates by NMR, which is a non-destructive powerful tool that identifies unequivocally organic molecules, such as the polymer additives.

The $^{1}H$ NMR spectrum of object (Figure 5) clearly presents a mixture of four organic compounds,

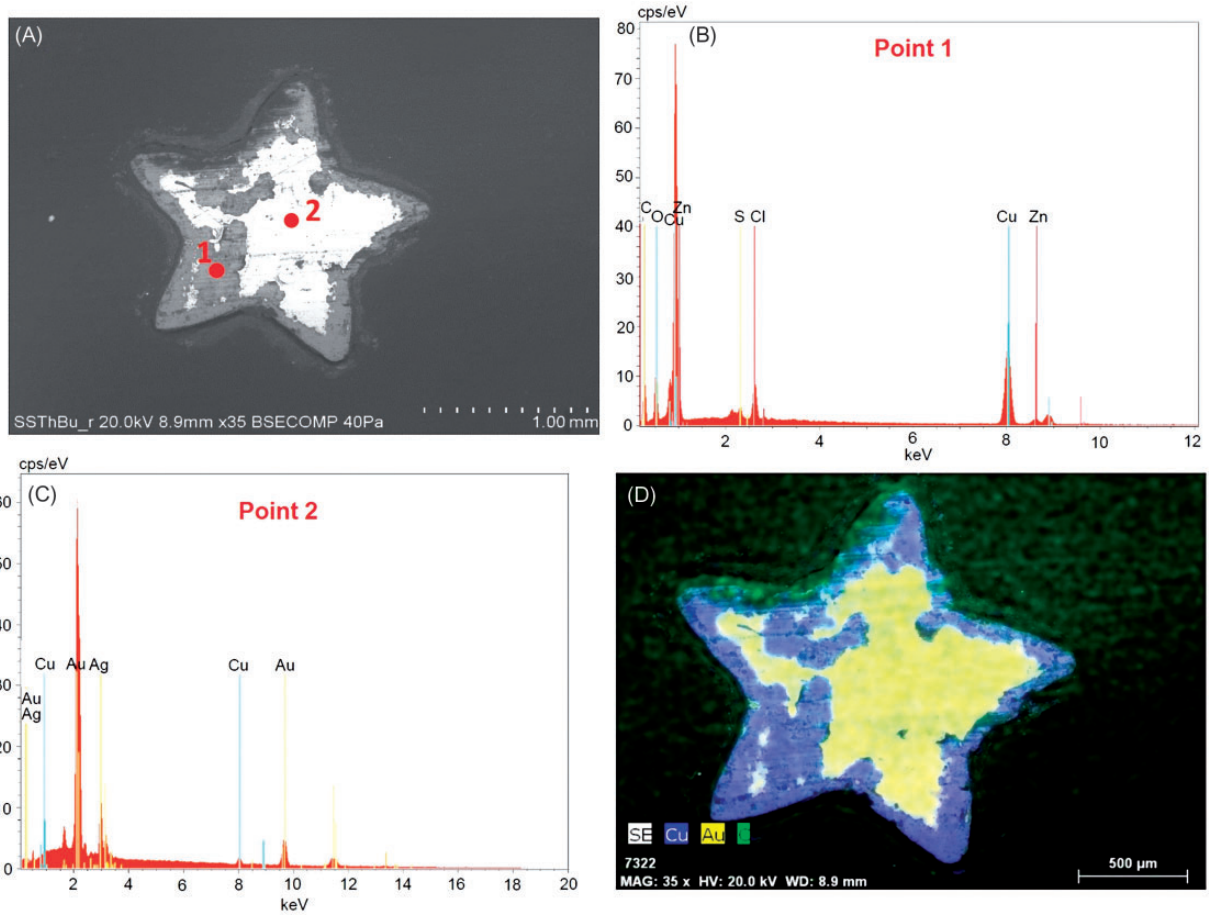


**Figure 3.** SEM micrograph (A) and elemental composition point analysis (B, C) and two-dimensional mapping of metal decorations (D).

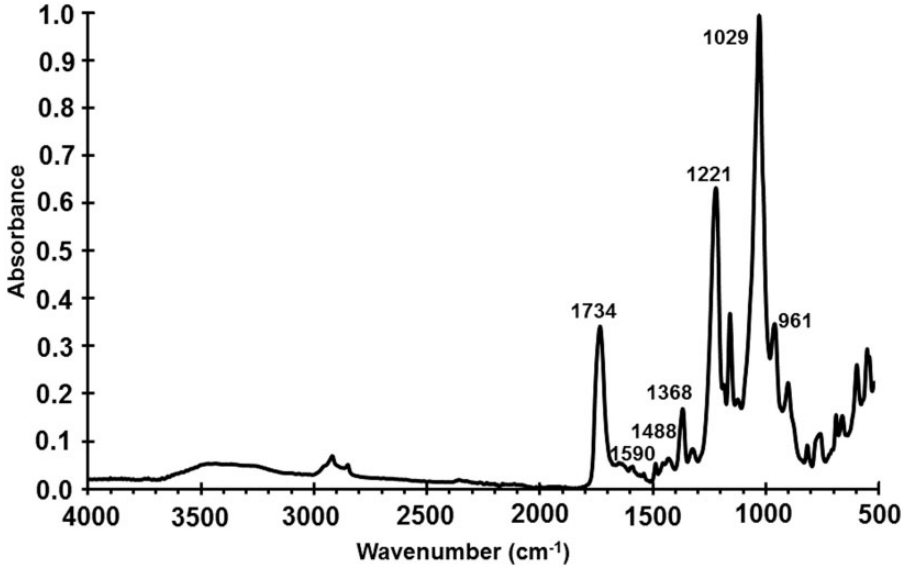


**Figure 4.** Attenuated total reflection Fourier transform infrared spectrum.

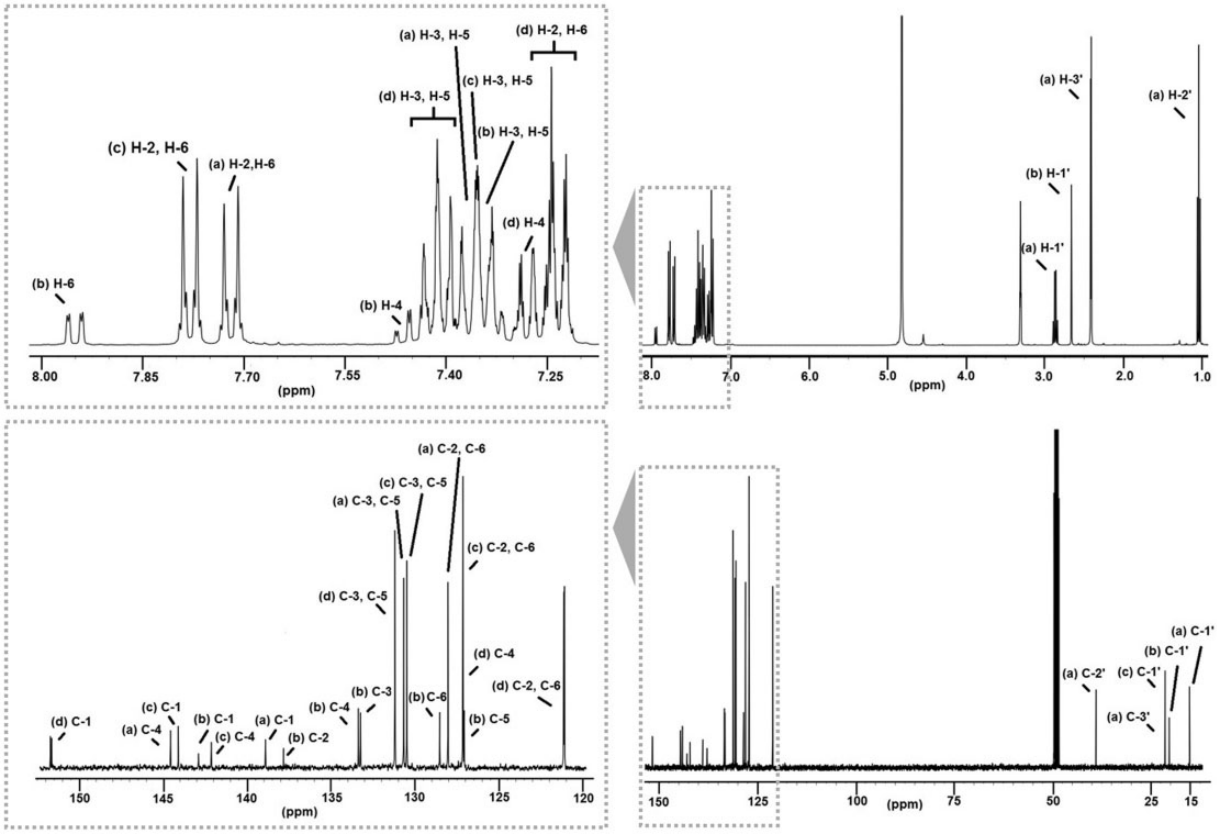


**Figure 5.** Top: $^{1}$H NMR spectrum (right) and 7.10–8.00 ppm expansion (left); Bottom: $^{13}$C NMR spectrum (right) and 120–153 ppm expansion (left).

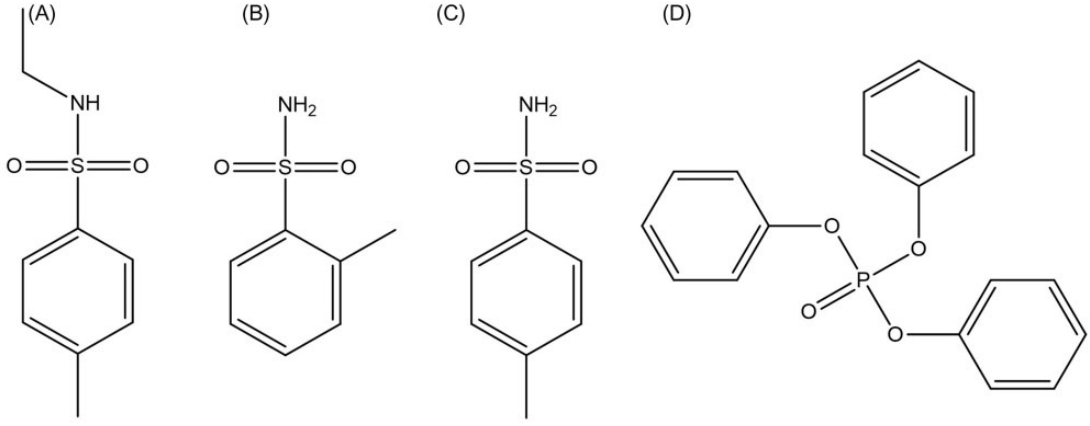


**Figure 6.** Additives identified in the analyzed samples. (A) *N*-ethyl-*p*-toluenesulfonamide, (B) *o*-toluenesulfonamide, (C) *p*-toluenesulfonamide, and (D) triphenylphosfate.

namely, *N*-ethyl-*p*-toluenesulfonamide, *o*-toluenesulfonamide, *p*-toluenesulfonamide, and triphenylphosfate (Figure 6).

*N*-ethyl-*p*-toluenesulfonamide (Figure 6a) presents a characteristic pattern of an *N*-ethyl group with a triplet and a quadruplet, at $\delta = 1.04$ and 2.87 ppm, respectively. The singlet of the amine proton appears at $\delta = 4.55$ ppm. The aromatic methyl group, a singlet, resonates at $\delta = 2.42$ ppm, and the four aromatic protons, in a system AA'BB', appear as two doublets of doublets at $\delta = 7.37$ ppm and $\delta = 7.72$ ppm, generated by H-3/H-5 and H-2/H-6, respectively.

**Table 1.** $^{13}C$ assignments of the additives.

| Additive | C | δ (ppm) |
|---|---|---|
| a | 1 | 138.91 |
| | 2 | 128.04 |
| | 3 | 130.68 |
| | 4 | 144.56 |
| | 5 | 130.68 |
| | 6 | 128.04 |
| | 1′ | 15.19 |
| | 2′ | 38.96 |
| | 3′ | 21.42 |
| b | 1 | 142.90 |
| | 2 | 137.84 |
| | 3 | 133.25 |
| | 4 | 133.38 |
| | 5 | 127.07 |
| | 6 | 128.53 |
| | 1′ | 20.33 |
| c | 1 | 144.11 |
| | 2 | 127.07 |
| | 3 | 130.49 |
| | 4 | 142.14 |
| | 5 | 130.49 |
| | 6 | 127.07 |
| | 1′ | 21.39 |
| d | 1 | 151.64, 151,72 |
| | 2 | 121.10, 121.15 |
| | 3 | 131.19, 131.20 |
| | 4 | 127.15 |
| | 5 | 131.19, 131.20 |
| | 6 | 121.10, 121.15 |

In Figure 6b, *o*-toluenesulfonamide presents the aromatic methyl group, a singlet, at $\delta = 2.66$ ppm. The four aromatic protons appear as a multiplet at $\delta = 7.30$–7.36 ppm generated by H-3/H-5, a double doublet of doublets at $\delta = 7.46$ ppm generated by H-4, and a doublet of doublets at $\delta = 7.95$ ppm generated by H-6.

In Figure 6c, *p*-toluenesulfonamide presents the aromatic methyl group, a singlet, at $\delta = 2.41$ ppm. The four aromatic protons appear in a system AA'BB'. The two *ortho* protons resonate as a doublet of doublets at $\delta = 7.35$ ppm and the *meta* protons as also a doublet of doublets at $\delta = 7.78$ ppm.

Triphenylphosfate (Figure 6d) presents three magnetically equivalent groups, with five aromatic protons each, in a system AA'BB'C. The two *orto* protons resonate as a multiplet at $\delta = 7.22$–7.25 ppm, the *meta* protons as a multiplet at $\delta = 7.27$–7.29 ppm, and the *para* proton also as a multiplet at $\delta = 7.39$–7.41 ppm.

The interpretation of $^{13}C$ NMR spectrum (Figure 5) allowed the unequivocally assignment of all carbon signals (Table 1), confirming the presence of the additives detected in $^{1}H$ NMR. The $^{13}C$ NMR technique proved to be a non-destructive analytical tool with high potential for the polymer additives characterization.

The new methodology allowed a deep insight on the chemical nature of the synthetic polymer and opens new analytical tools for the study of plastic objects in museum and cultural heritage contexts.

## Conclusion

This research started with a tortoise box with gold applications and ended up with a cellulose acetate box with brass applications. On a first glance, for the Portuguese Museum of Ancient Art this result was disastrous. However, major outcomes were indeed obtained:

1. The box was isolated from the rest of the collection avoiding possible contamination due to the release of acetic acid and other volatile compounds.
2. This work demonstrated that the use of NMR to analyze surface leachates complemented by in situ ATR FT-IR spectroscopy of the surface has an enormous potential for the plastics characterization on the field of cultural heritage. ATR FT-IR provided mainly the identification of the polymeric matrix and NMR the complete characterization of the additives, even isomeric mixtures.
3. This methodology avoids sampling of the object and allows its identification without any visible physical damage, representing an important advance for the study of plastic artefacts in museums.
4. The biological and enzymatic assays allowed the identification of the microorganisms present. In this case, it was possible to ascertain the role of filamentous fungi able to produce cellulolytic enzymes on the biodeterioration of the piece.

### Conflict of Interest

The authors report there are no conflicts of interest.

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