

Article

Health Risk Assessment in the Mass Recovery of Flood-Damaged Archival and Library Collections

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Abstract

Flood events have increased significantly in recent decades as a result of climate change, causing severe damage to cultural heritage and, in particular, to archival and library collections. Paper-based materials contaminated by floodwaters and alluvial mud are subject not only to physical and biological deterioration but also to potential hygienic and sanitary hazards associated with the persistence of human pathogens. Current emergency recovery strategies mainly focus on stabilizing materials through freezing, freeze-drying and mechanical cleaning, while health risk assessment is rarely addressed systematically. This pilot study proposes an innovative operational protocol that integrates microbiological indicators of hygienic relevance into the conservation workflow for assessing health risks during the remediation of flood-damaged archival and library materials. Its innovative character lies in shifting post-flood recovery from a predominantly material-stabilization approach toward a combined conservation and occupational-health framework, in which faecal contamination indicators, total microbial loads and selected viral targets are monitored before and after key remediation steps. The protocol was applied to collections from the Aurelio Saffi Municipal Library of Forlì (Italy), severely affected by the Emilia-Romagna floods of May 2023. The results indicate a substantial reduction in faecal contamination following freeze-drying, alongside the persistence of environmentally resistant microbial taxa. However, these findings should be regarded as preliminary and case-specific, and the proposed protocol will require validation through additional case studies, larger sample sets and controlled experimental trials before it can be considered broadly applicable. These findings highlight the need to integrate health risk considerations into conservation recovery workflows to support the safety of conservation professionals and users of recovered heritage materials.

Keywords: flood damage; climate change impacts; archival collections; library materials; health risk assessment; preventive conservation; freeze-drying



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1. Introduction

Climate change is increasing the frequency and intensity of extreme weather events, thereby amplifying flood-related risks to cultural heritage worldwide [1]. Among climate-

driven hazards, floods are among the most destructive because they can affect both immovable heritage and movable collections preserved in archives, libraries and museums [2]. Recent events, including the May 2023 floods in Emilia-Romagna, have shown how sudden and extensive inundations can compromise large quantities of documentary heritage within very short timeframes [3,4].

For archival and library collections, floodwaters and alluvial sediments cause severe and often irreversible damage to paper-based materials. Direct wetting, mud deposition and prolonged moisture exposure may lead to fibre weakening, deformation of bindings, adhesion between sheets, ink migration and loss of structural integrity [5,6]. In addition to these physical effects, flooded materials undergo complex post-event degradation processes: under partially anoxic conditions created by compacted mud, deteriorogenic bacteria can degrade cellulose-based supports, while moulds and actinomycetes may proliferate during uncontrolled drying [7,8].

Floods may also introduce biological contaminants of hygienic and sanitary concern into indoor environments and onto cultural assets [9,10]. The Italian National Institute for the prevention of workplace accidents has compiled an extensive list of pathogens associated with flood events to which affected populations and rescuers may be exposed [11]. Among these, viruses and microorganisms resistant to environmental stresses, such as *Escherichia coli*, *Mycobacterium* sp., *Staphylococcus aureus* and Hepatitis A virus (HAV), are of particular concern. Several studies reviewed by Jafari [12] also identify *Vibrio cholerae* and *Leptospira*, together with enterotoxigenic *E. coli* and HAV, among major flood-borne pathogens. Under high-humidity conditions, moulds growing on cellulosic substrates, including *Stachybotrys*, *Penicillium* and *Fusarium*, may produce mycotoxins with carcinogenic, immunotoxic, cytotoxic, neurotoxic, mutagenic and teratogenic effects [13,14]. These hazards are particularly relevant for conservation professionals, restorers, volunteers and other workers involved in handling, sorting, drying and cleaning contaminated materials.

The health impacts associated with floods have been widely investigated [15,16]. Common outcomes include gastrointestinal infections, skin ailments and respiratory issues, while a recent Italian study on the May 2023 flood in Emilia-Romagna reported significant correlations with specific clinical conditions in the resident population [17]. Although these studies mainly concern exposed communities, they underline the broader relevance of biological contamination in post-flood scenarios and the need to consider occupational exposure during cultural heritage recovery.

Over the years, various guidelines and directives have been issued in Italy for managing library and archival collections affected by flood events [18–20]. During major emergencies, such as the Emilia-Romagna flood, archives and libraries generally rely on mass remediation procedures, including freezing, dehydration or freeze-drying, and mechanical dedusting. These practices are primarily intended to stabilize wet materials, limit biodeterioration, remove mud and particulate matter, and enable subsequent conservation, restoration, storage and consultation.

However, the hygienic–sanitary dimension of these workflows remains insufficiently defined. Freezing, forced dehydration and mechanical removal of organic matter from porous materials such as paper can significantly reduce microbial contamination on flood-damaged items, but systematic studies confirming and quantifying these effects are lacking. Moreover, reference threshold values before and after remediation are not established for archival and library materials, making it difficult to determine when recovered items can be considered safe for operators and end-users.

The present study addresses this knowledge gap by introducing and applying a microbiologically based protocol for health risk assessment during the large-scale remediation of flood-damaged archival and library collections; however, this protocol should be regarded

as a preliminary proposal that will require validation by the scientific community and conservation practitioners through the analysis of additional case studies and/or experimental trials on artificially contaminated specimens. Using materials from the Aurelio Saffi Municipal Library of Forlì, severely affected by the 2023 Emilia-Romagna flood, the study evaluates indicators of faecal contamination, total microbial loads and a selected viral target before and after key remediation steps. Its novelty lies in integrating microbiological monitoring and occupational health considerations into conservation recovery workflows, thereby providing an operational framework for safer management of flood-contaminated documentary heritage.

2. Materials and Methods

2.1. Case Study

In May 2023, a major flood affected extensive areas of the Emilia-Romagna region (Italy) [3,4]. One of the most severely damaged sites was the building housing the General Municipal Archive of Forlì and decentralized deposits of the Aurelio Saffi Municipal Library and Civic Museums. The complex, located approximately 400 m from the Montone River, was inundated by more than 3.5 m of water and mud from the night of 16 May, sustaining severe structural damage, including the partial collapse of internal walls and shelving. Flood sediments had a mixed composition typical of urban flood scenarios, including sewage effluents, urban runoff and riverine inputs. The affected archival and library materials included administrative and historical records, periodicals, volumes and library collections, mostly dating from the twentieth century, together with some nineteenth-century holdings. From the earliest stages of the emergency, the National Fire and Rescue Service carried out technical inspections to assess the stability of the building and the extent of the damage, monitor air quality and implement initial safety measures [21]. Recovery operations began one week after the flood event and continued for two months.

Due to the large volume of affected materials, recovery activities were managed through mass procedures. Flooded items were prioritized, safely removed, inventoried and sealed in airtight plastic bags, each measuring 40 × 60 cm with a maximum thickness of 20 cm. The sealed bags were then placed in freezer containers at −22 °C to halt ongoing chemical and biological deterioration.

2.2. Remediation Workflow

Frozen materials were treated in batches by large-scale vacuum freeze-drying, beginning in September 2023. Prior to freeze-drying, partially thawed units underwent low-pressure surface washing to remove part of the adherent mud. Items were placed in a freeze-dryer specifically designed for archival and library materials (Book's Wind 1, Frati & Livi srl, Castel Maggiore (BO), Italy), with a capacity of up to 1 m³ per cycle. The procedure began with slow refreezing at −5 °C and a pressure of 4 mbar for approximately six hours. The system was then gradually heated until the internal shelves reached a maximum temperature of 65 °C over a total period of 65–70 h. During this phase, the water contained in the materials passed directly from the solid to the gaseous state, without liquefaction, thereby ensuring rapid and uniform drying. By the end of the first cycle, approximately 90% of the water present in the volumes had been removed. Additional rapid heating and cooling cycles then followed under variable pressure conditions, reaching minimum temperatures of −15 °C and maximum temperatures of 25 °C. The entire freeze-drying process was generally completed within five days [22]. Throughout the drying period, despite the high initial temperature reached by the freeze-dryer shelves, the temperature inside the materials remained limited, with peaks of 30–40 °C occurring only in the layers directly in contact with the shelves. It should nevertheless be emphasized that freeze-

drying is not an entirely innocuous process for paper-based materials. Severe dehydration, mostly limited to the layers in direct contact with the heated shelves, may affect cellulose crystallinity and, consequently, the mechanical properties of paper. For this reason, its use is particularly appropriate in emergency scenarios involving large quantities of materials, where rapid and effective stabilization could not otherwise be ensured within a sufficiently short timeframe.

Dried materials were subsequently subjected to mechanical dedusting on suction benches; the workflow concluded with flattening and restoration treatments where required.

2.3. Health Risk Assessment Protocol

The microbiological protocol targeted indicators relevant to human exposure in flood-derived matrices. In addition to total bacterial and fungal loads, indicators of recent and past faecal contamination were selected, including β -glucuronidase-positive *Escherichia coli*, Enterococci and sulfite-reducing Clostridia [23,24], together with yeasts and Hepatitis A virus (HAV). HAV was included in the protocol not as a general indicator of pathogenic viral contamination, but because local authorities had reported its possible presence in the alluvial mud. All parameters were examined before and after freeze-drying in nine Saffi collection archival units, except for HAV, which was analysed only in the pre-freeze-drying stage, since it was not detected in the initial six samples examined.

Additional analyses were conducted on two further library units from the same collection following dust removal and flattening procedures. For these units, total fungal (mesophilic and xerophilic) and bacterial loads were assessed, alongside an ATP test to determine residual microbiological activity present on the surfaces.

2.4. Sampling

A total of 11 archival units (periodicals, books, touristic maps and documents mainly composed of paper), all heavily soiled with flood mud, were selected for examination (Table 1).

Six units were selected in August 2023, with mud samples taken from the surfaces of the items while they were only partially thawed and unwashed. The collected samples were subsequently refrozen, pending freeze-drying, which was carried out in September 2023.

Table 1. Archival units examined during the different phases of the recovery.

		
Sample/archival Unit 01	Sample/archival Unit 02	Sample/archival Unit 03

Table 1. *Cont.*

Sample/archival Unit 04	Sample/archival Unit 05	Sample/archival Unit 06
Sample/archival Unit 07	Sample/archival Unit 08	Sample/archival Unit 09
Sample/archival Unit 10	Sample/archival Unit 11	

A further three archival units were selected from the frozen collection in September, immediately before freeze-drying. Here too, samples were taken from partially thawed material, but this time following the low-pressure surface washing. The samples were processed immediately through freeze-drying, without being refrozen in the freezer container.

At the end of the freeze-drying process in September 2023, all nine of these units were sampled simultaneously for microbiological analysis.

Sampling was conducted in a non-invasive manner, thereby ensuring the integrity of the items, by collecting flood residues using high-capacity big-size swabs. For the first six units sampled in August, the swabs were drawn across two adjacent 100 cm² areas: the first area was used for microbial group analysis, employing dry swabs with transport medium, while the second area was used to determine the presence of HAV, using swabs pre-moistened with PBS and without transport medium. For the three units sampled in

September, given the absence of HAV from the previous six samples, only one 100 cm² area was swabbed for determination of the six indicator microbial groups only.

After freeze-drying, sampling was conducted with identical swabs on a nearby 100 cm² section, positioned next to the areas chosen before freeze-drying, to analyze the microbial indicators group.

The two additional archival units examined after the final dust removal phase were sampled in November 2023 using standard sterile swabs pre-moistened with Ringer's Solution containing 20% glycerol, swabbing a 100 cm² area for the analysis of residual mesophilic and xerophilic fungal loads and total bacterial load. An additional pre-moistened swab (Ultrasnap, Hygiena LLC, Camarillo, CA, USA) was drawn across an adjacent 100 cm² area for ATP analysis using a compatible luminometer (Ensure, Hygiena LLC, Camarillo, CA, USA), to assess any remaining microbial activity on the surfaces.

2.5. Microbial Analysis

All indicator microbial groups were analysed at the IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy) microbiology laboratory.

Specifically, total mesophilic bacterial counts were determined using the culture-based method described in ISO 4833-2:2013 [25] on Plate Count Agar (BioKar Diagnostics, Allonne, France, supplied by DID S.p.A. (Milan, Italy)) at 30 °C. Beta-glucuronidase-positive *E. coli* were enumerated using the standard culture method ISO 16649-2:2001 [26] on Tryptone Bile X-Glucuronide agar (Oxoid, Thermo Fisher Scientific, Basingstoke, UK) at 44 °C. Enterococci were enumerated by plating samples on Kanamycin Aesculin Azide agar (Oxoid, Thermo Fisher Scientific, Basingstoke, UK) and incubating them at 37 °C for 48 h. Sulfite-reducing Clostridia and related genera were enumerated according to ISO 15213-1:2023 [27] on Iron Sulphite Agar (Microbiol S.r.l., Uta, Cagliari, Italy) under anaerobic conditions at 37 °C. Moulds and yeasts were enumerated by plating samples on Dichloran Rose Bengal Chloramphenicol agar (Microbiol S.r.l., Uta, Cagliari, Italy) medium at 25 °C. Finally, the presence/absence of hepatitis A virus (HAV) was determined according to ISO 15216-2:2019 [28] by real-time RT-PCR.

The dominant bacterial morphotypes detected on the different culture media were isolated and identified by MALDI-TOF mass spectrometry [29] using MALDI Biotyper[®] Sirius system (Bruker Daltonics GmbH & Co. KG, Brema, Germany).

Residual fungal and bacterial loads detected after the final dust removal phase were determined at the Biores Lab (Bologna, Italy) by culture analysis, using the following agar media: Dichloran Rose Bengal Chloramphenicol agar (Condalab, Madrid, Spain, distributed by KAIROSafe, Gorizia, Italy) for mesophilic fungal load, Dichloran 18% Glicerolo agar (Condalab, Madrid, Spain, distributed by KAIROSafe, Gorizia, Italy) for xerophilic fungal load and Tryptic Soy Agar Dichloran Rose Bengal Chloramphenicol agar) for total bacterial load. (Tryptic Soy Agar Condalab, Madrid, Spain, distributed by KAIROSafe, Gorizia, Italy) for total bacterial load. All the agar plates were incubated at 20 °C. The dominant isolates obtained in culture from the units sampled after the final dust removal phase were taxonomically characterised at the ISZLER microbiology laboratories using MALDI TOF MS. ATP analysis was used to estimate remaining microbial activity after dust removal on swab samples, using a bioluminometer (Ensure, Hygiena LLC, Camarillo, CA, USA) according to manufacturer instructions. This model reads ATP concentrations in Relative Light Units (RLU), where 1 RLU equals 10⁻¹⁵ moles of ATP.

All values reported refer to surface unit (CFU/100 cm²).

2.6. Choice of Reference Thresholds

In the absence of specific regulations for the hygienic-sanitary assessment of flood-affected materials, the approach adopted here should be regarded as a preliminary proposal rather than an established protocol. The proposed reference threshold values, derived from regulations pertaining to sectors involving the production, distribution and use of organic matrices not intended for human consumption, will require further evaluation, discussion and validation by the scientific community before they can be considered broadly applicable. Thresholds applicable to the food or healthcare sectors were deliberately not used, as these would be unnecessarily stringent for the context under consideration.

Specifically, regarding the thresholds for *E. coli* and Enterococci, reference was made to Regulation (EU) No. 142/2011 [30], which establishes sanitary rules for animal by-products. According to this regulation, manure intended for agricultural use must not exceed a limit of 10^3 CFU/g for both indicators. For Clostridia, the benchmark was the European Commission's "Working Document on Biological Treatment of Biowaste" [31], which proposes that compost can be considered hygienised when *Clostridium perfringens* is absent in 1 g of matrix. Although, in this case, the indicator chosen was the entire group of sulphur-oxidising Clostridia rather than solely *C. perfringens*, it was deemed prudent to retain the same reference value. The same threshold is also proposed for HAV. For total bacterial, fungal, and yeast loads, in the absence of specific references for environmental matrices not intended for human consumption, it is suggested to use the values commonly found on membranous surfaces of archival and library materials: on surfaces stored under standard conditions and not subject to biodeterioration, total loads usually do not exceed 5×10^1 /cm². Another possible approach for these parameters would be to regard as effective any sanitisation treatment capable of reducing the microbial load by at least 10^2 – 10^3 CFU (corresponding to a reduction of 99–99.9%).

For the ATP parameter, which was measured only after the final dust removal, the proposed reference values are again those typically found on membranous surfaces under standard conservation conditions. An ATP concentration of less than 10^{-13} moles/swab is considered not significant; concentrations between 10^{-13} and 3×10^{-13} moles/swab are regarded as marginally significant; values from 3×10^{-13} to 10^{-12} moles/swab are deemed significant; and levels above 10^{-12} moles/swab are classified as high.

The threshold values derived from sectors dealing with environmental matrices intended for agricultural use are based on weight, whereas the values measured in this study are referenced to surface area. To enable comparison, a weight-to-surface conversion was proposed as a methodological approximation. Using the technical data sheet for Whatman No. 1 paper as a benchmark [32], 100 cm² of clean paper surface was estimated to weigh 0.87 g. Assuming that muddy contamination contributes an additional fraction to the overall weight, 1 g of flood-affected paper material was approximated to a muddy flat sheet of about 100 cm². Therefore, Equation 1 g of matrix (mud, sediment, compost) = 100 cm² of flood-affected paper substrate was adopted. However, this conversion should be regarded as a provisional assumption rather than a validated equivalence, and it will require further assessment and validation by the scientific community before being used as a general criterion for defining threshold values.

2.7. Statistics

To statistically assess the effect of the treatments on the microbial indicators, all numerical values were first transformed into logarithmic units. The effect of the freeze-drying treatment was evaluated by comparing the mean values obtained from the nine paired samples before and after treatment using Student's *t*-test. To allow statistical analysis, values below the limit of detection (<LOD) were replaced by LOD/2. The effect of the final

dedusting phase on total bacteria e fungal (mesophilic) load, which was assessed on only two independent samples, was evaluated using a two-tailed Welch's *t*-test.

3. Results

3.1. Microbiological Loads Before Freeze-Drying

Overall, the pre-freeze-drying samples showed high total bacterial loads, reaching values above 10^8 CFU per sampled area, whereas moulds were comparatively low. Yeasts were below the detection limit in most samples, except for one sample in which yeasts were detected at low concentration (approximately 10^3 CFU per sampled area). Regarding faecal contamination indicators, enterococci exceeded the proposed reference threshold in the samples collected in August 2023, while *E. coli* exceeded the threshold in all three samples collected in September 2023. Sulfite-reducing Clostridia were detected in all samples, with mean values around 40 CFU per sampled area; one of the August samples exceeded 200 CFU per sampled area. The hepatitis A virus was not detected in any of the first six samples analysed in the pre-freeze-drying phase; consequently, it was not analysed in subsequent sampling rounds (Table 2).

Table 2. Microbial data obtained before the freeze-drying process. All data are expressed in CFU/100 cm².

Sample/ Archival Unit	Sampling Date	Bacterial Load	Moulds	Yeast	<i>E. coli</i>	Enterococci	Sulfite-Reducing Clostridia
01	August 2023	8.6×10^6 *	6.0×10^2	$<10^2$	4.0×10^1	2.5×10^4 *	5.0×10^1 *
02	August 2023	1.0×10^6 *	9.0×10^2	$<10^2$	4.0×10^1	1.4×10^3 *	9.0×10^1 *
03	August 2023	8.2×10^6 *	1.1×10^3	$<10^2$	2.0×10^1	3.8×10^3 *	5.0×10^1 *
04	August 2023	1.0×10^7 *	2.7×10^3	$<10^2$	1.3×10^2	5.9×10^3 *	3.0×10^1 *
05	August 2023	2.0×10^6 *	3.0×10^2	$<10^2$	1.2×10^2	5.8×10^3 *	4.0×10^1 *
06	August 2023	1.0×10^8 *	2.3×10^3	$<10^2$	2.0×10^2	3.0×10^4 *	2.0×10^2 *
07	September 2023	6.0×10^5 *	9.0×10^2	$<10^2$	9.0×10^3 *	$<10^2$	1.0×10^1 *
08	September 2023	6.0×10^5 *	7.0×10^2	$<10^2$	1.0×10^3 *	$<10^2$	3.0×10^1 *
09	September 2023	6.0×10^5 *	3.9×10^3	1.0×10^3	1.0×10^3 *	$<10^2$	1.0×10^1 *

* Values higher than the proposed thresholds.

MALDI-TOF MS analysis identified several bacterial taxa associated with soil and plant environments, including *Arthrobacter pascens*, *Chryseobacterium rhizospherae*, *Raoultella terrigena*, *Lactococcus lactis*, and *Pseudomonas brassicacearum*.

Within the enterococci-associated isolates, the dominant species were *Enterobacter ludwigii* and *Lelliottia amnigena*. Among the sulfite-reducing clostridia, the identified taxa included *Paraclostridium bifermentans*, *Paeniclostridium sordellii*, *Clostridium sporogenes*, and the pathogenic species *Clostridium perfringens*.

3.2. Effects of Freeze-Drying on Indicator Microorganisms

After vacuum freeze-drying (prior to mechanical dedusting), reductions were observed for several indicators (Table 3 and Figure 1). In the six units selected in August, total bacterial loads decreased by approximately 2 to 4 logarithmic units. In the three units selected in September, total bacterial loads decreased by about 1.5 logarithmic units; however, their initial loads (measured after low-pressure washing) were already markedly lower than those of the August units (around 6×10^5 CFU per sampled area). Notably, despite the observed reductions, total bacterial loads remained above the proposed reference threshold in all post-freeze-drying samples.

Moulds, already low before treatment, remained below the proposed reference level in all samples. Both *E. coli* and Sulfite-reducing Clostridia decreased below the detection limit in all cases. Enterococci decreased below the detection limit in most of the samples but remained detectable in three of the nine post-freeze-drying samples, although still below

the proposed threshold. In these three units, one sample showed a reduction slightly above 90%, another showed a reduction greater than 99.9%, whereas a third sample increased from below the detection limit to approximately 100 CFU per sampled area; this increase is compatible with accidental post-treatment contamination.

Table 3. Microbial data obtained after freeze-drying process. All data are expressed in CFU/100 cm².

Sample/ Archival Unit	Sampling Date	Bacterial Load	Fungal Load	Yeast	<i>E. coli</i>	Enterococci	Sulfite-Reducing Clostridia
01	August 2023	2.8×10^3	$<10^2$	$<10^2$	$<10^1$	$<10^2$	$<10^1$
02	August 2023	1.8×10^4 *	6.0×10^2	$<10^2$	$<10^1$	$<10^2$	$<10^1$
03	August 2023	4.2×10^3	2.0×10^2	$<10^2$	$<10^1$	$<10^2$	$<10^1$
04	August 2023	1.8×10^4 *	4.0×10^2	$<10^2$	$<10^1$	7.0×10^2	$<10^1$
05	August 2023	5.4×10^3 *	6.0×10^2	$<10^2$	$<10^1$	$<10^2$	$<10^1$
06	August 2023	1.4×10^4 *	5.0×10^2	$<10^2$	$<10^1$	2.0×10^2	$<10^1$
07	September 2023	1.4×10^4 *	4.0×10^2	$<10^2$	$<10^1$	$<10^2$	$<10^1$
08	September 2023	1.9×10^4 *	3.0×10^2	$<10^2$	$<10^1$	1.0×10^2	$<10^1$
09	September 2023	1.5×10^4 *	1.0×10^2	$<10^2$	$<10^1$	$<10^2$	$<10^1$

* Values higher than the proposed thresholds.

After freeze-drying, MALDI-TOF analysis still detected *Enterococcus casseliflavus* in several samples, indicating residual enterococcal contamination. Among the environmental microbiota, the dominant taxon was *Corynebacterium callunae*, together with a notable presence of *Cellulosimicrobium cellulans*, a species known for its marked cellulolytic activity.

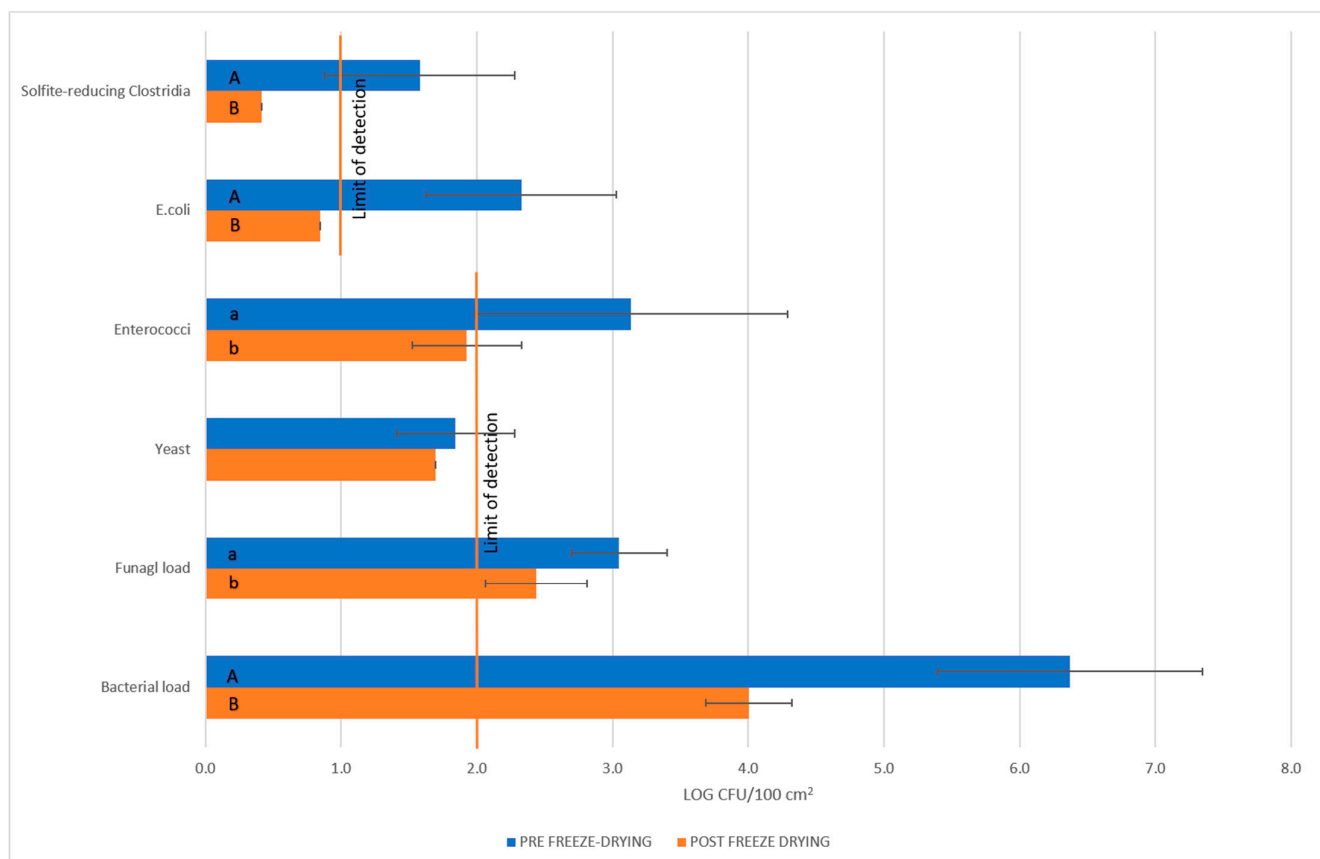


Figure 1. Microbial loads before and after freeze-drying. Values represent the mean of nine samples, and bars indicate standard deviation. Different letters within each microbial group indicate statistically significant differences between treatments: uppercase letters denote $p \leq 0.01$, while lowercase letters denote $p \leq 0.05$.

3.3. Microbial Loads After Mechanical Dedusting and Flattening

Two additional book units were analysed after the final mechanical dedusting/flattening phase (November 2023). In these items, no further significant reductions were observed for total fungal and total bacterial loads (Table 4 and Figure 2). The latter remained above the proposed reference threshold and were dominated by two isolates identified as *Carnobacterium viridans* and *Paenibacillus etheri*. In both samples, ATP levels remained above the proposed thresholds, indicating significant residual microbial activity.

Table 4. Microbial data obtained after dedusting process. Microbial loads are expressed in CFU/100 cm², ATP in mole/100 cm².

Sample/ Archival Unit	Sampling Date	Bacterial Load	Fungal Load on DRBC	Fungal Load on DG18	ATP
10	November 2023	4.5×10^5 *	1.0×10^3	2.0×10^3	1.1×10^{-12} *
11	November 2023	2.2×10^4 *	2.5×10^2	2.5×10^2	3.6×10^{-13} *

* Values higher than the proposed thresholds.

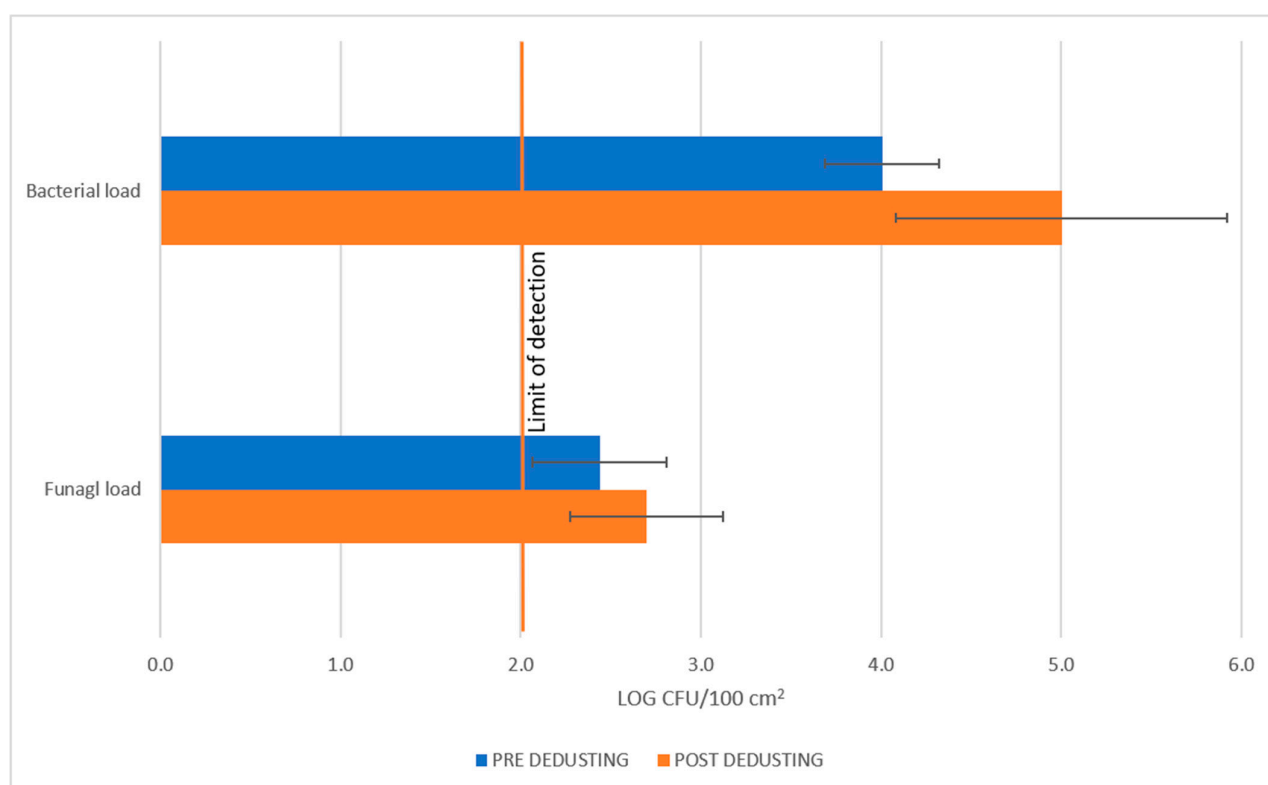


Figure 2. Microbial loads before and after mechanical dedusting. Pre-dedusting values represent the mean of nine samples, whereas post-dedusting values represent the mean of two independent samples. Bars indicate standard deviation. No statistically significant differences were observed between the mean values for either microbial group.

4. Discussion

The results highlight two complementary aspects of mass recovery workflows for flood-damaged paper-based collections: (i) vacuum freeze-drying can markedly reduce indicators of faecal contamination, and (ii) residual microbial communities may persist despite sequential stabilization and cleaning steps. The strong reductions observed for *E. coli* and enterococci, together with the disappearance of detectable sulfite-reducing clostridia after freeze-drying, suggest a potential role of dehydration-based treatments in mitigating acute health hazards associated with recent faecal contamination in the

specific conditions examined. While a reduction in *E. coli* and enterococci is expected, given their moderate resistance to heat, the result observed for clostridia is more noteworthy, as their spores are known to withstand even very high temperatures. Their apparent inactivation may be attributable to the repeated cycles of freezing, thawing and refreezing, combined with the subsequent dehydration process during lyophilization, which included multiple heating and cooling cycles. However, this interpretation should be considered a working hypothesis based on the present case study. Follow-up research using artificially contaminated mock samples and controlled comparisons of different freeze–thaw cycles will be required to verify this mechanism and assess the extent to which these findings can be generalized.

At the same time, the persistence of high total bacterial loads after freeze-drying indicates that the treatment is not sterilizing and should not be interpreted as a stand-alone guarantee of hygienic safety. MALDI-TOF MS identification showed a shift from a pre-treatment microbiota associated with soil, plants and flood-borne matrices to a post-freeze-drying bacterial community largely composed of Gram-positive taxa highly resistant to the stresses imposed by lyophilization, including *Corynebacterium callunae* and *Cellulosimicrobium cellulans*. The persistence of the latter, belonging to the taxonomic group of the Actinomycetes, is particularly relevant from a conservation perspective, because this species has documented cellulolytic potential [33] and may therefore contribute to residual biodeteriorative risk for paper-based archival and library materials, especially if post-treatment storage conditions allow renewed moisture availability or microbial regrowth. In parallel, MALDI-TOF MS also detected taxa associated with faecal indicators and hygienic concerns, including residual *Enterococcus casseliflavus* after freeze-drying and, in the pre-treatment phase, sulfite-reducing clostridia such as *Paraclostridium bifermentans*, *Paeniclostridium sordellii*, and *Clostridium sporogenes*, and the potentially pathogenic species *Clostridium perfringens*. The observation that post-treatment bacterial loads remained above the proposed reference threshold in all samples suggests that occupational exposure may still occur during subsequent handling (e.g., mechanical dedusting, sorting and rehousing), especially when large volumes of material are processed. The single post-freeze-drying sample in which enterococci increased to approximately 100 CFU per sampled area further illustrates the plausibility of incidental recontamination during post-treatment logistics, emphasizing the importance of controlled workflows and clean handling environments.

About viral risk, although HAV was not detected in the mud residues present on the examined materials, the possible presence of other viral pathogens potentially associated with flood sediments cannot be ruled out, such for example as in the context of the present case study gastrointestinal viruses (Norovirus, Rotavirus). As noted above, HAV was not included in the protocol as a general indicator of viral pathogenic contamination, but rather because local health authorities had reported its possible presence in the alluvial mud.

The absence of additional reductions after mechanical dedusting in the two items examined in November suggests that cleaning operations, while essential for conservation purposes, may have limited impact on viable microbial loads under certain conditions. However, these data should be interpreted with particular caution because the post-dedusting evaluation was based on a very limited number of samples. The predominance of *Carnobacterium viridans* and *Paenibacillus etheri* is consistent with the survival of environmentally resilient bacteria capable of tolerating stressful conditions. Both taxa are facultative anaerobes and can persist under extreme environments, including those encountered during freeze-drying. *C. viridans* is reported to remain metabolically active at temperatures below 5 °C [34], whereas *P. etheri*, similarly to related *Bacillus*-like genera, can form resistant spores with high heat resistance and able to germinate at refrigeration

temperature [35,36]; these traits provide plausible mechanisms for persistence throughout the remediation chain.

From the perspective of preventive conservation and occupational health, these findings support an integrated approach in which microbiological monitoring complements established conservation workflows. In practice, this implies (i) targeted indicator monitoring before and after key processing steps, (ii) the adoption of appropriate collective and personal protective measures during phases with high exposure potential (handling of muddy materials, pre-drying operations, dedusting), and (iii) strict post-recovery microclimatic control to prevent regrowth of residual microorganisms once the items are returned to storage or consultation environments.

5. Conclusions

Flood-contaminated archival and library materials may retain microbiological hazards during the early stages of recovery, even after initial freezing. In the case study examined here, vacuum freeze-drying was associated with a marked reduction in indicators of faecal contamination, including sulfite-reducing clostridia, but residual microbial communities were still detected after treatment. These observations suggest that freeze-drying may contribute to reducing some acute hygienic risks in emergency recovery workflows, while not providing complete microbiological safety. However, the evidence presented in this study should be interpreted with caution. The investigation was designed as a pilot application of a proposed health risk assessment protocol to a single flood-affected collection, and the number of samples, particularly after mechanical dedusting, was limited. For this reason, the results should be considered indicative of the specific conditions examined rather than directly generalisable to all flood-damaged archival and library materials. Further observations on additional real case studies, larger sample sets and controlled experimental trials on artificially contaminated paper-based materials will be necessary to validate the proposed protocol, test the weight-to-surface conversion used for threshold definition, and clarify the mechanisms responsible for microbial reduction or persistence during freeze-drying and subsequent cleaning steps. Within these limits, MALDI-TOF proved useful in characterizing the residual microbiota, documenting the replacement of clear faecal-indicator-associated taxa by predominantly environmental survivors, while still revealing occasional persistence of taxa of hygienic concern such as *Enterococcus casseliflavus*. From a conservation standpoint, the residual detection of *Cellulosimicrobium cellulans* is especially significant because its cellulolytic potential suggests that, even after stabilization, some recovered paper-based materials may retain a latent biodeteriorative risk if unsuitable storage conditions permit renewed microbial activity. Accordingly, targeted microbiological monitoring, appropriate occupational safety measures and strict control of microclimatic conditions after reintegration are recommended as precautionary measures to support long-term safety for conservation professionals and users.

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