

## Article

# Investigations of the Surface of Heritage Objects and Green Bioremediation: Case Study of Artefacts from Maramureş, Romania

Dorina Camelia Ilieş <sup>1,\*</sup> , Nicolaie Hodor <sup>2</sup>, Liliana Indrie <sup>3</sup>, Paula Dejeu <sup>4</sup>, Alexandru Ilieş <sup>1</sup>, Adina Albu <sup>3</sup>, Tudor Caciora <sup>1,\*</sup> , Marin Ilieş <sup>5</sup>, Lucian Barbu-Tudoran <sup>6,7</sup> and Vasile Grama <sup>1</sup> 

- <sup>1</sup> Department of Geography, Tourism and Territorial Planning, Faculty of Geography, Tourism and Sport, University of Oradea, 1 Universitatii Street, 410087 Oradea, Romania; dilies@uoradea.ro (D.C.I.); ailies@uoradea.ro (A.I.); vgrama@uoradea.ro (V.G.)
  - <sup>2</sup> Faculty of Geography, “Babes-Bolyai” University, 5–6 Clinicilor, 400090 Cluj Napoca, Romania; nicolaie.hodor@ubbcluj.ro
  - <sup>3</sup> Department of Textile, Leather and Industrial Management, Faculty of Energy Engineering and Industrial Management, University of Oradea, B. St. Delavrancea Str. No. 4, 410058 Oradea, Romania; lindrie@uoradea.ro (L.I.); aalbu@uoradea.ro (A.A.)
  - <sup>4</sup> Medical Laboratory Service M.D., Bethany Medical Clinic, 410004 Oradea, Romania; office@betania-centrumedical.ro
  - <sup>5</sup> Faculty of Geography Extension, “Babes-Bolyai” University, 6 Avram Iancu Street, 435500 Sighetu Marmatiei, Romania; marin.ilies@ubbcluj.ro
  - <sup>6</sup> Electron Microscopy Laboratory ‘Prof. C. Craciun’, Faculty of Biology and Geology, “Babes-Bolyai” University, 5–7 Clinicilor Str., 400006 Cluj-Napoca, Romania; lucian.barbu@itim-cj.ro
  - <sup>7</sup> Electron Microscopy Integrated Laboratory, National Institute for R&D of Isotopic and Molecular Technologies, 67–103 Donat Str., 400293 Cluj-Napoca, Romania
- \* Correspondence: caciora.tudoriulian@student.uoradea.ro; Tel.: +40-740941144



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**Abstract:** Old textiles are important elements of the cultural heritage. As a result of their composition mostly of natural elements old textiles are extremely prone to physical and chemical degradation due to fungal action. The treatments usually applied for the cleaning of heritage textiles target the use of synthetic fungicides, which are potentially harmful to both human health and the environment. Numerous studies highlight as an alternative to the use of conventional antifungals, the employment of essential oils and plant extracts, which are environmentally friendly and which have no adverse effects on human health. Against this background the present study aims to test six essential oils (*Lavandula angustifolia*, *Citrus limon*, *Mentha piperita*, *Marjoram*, *Melaleuca alternifolia*, *Origanum vulgare*) to establish their inhibitory effects against fungi identified on an old piece of traditional Romanian clothing from Maramureş. For the study, the types of fungi present on the objects was determined primarily through the open plates technique and microscopic identification. After identification, the essential oils were applied to the delimited surfaces, and their effects observed up to 32 days after application. The results show that these essential oils have a strong inhibitory effect on such fungal genera as *Penicillium* sp., *Cladosporium* sp., *Aspergillus* spp., *Candida guilliermondii*, *Botrytis* sp., *Mucor* sp., having no observable side-effects on the physical properties of the materials concerned. The antimicrobial effects that essential oils and plant extracts have in the short term must be tested in future to ensure the enhanced preservation of heritage textiles and the health integrity of the restorers and visitors who view them in museums, collections or exhibitions.

**Keywords:** cultural heritage; materials; fungi; essential oils; antifungal; inhibitory effects

## 1. Introduction

The traditional folk costume from Maramureş is an important component of the Romanian cultural heritage. Certain elements of the peasant’s traditional garments are two thousand years old, as evidenced by the scenes on Trajan’s Column in Rome, Italy

(113 AC), those on the Monument to Adamclisi, *Tropaeum Traiani* (108–109 AC), from Dobruja, Romania and some other important archaeological discoveries from Romania. The preservation and conservation of popular traditional cloths is of great importance, especially now, when the modernisation of society and globalisation generate a growing standardization and lack of differentiation in the appearance of outfits [1,2], most especially those for men. While Maramureş celebration clothes still retain their former appearance, recently some materials and colours have come to be produced by relatively advanced and modernised techniques. The traditional objects are those most subject to degradation, due to the numerous insects, moths, fungi, bacteria and mechanical forces, among others, to which they are subjected over the years. At the same time traditional methods of preservation and conservation can be lost. Therefore, the preservation, in good condition, of the old components of traditional garments has acquired increasing importance, for the use, the identity and the pride of the future inhabitants of the region/country. Arguably, Maramureş has become an identity brand, symbolising the art and the creative power, as well as the youth, vigour and elegance of the Romanian inhabitants of Maramureş.

Under investigation is an aged garment object which is mainly made up of natural sheep's wool; for the popular motifs that adorn it materials as sheepskin, cotton yarn, pearly beads and hemp yarn also are used. All of these materials are organic, undergoing chemical processing to give them a long life, stability and elasticity, if they are stored and used properly. Historically, the causes of damage to natural materials vary over time. Biological damage is a factor that makes the leather and furs particularly vulnerable. Due to the acidity of the skin under conditions of high humidity, the development potential for colonies of microorganisms is very high, with them adhering to the substrate along with dust particles and other environmental substances. Furs and wool can be attacked by insects such as moth larvae, beetles and others, and specific microclimatic conditions, characterised by conditions of high humidity, low intensity of light and others, favour their multiplication. Bio-damage can lead, in time, to the complete destruction of the object. Physical and chemical damage is caused by natural factors, with chemical damage being faster than physical damage, with the former's effects being profound and irreversible [3]. Deterioration can be caused by natural factors [4]. Temperature levels can pose problems that are difficult to solve in relation to the conservation of collections if favouring the development of moulds (along with high humidity), along with insects and rodents, and accelerating chemical damage to materials. The amount of damage incurred is increased when the materials are present in a relative humidity below 50%, under which conditions they tend to become dry and brittle, and their physical strength decreases. At high humidity or on contact with aqueous solutions, the collagen present is destroyed, with negative effects occurring in terms of destruction of the structure and an increase in the hydrophilicity of the fibres involved. Light, due to photochemical effects, can also cause significant degradation, in terms of the weakening of the resistance of the material substrate, friability, chromatic changes, rupture of collagen fibres and others. Ultraviolet light should be removed without affecting the lighting concerned. The presence of dust promotes biological attacks on the skin and furs. Damage to the objects due to anthropogenic causes can also occur, as a result of improper storage and exposure (e.g., materials being placed directly on top of one another, wrinkled, placed in contact with metal objects, fixed with needles and/or nails, the improper affixing of labels among others) [5,6].

Under certain environmental conditions the colonization of microorganisms is possible with the combination of factors including: temperature, humidity fluctuation, natural or artificial lighting (favorable premises for the installation and evolution of microorganisms are improper exposure to sunlight and weather [7–9], dust content and carbon dioxide high values or when nutrients are favorable); these can induce very frequent biodeterioration, alteration processes [10–12]. Wool fibers are most susceptible to attack by bacteria and fungi, especially in conditions where moisture can be accentuated due to the hygroscopic properties of wool. The microorganisms most frequently [13] mentioned in degradation of wool and other protein fibers are bacteria which belongs to the genus:

*Bacillus*, *Proteus*, *Actinomycetes* (*Streptomyces* sp.) and micro-fungi: *Aspergillus*, *Fusarium* and *Trichoderma*, *Penicillium*.

The biodeteriogens represented by fungi can lead to the deterioration of the cultural heritage organic composition materials, with their hyphae penetrating the substrate material favouring the germination of spores. The possible physical damage is likely to be colouring/discoloration, the smell of mould, fissures, fragment detachments, fragility and the variation of dyeing properties, among others [13–17]. Possible chemical damage and changes can be wrought by fungal-derived carboxylic acids, including oxalic, citric, succinic, formic, malic, acetic, fumaric and others. In addition, chemical changes can also be reflected in aesthetical damages to the cultural materials concerned, such as in the form of the discoloration and deterioration of the surfaces involved, leading to the appearance of stains that may alter the original colour of the garment [18–21]. The presence of organic residues (e.g., glue, dirt, dust) may accelerate the processes of degradation, with the aesthetical changes and chromatic alteration taking place. At the same time, they can lead to the loss of strength and elongation specific to the material, oxidation, discoloration or coloration, due to the pigments involved and the modification of the molecular structures present [22].

Remediation can be realised using toxic materials (e.g., ethylene oxide [EtO], gamma rays, etc.), but the use of such materials can significantly affect the biodiversity and ecological systems concerned. Therefore, specialists in the field try to apply environmentally and ecologically friendly biocides instead. The use of antifungal natural extracts (i.e., essential oils [EOs]) has always been a viable alternative to the use of harmful chemicals, with, for example, plant extracts (*Allium ursinum* and *Ocimum basilicum*) having been tested as successful antifungals [23].

EOs (e.g., lemon, spearmint, rosemary, fennel, marjoram, pin, eucalyptus, etc.) can successfully serve as part of a green bioremediation procedure to be applied in the sustainable conservation of artworks. Having been tested against microbial colonies isolated from different substrates using green potential strategies, they have been found to act as a sound alternative to traditional procedures in terms of their selective action, human safety and impacts on artworks [20,24–41]. The antifungal activity of *Origanum vulgare*, *Rosmarinus officinalis* and *Lavandula angustifolia* EOs has been tested, with positive results having been achieved against fungi isolated on stone (*Bipolaris* sp.) and against *Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp. on different objects by Stupar et al. [42], Savković et al. [43] and Bayramoğlu [44], among others. Some relevant studies in the field [45–50] confirm that thymus EO has been found to have good antimicrobial activity against *Bacillus*, *Staphylococcus*, *Fusarium* and *Aspergillus* spp., in comparison to the effects of EO with commercial biocide. Radwan et al. [49] point out the inhibitory effect of thyme, clove and cinnamon EOs on *Candida albicans* and different finds of mould. The EOs of eucalyptus and lavender tested with good results as natural preservatives for leather [51], with oregano EO being successfully used as a bactericidal agent in the leather industry by Bayramoğlu et al. [26,44]. Combinations of the EOs [52], *Thymus vulgaris* and *Pimpinella anisum* and methanol extracts, have shown outstanding antibacterial properties against the pathogenic bacteria, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, *Salmonella typhi*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. EOs obtained from *Matricaria chamomilla* have been tested [53] as environmentally friendly in cultural heritage environments, against *Aspergillus* spp.; similarly, Mahilrajan et al. [54] pinpoint in the same study, the intense antifungal activity of camphor oil, in comparison with other tested oils. Pepa et al. [55] point out that EOs from southern Italy, derived from *Origanum*, tested with the minimal inhibitory concentrations, *in vitro*, with evident antibacterial and antifungal activity. EOs like *Origanum vulgare* and *Thymus vulgaris* have also been applied by Palla et al. [56] to emphasise the green remediation in the biodeterioration of cultural heritage artworks, which is induced by fungal colonisation (e.g., by that of *Aspergillus flavus*). The above-mentioned authors concluded that valid green conservation strategies

(with no negative effects for human health and no environmental pollution) could possibly replace traditional biocides.

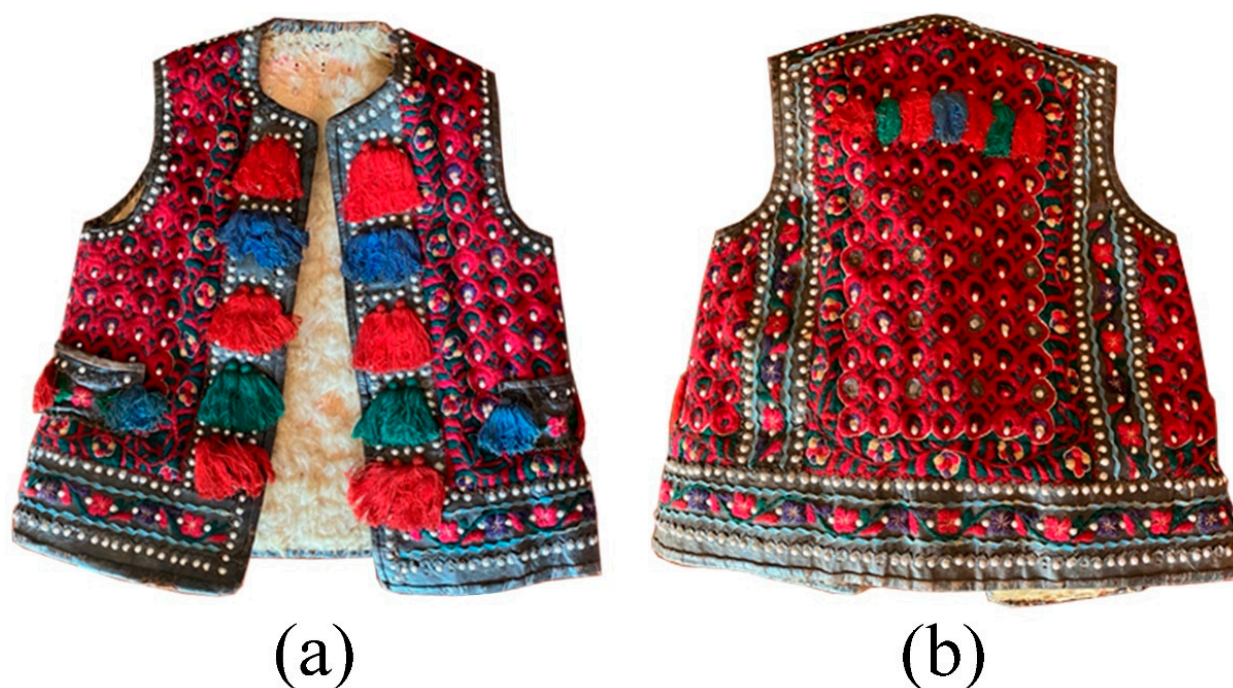
In Romania, recently, an EO isolated from thyme (*Thymus vulgaris*) was assayed for antifungal activity against *Candida albicans* and *Aspergillus niger* on sheepskins, with it being shown to have outstanding antifungal proprieties [57–59]. Moreover, the studies by Niculescu et al. [60,61] and Marcu et al. [62] have emphasised the possibility of using antifungal ecological materials to treat cultural heritage objects made of natural leather.

Following the above, this research aimed to investigate the microbial load of an old heritage object, made of natural animal sheepskin, which metabolic activity could generate, thus accelerating the biodegradation process, and the loading of the aerosol with biological particles, like spores, toxins and allergens, as well as other harmful substances. Such action can cause problems regarding the health of workers and users and the application of natural biodegraders. Consequently, the antifungal properties of some EOs of *Lavandula angustifolia*, *Citrus limon*, *Mentha piperita*, *Marjoram*, *Melaleuca alternifolia*, *Origanum vulgare* were tested, with the results of their applications being recorded at certain time intervals.

## 2. Materials and Methods

### 2.1. Investigated Object

The object investigated was a handmade, short coat for young men, lined with sheepskin from the Mara Valley, Maramureş, Romania (Figure 1) [63–66], about 80 to 100 years old. The men's garment was created to cover the torso, with it being worn most often on important holidays or on Sundays.



**Figure 1.** Traditional Romanian men's coat; (a)—front view; (b)—back view.

The coat was made of hemp thread (also called '*pânză de tort*' in Romanian). The coat's lining consisted of sewn tanned lambskin, of curly wool. On all its edges and at the seams, an intensely dark-hued strip ('*cipcă*') or hem of red leather was applied, conveying a sense of perfect regularity and symmetry. On the inside, there was a large pocket on the left side, facilitating access by the right hand. The coat was decorated by hand-sewing (similar to embroidery), with coloured thread, and by applying decorative elements made of tanned leather, over the whole bridge of the coat, consisting of the tree of life, rose flowers called '*ruji*', stitched flowers encircling mirrors and pseudo zig-zag patterning. In the middle

of some rose flowers without mirrors, special metal buttons ('*bumbi*') had been placed, rounded and extended, with some fasteners facing inwards, which helped to affix them to the fabric structure, by means of their being bent. Near the free edge of the wings and on the back of the coat, close to the shoulders are several ornamental tassels (bell-shaped tassels) arranged symmetrically, in red, green and blue colours.

In the last half century, other examples of such fur coats (from Mara) have begun to be worn in other parts of Maramureş, becoming an identity brand [67–70], symbolising the art and creative power of the youth, as well as the vigour and elegance of the Romanians in Maramureş.

## 2.2. Analytical Methods

The procedure followed in the current study required the following materials: a delimiting frame; sterile swabs on a wooden rod; and six EOs with 100% purity from the Young Living Essential Oils, used for their antifungal properties [71–79]. The types of EOs employed were: *Mentha piperita*, *Lavandula angustifolia*, *Citrus limon*, *Melaleuca alternifolia*, *Marjoram* and *Origanum vulgare*. The products used consisted of: *Mentha piperita*, batch 103519, expiry date 7 August 2021; *Lavandula angustifolia*, batch 87491, expiry date 7 August 2021; *Citrus limon*, batch 103517, expiry date 7 August 2021; *Tea Tree*, batch 89075, expiry date October 2021; *Marjoram*, batch 86942, expiry date July 2022; and *Oregano*, batch 89094, expiry date September 2022. The material resources used consisted of: Sabouraud sterile culture medium for the yeast and mould isolation; an incubator ICT 18/FALC with a temperature range between 5 and 80 °C; a microbiological hood; glass slides and slides for the microscopic identification; microbiological handles; an optical microscope Micros Austria with binocular head series BIM-105B; an API yeast identification kit<sup>®</sup> 20C AUX; and a densitometer.

Six different areas on the face of the traditional coat were examined, as shown in Figure 2. The working areas were delimited by means of a metal dial with a size of 25 cm<sup>2</sup>. Small fragments (millimetres) of fur and sheepskin were removed with tweezers previously sterilized by the incandescent method for testing of the fungi concerned; so as to observe the effects that they might have had on the individual strands of material. The tests were conducted both before and after applying the EOs to each delimited area, although the primary samples were considered as blank references.

After delimiting the surfaces on which the in-situ procedures were to be performed, the samples of the surface were taken. The samples were extracted using sterile swabs from all six areas of the sheepskin coat, both before and after the application of the Eos (see Table 1). Ten drops (the equivalent to 300 µL) of the corresponding EO were applied directly to the tested material surface in the centre of each delimited area [75]. After the application of the EOs, three samples were taken for each of the six different work areas delimited on the area of sheepskin to which the EOs were applied. The three samples were taken at pre-set intervals, with the first sample being taken 30 min after the administration of the EOs, the second test being administered after 24 h and the third after 48 h. Table 1 also shows the type of material examined and the type of EO applied to each of the delimited areas. The EO used for each type of material concerned was selected in accordance with the recommendations made in the literature, especially that of Palla et al. [56], which state that certain EOs tend to be more effective than others when applied to certain types of material, such as those which constitute heritage objects.

It must be noted that the air in the room in which the sheepskin coat was stored and examined was not circulated, and neither was the room ventilated for 48 h during the entire examination, so as to avoid contamination with other types of spores from the atmosphere that could, otherwise, have been introduced by currents of air moving through the room. The temperature was maintained between 22 °C and 24 °C, while the humidity was kept between 52% and 55%.



**Figure 2.** Delimitation of the six work areas on the front of the coat, indicating the type of essential oil applied to each (A)—*Lavandula angustifolia*; (B)—*Citrus lemon*; (C)—*Mentha piperita*; (D)—*Melaleuca alternifolia*; (E)—*Marjoram*; (F)—*Origanum vulgare*.

**Table 1.** Areas of the sheepskin coat examined, and to which essential oils applied.

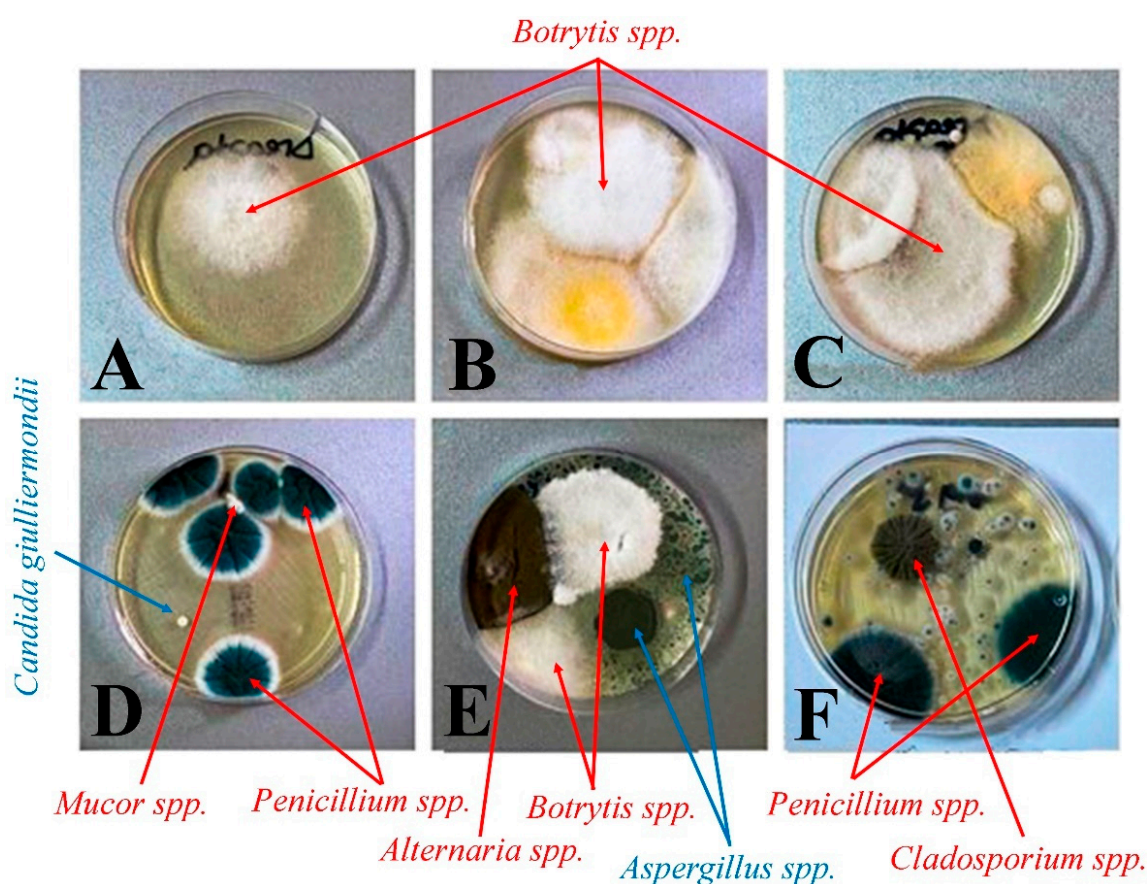
Examined Area (Figure 2)	Applied Essential Oil	Material Type
A	<i>Lavandula angustifolia</i> (lavender)	Cotton yarn
B	<i>Citrus limon</i> (limon)	Leather-cotton yarn mix
C	<i>Mentha piperita</i> (mint)	Leather
D	<i>Melaleuca alternifolia</i> (tea tree)	Wool
E	<i>Marjoram</i> (marjoram)	Cotton yarn
F	<i>Origanum vulgare</i> (oregano)	Pearly beads

With the EOs having been applied directly to the examined surfaces, all the samples were seeded on sterile Sabouraud culture media, with the plates used being incubated for 30 days at a temperature of 28 °C. The third stage of the procedure involved isolating and identifying the fungi developed on culture media. The plates were evaluated daily to observe their evolution in terms of the changes in the appearance of the colonies concerned, with the evaluation undertaken sequentially being that of shape, texture, consistency, diameter, colour and contour.

The moulds present were identified after evaluating the macroscopic and microscopic characteristics involved, and the yeast species was determined, using the API<sup>®</sup> 20 °C AUX6 identification kit, after examination of the 19 biochemical assimilation reactions that took place. The technique of working on the latter involved preparing the yeast suspension solution, calibrated at 2 McFarland, using a densitometer, and thereafter pipetting 100 µL into each microdwell of the gallery. After incubating the API gallery at 29 °C ± 2 °C for 72 h, the results were read, with a final result being obtained after comparing each well with the negative control. The final result was a 7-digit numerical profile, which was decoded using Apiweb<sup>™</sup> computer software, so as to identify the corresponding yeast species.

### 3. Results

Fungal colonies were developed rapidly on Sabouraud media, from samples taken before the application of EOs. At 72 h of incubation, the first fungal colonies could be macroscopically visualised. After 10 days of incubation, the plates were completely invaded. Figure 3 shows the plates on the seventh day of incubation; each image indicates the examined area of the sheepskin coat. The high degree of fungal contamination of the coat can easily be observed.



**Figure 3.** Plates seeded with primary samples (before application of essential oils). Reading taken after 7 days of incubation (A)—*Lavandula angustifolia*; (B)—*Cistrus lemon*; (C)—*Mentha piperita*; (D)—*Melaleuca alternifolia*; (E)—*Marjoram*; (F)—*Origanum vulgare*.

In the above-mentioned way, seven different types of colonies were identified [80,81], with six being from the mould class (*Alternaria* sp., *Aspergillus* sp., *Botrytis* sp., *Cladosporium* sp., *Mucor* sp. and *Penicillium* sp.) and one from the yeast class (*Candida guilliermondii*) (Table 2).

As previously stated the number of samples taken after applying the EOs was 3 sets of 6, taken 30 min, 24 h and 48 h after applying the corresponding EO. The plates were monitored for 30 days in order to evaluate the inhibitory effects of the EOs. Figure 4 shows the plates, as read on day 14 of the incubation. The inhibitory effects of the EOs are evident. The only plate on which a fungal colony developed is the one corresponding to the inner area of the sheepskin coat, present in the sample taken 48 h after applying the EO of *Melaleuca alternifolia* (tea tree). The isolated fungal colony consisted of *Cladosporium* sp., which became macroscopically visible on day 5 of incubation. It was observed that this type of mould was not isolated from the primary sample, as is detailed in the discussion section below.

Table 2. Identified fungi and the effect of essential oils.


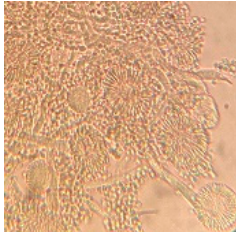

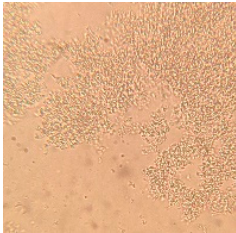
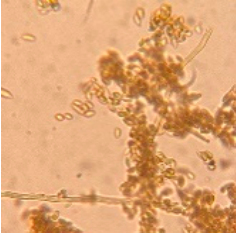

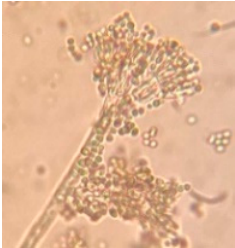
Microscopic Image	Genus Name	The Examined Area of the Sheepskin Coat from Which It Was Isolated (Figure 2)	Applied Essential Oils—Effect
	<i>Alternaria</i> sp.	Area E	<i>Marjoram</i> —inhibitory
	<i>Aspergillus</i> sp.	Area E	<i>Marjoram</i> —inhibitory
	<i>Botrytis</i> sp.	Area A	<i>Lavandula angustifolia</i> —inhibitory
		Area B	<i>Citrus limon</i> —inhibitory
		Area C	<i>Mentha piperita</i> —inhibitory
		Area E	<i>Marjoram</i> —inhibitory
	<i>Candida guilliermondii</i> Profile API: 6702377	Area D	<i>Melaleuca alternifolia</i> —inhibitory
	<i>Cladosporium</i> sp.	Area F	<i>Origanum vulgare</i> —inhibitory
	<i>Mucor</i> sp.	Area D	<i>Melaleuca alternifolia</i> —inhibitory

Table 2. Cont.

Microscopic Image	Genus Name	The Examined Area of the Sheepskin Coat from Which It Was Isolated (Figure 2)	Applied Essential Oils—Effect
	<i>Penicillium</i> sp.	Area D Area F	<i>Melaleuca alternifolia</i> —inhibitory <i>Origanum vulgare</i> —inhibitory

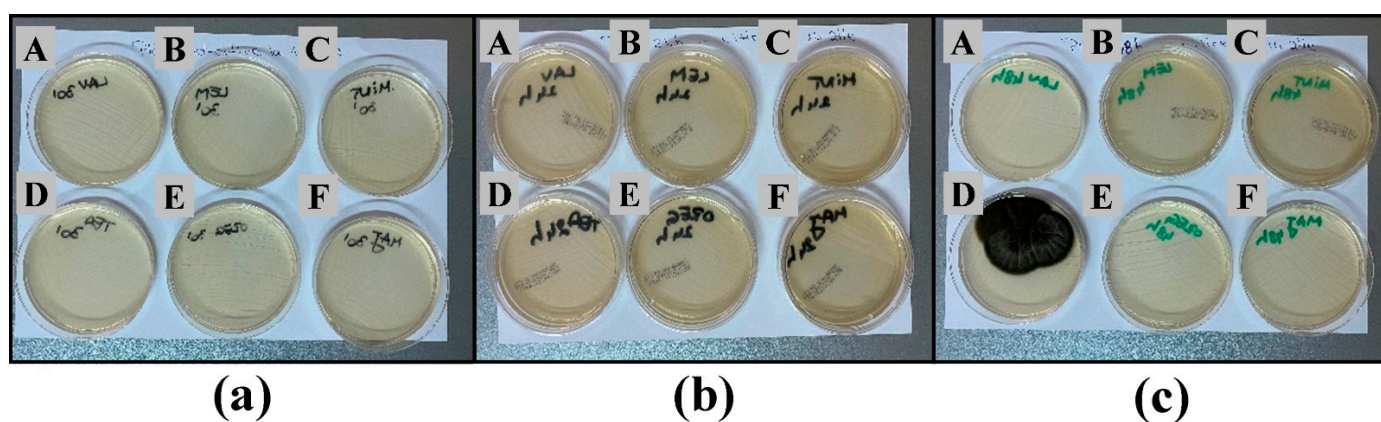


Figure 4. The plates read on day 14 of incubation: the samples at (a) 30 min, (b) 24 h and (c) 48 h.

Figure 5a–c show the plates, as read on day 30 of the incubation. The developed colonies became macroscopically visible after between 20 days to 25 days of incubation.

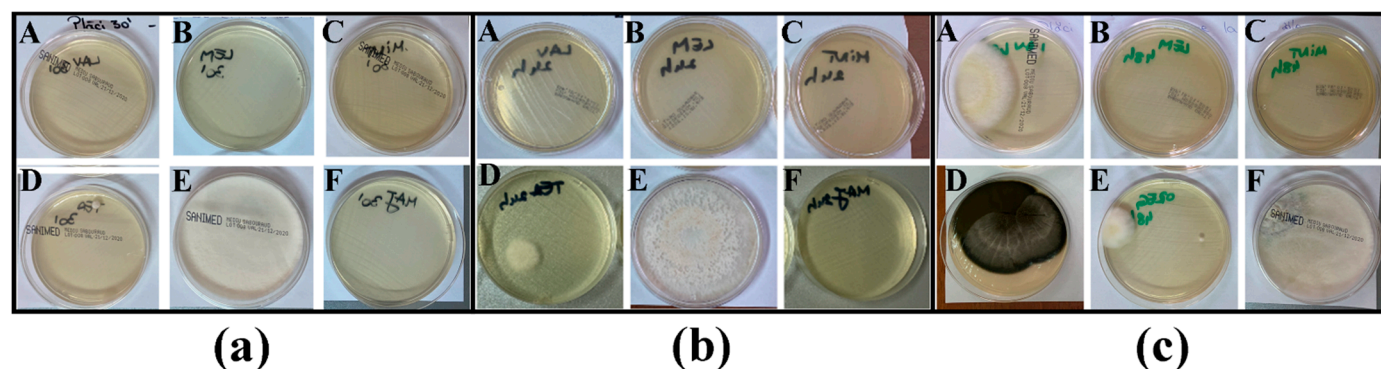


Figure 5. The plates read on day 30 of incubation: samples at (a) 30 min, (b) 24 h and (c) 48 h.

The results, obtained after the samples from the examined areas were examined at 30 min, 24 h and 48 h following the application of the EOs, are presented in Table 3.

The existing difference was due to the duration of the effect that they had on certain types of fungi as shown on Table 4. It is evident that some EOs (e.g., *Lavandula angustifolia*, *Melaleuca alternifolia*, *Marjoram*, *Origanum vulgare*) inhibited the action of some fungal genera up to a period of 22 days, whereas on others the effect lasted for a period of at least 30 to 32 days (Table 4).

**Table 3.** Interpretation of the seeded plates, with samples taken 30 min, 24 h and 48 h after applying the essential oil.

Samples Taken	Examined Area	Essential Oil	Fungal Colony Growth
30 min after application of the essential oils	Area A	<i>Lavandula angustifolia</i>	Absent
	Area B	<i>Citrus limon</i>	Absent
	Area C	<i>Mentha piperita</i>	Absent
	Area D	<i>Melaleuca alternifolia</i>	<i>Mucor</i> sp.
	Area E	<i>Marjoram</i>	Absent
	Area F	<i>Origanum vulgare</i>	<i>Botrytis</i> sp.
24 h after the application of the essential oils	Area A	<i>Lavandula angustifolia</i>	Absent
	Area B	<i>Citrus limon</i>	Absent
	Area C	<i>Mentha piperita</i>	Absent
	Area D	<i>Melaleuca alternifolia</i>	<i>Mucor</i> sp.
	Area E	<i>Marjoram</i>	Absent
	Area F	<i>Origanum vulgare</i>	<i>Botrytis</i> sp.
48 h after the application of the essential oils	Area A	<i>Lavandula angustifolia</i>	<i>Botrytis</i> sp.
	Area B	<i>Citrus limon</i>	Absent
	Area C	<i>Mentha piperita</i>	Absent
	Area D	<i>Melaleuca alternifolia</i>	<i>Cladosporium</i> sp.
	Area E	<i>Marjoram</i>	<i>Botrytis</i> sp.
	Area F	<i>Origanum vulgare</i>	<i>Cladosporium</i> sp. <i>Botrytis</i> sp.

**Table 4.** Fungal species inhibited by tested essential oils and the tested duration of their inhibitory effect.

Essential Oil Used	Inhibited Fungal Species	Duration of the Inhibitory Effect from the Moment of Application
<i>Lavandula angustifolia</i>	<i>Botrytis</i> sp.	Up to 22 days
<i>Citrus limon</i>	<i>Botrytis</i> sp.	Minimum of 32 days
<i>Mentha piperita</i>	<i>Botrytis</i> sp.	Minimum of 32 days
<i>Melaleuca alternifolia</i>	<i>Candida guilliermondii</i>	Minimum of 32 days
	<i>Mucor</i> sp.	Up to 22 days
	<i>Penicillium</i> sp.	Minimum of 30 days
<i>Marjoram</i>	<i>Alternaria</i> sp.	Minimum of 30 days
	<i>Aspergillus</i> sp.	Minimum of 30 days
	<i>Botrytis</i> sp.	Up to 22 days
<i>Origanum vulgare</i>	<i>Cladosporium</i> sp.	Minimum of 30 days
	<i>Botrytis</i> sp.	Up to 22 days
	<i>Penicillium</i> sp.	Minimum of 30 days

#### 4. Discussion

After the tests were performed on the delimited surfaces of the 80- to 100-year-old clothing object, the conclusion was drawn that all the EOs had a strong inhibitory effect on the fungi present, even if they were applied on different materials (cotton yard, leather, wool, pearly beads). This is due to the fungicidal effect of EOs, which persists in some cases over 30 days after application. Similar results were obtained by different authors [82–85] following the application of these extracts on various materials. Most EOs (except for *Citrus limon* and *Mentha piperita*) were observed to have relatively little effect on *Botrytis* sp., which might indicate some resistance of this type of fungus to the action of the substances concerned.

It was revealed that the genus *Cladosporium* sp. developed at 5 days of incubation on the culture medium shown with the sample taken 48 h after the application of the EO, without the species involved having been isolated from the primary sample (taken before the application of the EO). The sample concerned was taken from the wool threads of the inner region of the coat. Woollen garments seem to be easily contaminated with fungal spores, with the degree of fungal contamination being most likely to differ from layer to

layer—superficial, intermediate and basal. The spores that are present in the lower layers may rise to the surface over time, and, if the EO is applied superficially or in insufficient quantity, its inhibitory effect is diminished. Moreover, in the case of studies like the present, the environmental conditions present in the room (temperature, humidity, air currents) must be taken into account, as well as the sampling procedure itself. All such factors form a set of features that can interfere with the final results.

The fungal action identified on the garment investigated could be pathological for humans, especially for the personnel handling the garment. Although the fungi of the *Aspergillus* spp. family are generally harmless, they can cause various pathologies in those with compromised immune systems, underlying lung disease or asthma, who inhale the relevant fungal spores. The pathologies resulting from infections with such a fungus, which usually affect the respiratory system, have greatly varying signs and severity. In some (such as asthmatics), the spores trigger an allergic reaction, whereas others tend to develop mild to severe lung infections. The most serious form of aspergillosis, invasive aspergillosis, occurs when the infection spreads through the blood vessels, with the signs and symptoms of the infection varying, depending on the type of pathology that the patients concerned develop, and with it not being contagious. Daily exposure to *Aspergillus* is rarely a problem for people with healthy immune systems [86–88]. Fungi from the *Candida guilliermondii* family appear to be one of the most common pathogens in localised infections of the nails (onychomycosis) and/or of the skin on the toes. In patients with low immunity, infections with such a fungus can be a potential cause of fungal infections in the blood, namely fungemia, especially in patients with haematological malignancies, organ transplants and central venous catheters. This type of fungus is one of the most common opportunistic agents in severely immunocompromised patients. In such cases, depending on the associated pathologies, fungemia can prove fatal [89]. Fungal infections of the *Cladosporium* spp. family either aggravate the symptoms of an existing asthma, or are associated with atopy located mainly in the upper respiratory tract (allergic rhinitis/sinusitis) and less often in the skin (superficial or deep skin lesions). The impact of patients' symptoms (dyspnoea, sneezing, stuffy nose, pruritus, nasal/ocular secretions) was significantly correlated with spore concentrations. In patients with weakened immune systems, infections with this type of fungus can even cause disseminated infections, called fungemia [90–92]. Fungi of the genus *Penicillium* spp., which are occasionally the cause of infection in humans, can be isolated from patients with keratitis, endophthalmitis, otomycosis, necrotising esophagitis, pneumonia, endocarditis, peritonitis and urinary tract infections. Most *Penicillium* infections occur in immunosuppressed hosts, and the mechanism of infection with this type of fungus is either by means of inhalation (most commonly resulting in fungemia) or post-traumatic [93,94].

## 5. Conclusions

The clothing objects that make up the cultural heritage are prone to deterioration, due to fungal action, especially if they are stored or exposed in environments that lack careful adjustment of the internal microclimate parameters. In addition to the harmful effects that fungi can have on heritage objects, people (restorers, museographers, collectors, visitors) who come in contact with such infested objects can develop specific conditions. Thus, treating historical textiles in the most responsible way possible becomes a dual purpose issue; on the one hand, it is necessary to preserve the materials under the best possible conditions, and, on the other hand, it is necessary to ensure the integrity of the health of those who are interested in them. The current study demonstrates that EOs are one of the most viable solutions to help ensure responsible treatment given that their antifungal properties can exceed 30 days of use, and with the products being both ecofriendly and accessible in terms of cost. The six EOs (*Lavandula angustifolia*, *Citrus limon*, *Mentha piperita*, *Melaleuca alternifolia*, *Marjoram*, *Origanum vulgare*) applied on different types of materials served to inhibit the presence of six different fungal colonies (*Alternaria* sp., *Aspergillus* sp., *Botrytis* sp., *Cladosporium* sp., *Mucor* sp. and *Penicillium* sp.) and a class of yeast (*Candida*

*guilliermondii*) identified as being on the traditional coat. Simultaneously, the EOs had a strong antifungal effect on several materials (cotton, leather, wool) that formed part of the object investigated. Thus, all of the products studied were proven to be effective on certain surfaces and against certain types of fungi, in terms of both their cleaning and their protection effects. Prospects for the future must include the use of natural antifungals for the treatment of heritage objects; but at the same time, the tests must also aim at determining the possible negative effects that they may have on fragile objects.

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