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A review of indoor microbial growth across building materials and sampling and analysis methods

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ABSTRACT

Microorganisms from damp indoor environments are known to be one of the main causes of the degradation of indoor air quality and can be serious health hazards to occupants because of the production of airborne particles. Surfaces of building materials (plasterboard, mortar, etc.) are generally highly porous and rough. In damp environments, these materials can provide an environment favourable to proliferation and growth of microorganisms. Sampling of microbial communities on building materials, in addition to air sampling, is thus necessary to evaluate microbial proliferation indoors.

The present paper aims to (i) summarise and compare the different methods used for sampling and analysing microbial growth on building materials and (ii) make a synthesis on the colonising microbial communities and the building materials parameters (humidity, chemical composition, pH, etc.) affecting their growth.

With regards to methods, our investigations focused exclusively on studies dealing with building materials. When available, studies comparing the efficiency of methods on building materials were discussed. In-situ sampling campaigns were reviewed and the microorganisms identified on building materials were listed. Factors determining bio-receptivity of materials were also examined on the basis of studies performed on various types of materials (including building materials).

The microorganisms the most frequently detected on indoor building materials are (i) fungi genera *Cladosporium*, *Penicillium*, *Aspergillus* and *Stachybotrys*, and (ii) Gram negative bacteria and mycobacteria. Some correlations between microbial genera/species and the type material can also be outlined. The water activity, the chemical composition, the pH and the physical properties of surfaces are parameters influencing microbial growth on materials. The particular behaviour of porous materials in terms of water sorption and the effect of water on microbial proliferation are underlined.

In the future, the standardisation of methods for sampling, analysis and laboratory testing will be helpful in the assessment of microbial proliferation in building materials. Moreover, investigations on the impact of the material's mineralogy and its surface properties on growth will be necessary for a better understanding and predicting of microbial proliferation on these substrates.

1. Introduction

The degradation of indoor air quality induced by microorganisms (moulds, bacteria, fungi) is of growing concern to international health organisations [1–3]. In Northern Europe and North America, it is estimated that between 20 and 40% of buildings are contaminated by indoor mould [2]. The World Health Organisation has already published guidelines for indoor air quality related to humidity and mould [1].

Several hundreds of fungal and bacterial species can be found in indoor environments [2,4,5]. Fungi, mainly *Cladosporium sphaerospermum*, *Penicilium chrysogenum*, *Aspergillus niger*, *Aspergillus versicolor*, *Alternaria alternata*, *Stachybotrys chartarum*, and bacteria, mainly large groups of Gram negative bacteria and mycobacteria are all microorganisms usually found inside dwellings and other buildings. They may produce contaminants, i.e. aerial particles such as spores, allergens, toxins and other metabolites that can

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contribute to the degradation of indoor air quality and be serious health hazards to occupants [6–13]. The most significant health troubles experienced by exposed people include irritations and toxic effects, superficial and systemic infections, allergies and other respiratory and skin diseases [14–21]. The resulting social and economic impact is very significant [17,22]. For example, in the USA, Mudarri and al. estimate that more than 4.5 million cases of asthma result from exposure to damp and mould and the annual economic cost is approximately $3.5 billion [21].

According to F. Squinazzi, indoor air micro-organisms have four main sources [23]:

- humans, through the production of saliva, nasal droplets and skin flakes; contaminated water tanks (showers, mist blowers and sprayers, etc.) which spread micro-droplets in the atmosphere;
- dusts induced by activity in buildings and that become suspended in the air;
- wet surfaces, which become major sites of microbial growth once contaminated by contact with a source of microorganisms (human, animal, clothing, dust, etc.).

The direct evaluation of air samples to estimate health risks to occupants has been widely reported over the last few years [17,24–26]. The extent of exposure to these microbial airborne particles and the associated risks are related to many parameters, such as genera/species of microorganisms (which determine a part of the contaminants), exposure pathway (inhalation or contact with skin/eyes) and environmental conditions (convection, etc.), total area of microbial growth, aerosolisation of contaminants, etc. [27]. Many authors have suggested that aerial samples are not sufficient to describe the entire microflora present inside buildings, especially in water-damaged buildings [28–30] and identifying microorganisms established on building materials of the indoor environment, collected by surface sampling, has been shown to provide relevant information about the potential sources of airborne microbial contaminants [29,31]. In addition, species producing mucilaginous spores, that remain attached to substrates, require the use of surface sampling methods to draw up an inventory of the full microbial biodiversity [29]. Although microbial communities on surfaces are nor directly correlated with health troubles of the occupants, the French High Council for Public Health recommends sampling such communities on building materials, in addition to air sampling, in order to evaluate microbial proliferation indoors [2].

Swab, adhesive and contact plate sampling, along with bulk sampling, are techniques commonly used on the surface of building materials to collect microorganisms and microbial contaminants prior to analysis. The sampling method, in addition to the analysis method, e.g. culture, observation, chemical or molecular method used for microbial quantification or identification, will have an influence on the pattern prevalence in the results. Studies investigating the microbial growth on building materials, including laboratory testing, report the impact of several factors on the microbial development. One of the main factors is the water available for microorganisms. Available water is responsible for microorganism germination and growth on various types of building materials [27,32–35]. The chemical composition of the substrate, here building materials, also influences growth, as it is a potential nutrient supply for microorganisms [8,32,36,37]. Studies reveal that some specific taxa are detected more frequently than others on certain building materials [11,27,38]. In the particular framework of building materials, porosity and roughness are fundamental parameters as they can promote water absorption and dust attachment. Various studies point out that these physical parameters have a significant impact on the colonisation of materials by microorganisms, for example by promoting attachment in the asperities [39–41] or supplying moisture and nutrients [36,37,41].

This review first describes the various methods for sampling and analysis in studies dealing with microbial growth on building materials. These methods, commonly used in microbiology, are applied to particular materials here, such as gypsum board, mortar, concrete, etc., that are all porous materials but with very different compositions. The microorganisms commonly found are then presented. In a second part, the specific procedures related to the exposure of building materials to microorganisms in laboratory conditions are presented. Different parameters that govern microbial growth on these materials are also discussed. The present paper aims to outline the microbiological methods used for assessing microbial growth on building materials and to emphasise, in addition to the conclusions of the relevant studies, the need to adapt existing standards and methods for these types of rough and porous materials with particular chemical compositions.

2. Methodologies for characterising microbial communities on building materials

The following section aims to give a comprehensive list of the methods used in microbial investigations on building materials. Concerning both sampling and analysis, only the methods carried out on building materials are reported here. Regarding sampling, methods used a) in-situ and b) in laboratory experiments are described. When available, studies comparing the efficiency of the method with respect to building materials are also reported.

2.1. Micobial sampling methods

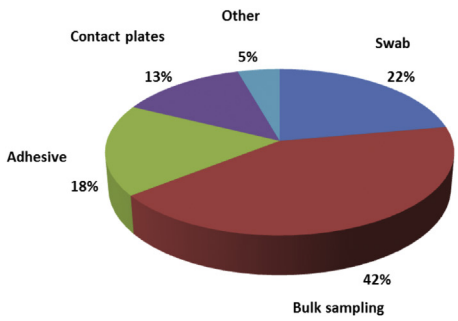
Different methods exist for sampling microbial populations on materials: swab, bulk, adhesive, contact plate, etc. but the *in-situ* collecting process has not been well standardised yet. Moreover, although many of these methods have been tested to evaluate their collecting efficiency on non-porous and non-absorbent surfaces (glass, steel, plastic, etc.), few studies have concerned construction materials such as concrete, coatings, mortar, and gypsum board, which are porous, rough and more or less dusty materials. The "Mould in the home" working group of the French High Council for Public Health has issued methodological recommendations for sampling on surfaces of building materials and suggests the use of at least two of the following surface sampling methods: swab, bulk sampling, adhesive tape and agar contact (imprint methods) [2].

Fig. 1 shows the frequency of use of various techniques in studies carried out on construction materials (for 33 studies considered).

2.1.1. Swab

Swab sampling consists of rubbing a contaminated surface area with a sterile gauze swab generally dipped in physiological solution. It is a relatively low cost system allowing samples to be collected under all circumstances. Swabbing is usually chosen when imprint or tape methods are impossible owing to difficulties in accessing the surface [6,42], for example when samples are collected in corners of walls or under window sills [10,30,43,45,46]. Several studies point out the influence of many parameters on the efficiency of the swab sampling method, including: handling by the operator [2], swab type (cotton, foam, viscosin, polyester, nylon) and whether the swab is wet or not [47,69–71].

In addition, Buttner et al. [44] highlighted the major influence of the substrate material properties on the sampling efficiency. They compared the recovery efficiency between swab and sponge sampling on different materials. They quantified microorganisms by



Sampling method	References
Swab	[6,10,30,42–48]
Bulk	[9,11,20,30,43,48–62]
Adhesive tape	[6,11,27,50,63–66]
Contact plate	[6,28,42,50,63,67]
Carpet stamp	[68]
Vacuum filter sock	[61]

Fig. 1. Surface sampling methods used in studies on construction materials. Percentage of use calculated on 33 studies.

PCR analysis[1] and calculated efficiency by dividing the number of cells collected by the number of cells inoculated. The authors explained that the estimated recovery efficiencies were affected by the sampling method and the material of the sampled surface. Also, the largest values were found for smooth, non-porous material: 52% and 47% for glass and 29% and 11% for wood laminate, using swab and sponge sampling respectively. In contrast, recovery efficiencies were only around 0.8% and 0.7% for concrete.

2.1.2. Bulk sampling

Bulk sampling is a destructive method in which samples are directly removed from the surface to be analysed, by scratching, scraping or coring of small pieces of the material (0.3–5 g). It is the most widely used sampling technique in microbial assessment on building materials (Fig. 1) [9,11,20,30,43,48–62]. Microorganisms can be isolated by bulk sampling in two ways: (i) direct plating of the sample onto a culture medium, (ii) microbial solution plating onto a culture medium [72]. In the latter case, bulk samples are first dipped in a physiological saline solution or rinsed with solvents according to various protocols to extract the microorganisms; dilution steps are then possible before plating. Samples can also be removed so as to be properly observed under a microscope [11].

2.1.3. Adhesive tape sampling

An adhesive tape is applied to the contaminated surface. The surface should preferably be flat and dry before the sampling. Then, it is possible to inoculate the microorganisms onto plates by applying the tape to a solid culture medium [50,63,66] or to observe them with a microscope in order to identify them and/or perform semi quantification [6,11,27,64,65,73].

2.1.4. Contact plate sampling (imprint methods)

A culture medium is directly pressed against the surface for enough time to allow the adhesion of microorganisms. Then, the plates are protected from air contamination by a lid and incubated [6,28,42,50,63,67]. Some studies have shown that the extractability of microorganisms depends on various parameters, notably time and pressure on the plate [2,6]. For this reason commercial applicators are usually designed for a defined time and pressure.

2.1.5. Other methods

Shirakawa et al. performed fungal isolation using the Mariat and Adan-Campos carpet-stamp technique [74]. It consists of rubbing a small piece of sterilised wool against the surface to be tested [68]. This method is more often used in the medical field to isolate fungi, e.g. in cases of mycosis on skin. The wool is then placed on a culture medium.

In another study, Brown et al. [61] evaluated the sampling efficiency of a vacuum filter sock method on *Bacillus atrophaeus* spores. Spores were collected from the contaminated surface with a vacuum pump system and retained on a filter sock, then extracted by sonication. The collection efficiency was calculated as the ratio between the number of Colony Forming Units enumerated from the filter sock sample and the number of CFU enumerated from a reference stainless steel coupon from which spores were directly extracted by sonication. Results showed between 19% and 29% of collection efficiency for stainless steel, painted wallboard, carpet and concrete. The authors mention that these differences in efficiency between the various materials are not statistically significant. Although the technique is not as efficient as swab methods, it provides the capability to sample a larger area. It should be noted that the detection limit was between 105 and 160 CFU per 100 cm^2 for all material tested [61]. Many authors emphasise the need for standardisation of the protocols for microorganism sampling on construction materials [2,6,10,48,58]. At present, results can be influenced by the operator and many other factors, including the sampling technique itself and its different steps (sampling location, pressure applied, conservation of strains, etc.), the analysis method (observation, chemical, molecular, etc.) and/or the chosen culture medium. There are far too few studies that compare the collection efficiency of the various techniques applied to given building materials and few papers that deal with the influence of the material type. Moreover, the number of microorganisms collected from a surface is likely to depend on the species and the stage reached in the adhesion and biofilm formation process. This aspect has also been little studied to date.

2.2. Analytical methods

Many analytical methods may be used to carry out quantitative or qualitative assessments of microorganisms on a substrate. The choice of an appropriate method for microbial analysis depends not only on its duration and cost but especially on the aim of the investigation. The following section describes the main analytical methods found in the literature concerning microbial growth on building materials: culture-based methods, observation methods, chemical methods, and molecular biological methods.

2.2.1. Culture-based methods

Microorganisms may be cultured prior to any analysis for quantitative and/or qualitative microbial assessment of surfaces, depending on the aim of the study. The culture medium has a major impact on microorganism growth. Owing to their specific chemical nature, some culture media, called selective media, can be used to isolate selected species/genera by promoting their growth at the expense of other microorganisms. Samson and co-workers recommend the use of specific culture media depending on the

[1] See Section 2.2 on the different methods of analysis.

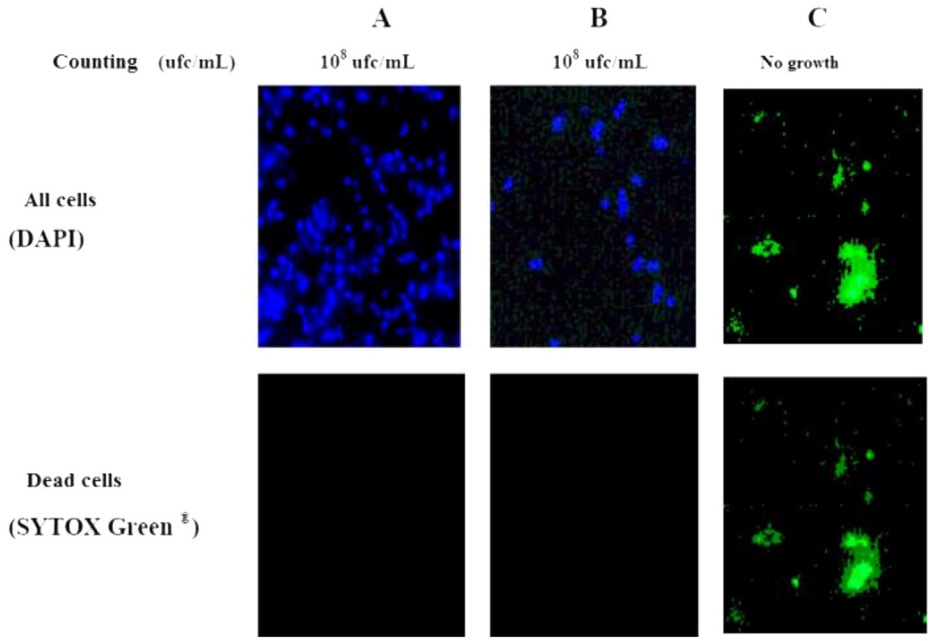


Fig. 2. Observations using epiflurescence microscope (×40) of Listeria monocytogenes 10357 (A: Stationary phase of cell growth; B: Disinfection control; C: Disinfection testing) [84].

type of analysis and the microorganisms to be studied [14]. Culture-based methods are widely used and are recommended by various standards. Quantitative assessment can be achieved by counting the number of active Colony Forming Units (CFUs) developed on a plate. This number is considered to represent the number of cells (or spores) initially presents on a sample and can be related to a given mass, volume or surface of the sample. Although direct identification and quantification by culture-based methods are quite simple to perform, in most cases, they are relatively time-consuming.

In recent years, authors have agreed that the exclusive use of culture-based methods is not sufficient to characterise a contaminated area with high accuracy, because of the many possibilities for introducing bias. These methods are usually more sensitive than other analytical methods to the sampling quality [45] and they only detect fractions of all the microorganisms present on a sample [2,6,75–82]. In particular, they detect active forms that are capable of growth but not slow-growing microorganisms or inactive forms (viable non-culturable) or non-viable forms. In addition, isolation prior to identification requires various types of cultures to be implemented because of the different nutritional and environmental needs of a microbial population and therefore induces a heavier work load.

An *in-situ* sampling campaign by Santucci et al. [6] showed that fungal patterns identified after culture-based methods following swab and imprint sampling were different from those found by direct observations on adhesive tapes. The identification of genera after culture reached 87% of the number identified by direct observations. On the other hand, direct observations identified only 42% of the genera identified after culture only [6].

Quantitative assessment tends to underestimate populations and especially inter-species ratios. The advantages of qualitative assessment are the isolation and preservation of strains. Identification by simple visual observation is also possible and quite accurate [6].

2.2.2. Observation methods

Quantitative measurement of microbial communities on samples is based on direct counting (CFU, fungal propagules etc.) or on tagging with fluorescent stains followed by image analysis to semi-quantitatively estimate the proliferation on surfaces (Fig. 2). Fungal identification, at species or genus level, can also be achieved through the observation of specific morphological features. Such identification requires particular skills [2,6,73]. Samson and Flannigan are widely quoted for their detailed descriptions of fungi (Fig. 3) and their identification method based on morphological observations [15,83].

Due to the limited diversity of bacterial morphologies, their identification by observation is rare. Populations can be classified by Gram staining[2] but strict identification of a genus or a species is usually achieved by chemical (in reaction tubes) or molecular biological analysis.

Whether the cells are culturable or non-culturable, viable or dead, direct observation methods using microscopes and/or fluorescent dyes can show the whole microflora adhering to a substrate.

For example, optical microscopy (bright/dark field, phase contrast, fluorescence) enables microbial cells to be detected on a substrate up to a maximum resolution of approximately 0.2 μm [85]. A microscope may be fitted with a haemocytometer, which is commonly used by microbiologists. This device consists of a glass slide divided into chambers with a grid having known bounded areas. After dropping a microbial suspension onto the slide and waiting for microorganism sedimentation, it is possible to count the number of cells in a specific volume or area and therefore estimate the initial concentration of cells in the suspension [54,66,86].

During recent years, some studies in microbiology have used epifluorescence microscopes. The principle is based on the irradiation of a fluorochrome, which is fixed to the DNA (deoxyribonucleic acid) by an operator, with specific wavelengths of light. The advantages are rapid and representative assessments of adhered biomass [84] or the concentration of spores in a fluid [86].

[2] Staining method for differentiating bacterial species into two groups: Gram+ and Gram− depending the chemical composition of cell wall.

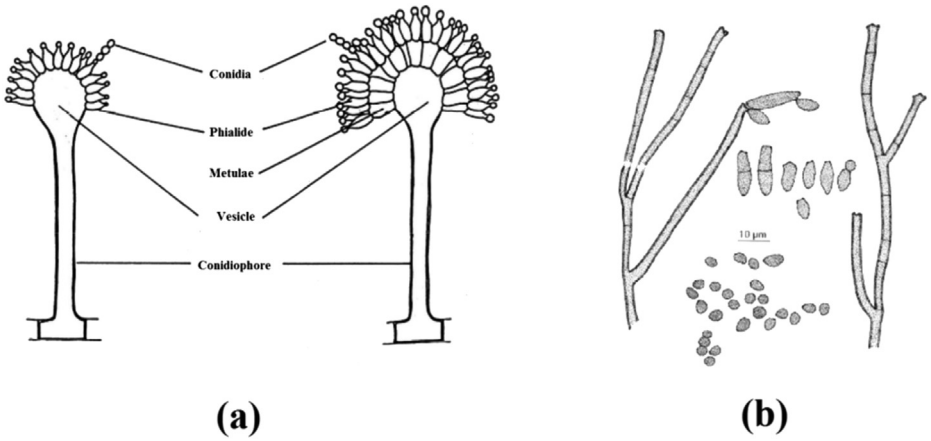


Fig. 3. Illustration of the typical morphology of the genera *Aspergillus* (a) and *Cladosporium* (b) [83].

In addition, the use of such a device to study building materials could be attractive because the observations do not require a transparent substrate. In her works, Allion developed a quick procedure to evaluate the viability of microorganism *in-situ* by direct tagging of adherent cells. However, thick clusters are quite difficult to observe, as are fungi in the filamentary state of growth. Some microorganisms can also resist tagging. Other works by Méheust have combined epifluorescence and flow cytometry technology, generally employed for microbial assessment of water or wastewater, in order to quantify fungal populations on surface samples collected in a hospital [47]. Here, the principle is based on the high-speed scrolling of microbial cells in a liquid stream through a laser beam (single wavelength). Results are obtained from the light re-emitted by the cells. The technique differentiates between viable and non-viable cells but cannot be used for every kind of environment because the signal can be perturbed by dust.

Finally, electron microscopes (transmission, scanning, confocal) have also been used. Like epifluorescence microscopes, these devices do not need a transparent substrate and are therefore commonly used for microbial investigation on building materials. Observations of surfaces and cross sections may show damage due to the penetration of fungal hyphae inside the matrix [66,87]. The technique can also be used to estimate the number of fungal propagules from adhesive tapes [11] or to observe the fungal growth directly on the substrate through Scanning Electron Microscope observations [38,88]. Adan carries out Low Temperature Scanning Electron Microscope observations to study fungal growth on gypsum-based finishes [88].

2.2.3. Chemical methods

Various chemical methods can give much information related to microorganisms. They are most often used to estimate the metabolic activity and thereby the toxicity potential of a microbial population on a substrate. The relevance of methods involving the measurement of chemical components from microbial cells depends on the choice of the components to be considered. Two possibilities are:

- *Measurement of the chemical components composing the microbial cells* such as components that form the mycelium cells for fungi (ergosterol, chitin) [37,53,55], adenosine triphosphate (ATP) which is an energy-producing molecule, and polysaccharides of the cell walls (β-D-glucane) [11]. The quantity of components can be linked to the number of microorganisms or it can be correlated with the type of microbial species. These methods are also suitable when microorganisms are in an inactive form.

  Since Seitz's works, the ergosterol content is widely determined to monitor microbial growth in food industry studies [89] and, as shown in Table 1, in studies on building materials. This method is widely believed to provide good estimates of fungal biomass [53], and numbers of spores and CFUs [5,90]. However, some authors point out that the estimation of the ergosterol content of materials depends on many factors, such as the type of material, the moisture content, the microorganism species and age, and the growth conditions [53,90–92]. According to Nout et al., identifying fungal biomass grown on natural substrate by a comparative quantification of the ergosterol produced by the fungi in culture is not possible because of variations induced by the testing parameters (age of strains, medium used, air stream). In contrast, temperature does not seem to have a significant effect on ergosterol production [91].
- *Measurement of chemical compounds produced by microorganisms* such as nitric oxide [86], various toxins (endotoxins,

Table 1
Compounds/components and techniques for chemical analysis of microorganisms collected on building materials.

Microbial metabolites – cell chemical compounds analysed	Techniques	Microorganisms	References
Endotoxins	LAL[a]	Fungi	[11]
Mycotoxins	HPLC[b], TLC[c], GC[d], GC-MS[e], ESI-MS[f]	Fungi	[7,9,49,93]
Cytokines	ELISA	Fungi	[86]
Nitric oxide (NO)	Griess test[g]	Fungi	[86]
Glucans	LAL, Hydrolysis	Fungi	[11,37]
Chitins	Hydrolysis, IC[h]	Fungi	[37]
Ergosterol	HPLC, TLC, GC, GC–MS	Fungi	[5,7,52,53,55, 60,62]
3-Hydroxy-fatty-acids	GC–MS	Gram– bacteria	[55]
ATP[i]	Bioluminescence	Bacteria	[46]

[a] Limulus amebocyte lysate.
[b] High-pressure liquid chromatography.
[c] Thin layer chromatography.
[d] Gas chromatography.
[e] Gas chromatography–mass spectrometry.
[f] Electrospray ionisation-Mass spectrometry.
[g] A test using Griess reagent which detect the presence of organic nitrite compounds.
[h] Ion chromatography.
[i] Adenosine Triphosphate.

mycotoxins, etc.) and other metabolites sampled from a surface [9,11,93]. This is an indirect method for assessing the metabolic (or biological) activity of microorganisms and thus estimating the microbial population. This type of method is generally used to assess the quantity of potentially deleterious compounds (metabolites on substrates or volatile compounds) and to deduce the pathogenic potential of the environment sampled and the resulting health hazard.

According to Tuomi et al., in most cases, there is no significant correlation between the presence of fungal species and the expected compounds [9]. Moreover, various metabolites can be produced by a single species [93]. Production can occur at specific times of microorganism growth (e.g. secondary metabolites are generally produced in the latest stages of growth). Analysis of microbially produced chemical compounds reveals more about the cell state at a given moment than about the number of microorganisms.

After culture and biophysical isolation of microorganims, biochemical methods are used in qualitative investigations of the reactions generated upon contact with specific substrates (Analytical Profile Index type system) [37,67].

There are also immunological methods based on the interaction between an antigen and specific tagged antibodies (animal or human) that enable the antigen–antibody complexes so formed to be detected and quantified. Muretoniemi et al. used the **ELISA** method (Enzyme-Linked Immunosorbent Assay) in order to evaluate metabolic activity through the cytokine level [86]. The **LAL** method (Limulus Amebocyte Lysate) was used on building materials by Andersson [11] for endotoxin measurements on water-damaged building materials. These tests are convenient by their relative ease of implementation and their low price.

The chemical compounds/components and techniques for evaluating surface contamination are presented in Table 1. Chromatography (thin layer, high performance liquid, gas, ionic) and mass spectrometry are the main analytical techniques employed for these measurements.

2.2.4. *Molecular biological methods*

Methods using recombinant DNA are based on the isolation of specific DNA sequences in order to target a particular phenotype, which is the signature of a group of microorganisms.

Since it was invented in the 1980s by K. Mullis, PCR (polymerase chain reaction) has become an essential tool in most studies of microorganisms [85] as PCR-based methods enable the detection, identification and even quantification (Rt-PCR) of microorganisms present in a sample. The process is based on the use of two primers, the function of which is to bind to a DNA region that is specific to a species or a larger group.

These methods can be expensive but they offer rapid and sensitive assessment of cultivable and non-cultivable organisms. On the other hand, no distinction is made between viable and dead cells. In their work on fungal contamination of moisture-damaged dwellings, Bellanger et al. found *Stachybotrys chartarum* on 21 samples using Rt-PCR while only one was isolated with a culture-based method [10]. This targeting approach requires some preliminary knowledge of the organisms likely to be present on the substrate and a data bank to select DNA sequences and the corresponding primers. Some authors have scanned a large diversity of prokaryotes by targeting the DNA 16S region (18S for eukaryotes) [11,56,59,86] while other studies have selected more specific regions such as ITS (internal transcribed spacer) for fungi [94–98]. This highlights the interest of coupling PCR with other techniques such as RFLP (restriction fragment length polymorphism) to add a degree of specificity. According to several studies, molecular biological methods give a more accurate view of microbial communities than culture-based methods alone [44,56].

Microbiological methods are relatively numerous and varied. Regardless of the method used, it is essential to distinguish two analytical approaches: targeting specific species or analysis of the overall population. Targeting is generally more time consuming. Overall analysis is faster but it has a much higher limit of detection and may thus not detect populations present in smaller quantities. In the 1990s, for example, studies showed that the use of PCR coupled with denaturing gradient gel electrophoresis (DGGE) detected microbial populations that made up at least 1% of the total community [99,100]. Assuming that the total community contained 10^6 microbial cells, this technique enabled populations of 10^4 cells to be detected, but any population with a smaller number of cells was not detected. To remedy this, species targeting approaches are necessary, using either a molecular biological approach or several selective media (culture and observations).

Overall, several methods are available for sampling and analysing microbial agents on building materials and the results obtained are linked to the method chosen: for example, a chosen culture medium could promote the growth of one species at the expense of another and lead to some microorganisms being masked in the measurement. For sampling and analysis processes, particular attention must be paid to the handling of samples. The need for methodological standardisation has been raised by many authors. For example, various measures can be found in the literature for quantitative assessment, which makes the comparison of results quite difficult. Criteria such as surface coverage, amount per square meter, toxicity potential, etc., should be unified to evaluate microbial contamination of building materials.

3. Overview of *in-situ* sampling campaigns and microorganisms identified on building materials

Since the late 1990s, *in-situ* prospection studies have been carried out in order to better understand the links between microorganisms in an indoor environment and health hazards for the occupants. Although a direct correlation between surface samples and occupants' diseases is difficult to establish, various authors point out that an estimation of the level of contamination of building materials would provide a good picture of potential hazard sources for people exposed, either by identification or by quantification of the genera/species and contaminants involved [11,30,58]. Moreover, the prevalence of microbial patterns related to specific materials should give information that would be helpful in the prevention of microbial contamination.

Some authors have reported various factors likely to be involved in the microbial contamination of building materials, such as humidity and material type (gypsum board, wallpaper, mortar, paint, etc.). It should be noted that most studies available in the literature focus on damp buildings and water-damaged building materials when investigating the presence of microorganisms. Humidity is believed to have an impact on microbial growth by increasing both the concentration and diversity of microorganisms on water-damaged surfaces [6,11,65]. Correlations between building material types and microorganisms present have been investigated in some studies. Species belonging to the genus *Penicillium* are the most frequently recovered microorganisms in all kinds of building materials [9,11,27,28,58,59]. *Aspergillus* species are commonly found on ceramic-type materials (concrete, mortar) and paints and glues [27,28,58]. The unexpectedly high occurrence of *Stachybotrys*, especially *S. chartarum*, in gypsum-type materials has also been mentioned [11,28,38,58]. According to Andersson et al., a synergistic relationship with potential dinitrogen fixers, also found in large amounts in these materials, may explain the massive

development of Stachybotrys in such a nutrient-poor environment [11]. Mycobacteria and Streptomyces were also widely found on these materials [11,13,59]. However, some studies bring out correlations between microorganisms and the location of sampling but not with the nature of the materials. Only the sampling locations, such as "walls", "ceiling" or "floor", are specified and no qualitative indications are provided concerning surfaces [6,10,45].

Table 2 summarises the different genera and species identified in situ (dwellings, schools or other buildings) on different materials in 9 studies found in the literature. The identification method is also mentioned. This is not an exhaustive list but it shows the microorganisms most frequently isolated and identified on indoor surface samples. *Cladosporium*, *Penicillium*, *Aspergillus* and *Stachybotrys genera* are the most frequently isolated whatever the technique, the environmental condition or the type of material. This classification of mould prevalence on surfaces was confirmed by a report by the International Energy Agency. Similarities can be observed with results from air samples [73]. Associated species are most commonly *C. sphaerospermum*, *P. chrysogenum*, *A. Niger*, *A. versicolor*, *S. chartarum*. Some of them, because of their well-known toxic and allergenic roles, are included among the potentially pathogenic species listed by the French Higher Council for Public Health and the France Environment Health Association [2,21,101].

Depending on the methodology followed, the study of samples from indoor building materials allowed several hypotheses to be put forward about the microbial communities present and potential contaminants. Field observations also led to hypotheses on factors influencing growth, such as moisture and material type. Laboratory testing on microbial growth allowed the field hypotheses about microbial growth on building materials to be confirmed or infirmed. The laboratory testing conditions include many factors influencing growth (%RH, temperature, nutrient supplies, etc.) and thus require particular attention.

4. Laboratory testing protocols: exposure of building materials to microorganisms

In addition to in-situ sampling campaigns, laboratory testing for microbial growth is also necessary to understand the phenomena governing the development of microorganisms on building materials. Various types of tests can be performed, depending on whether the goal of the study is to highlight the microbicidal effect of a given material or simply to observe its behaviour (resistance/receptivity) relative to microbial growth. The choice of some experimental parameters such as microbial strains, moisture, inoculation technique, etc. is defined by the type of test to be conducted.

4.1. Standards

Microbial growth in general and fungal growth in particular can take an extremely long time (from several days to several months), so standards generally recommend optimal growth conditions, i.e. high relative humidity, temperature around 30 °C and nutrient input in order to limit the time for reading and interpreting. However, this approach differs from natural growth conditions. Table 3 gives an overview of existing standards on microbial growth testing in laboratories. The table compares parameters of each standard and type of testing (antibacterial activity, fungus resistance, biodeterioration, etc.). The results are generally evaluated by visual inspection of the inoculated area or by measuring the mass variation of the samples. The table also shows that high temperature and humidity are always specified, whatever the test.

The standards for antibacterial activity testing recommend short durations (few hours) and control of the contaminated area is achieved by putting a transparent film (or glass) with a defined surface area over the inoculum.

4.2. Selection of strains

Microbial strains for testing can be recommended by standards or chosen because they satisfy specific criteria (resistance, acid production, occurrence in specific environments, etc.). The strains to be used during the test can either be supplied by a specialised laboratory ("collection strains") [5,46,86,102–104], or come from {in-situ} sampling ("wild strains") [68,86,103,105,106]. Allion suggests that the nature of the strain, "collection" or "wild", has an influence on the composition of the cytoplasmic membrane and thus might affect the bio-adhesive behaviour of the microorganisms toward some disinfectants [84].

4.3. Inoculation

Fig. 4 presents the most widespread inoculation techniques and their frequency of use as estimated from twenty publications. Droplet (by pipetting) and spraying are the most common techniques. The pipette allows a specific amount of cell suspension to be dripped on to the surface of a material [36,46,68,102,105,107], whereas spraying, dry or wet, produces a relatively homogeneous distribution but a less accurate amount of suspension, over a large area [5,61,87,104,108,109].

To overcome the lack of uniformity of cell distribution due to inoculation by droplet, some standards recommend applying a transparent plastic film or a glass slide directly on the inoculum. The inoculum then spreads under the film (glass), which forms a controlled cellular distribution surface [110,111].

In his work on fungal resistance tests for interior finishes, Adan objects to the use of aqueous suspensions for inoculation by explaining that they may cause an initial disequilibrium between the porous substrate and the adjacent air and provide favourable humidity conditions for fungal growth [88]. He transferred dry conidia by brushing the sample surface using dry sterile cotton swabs.

Hoang et al. developed a natural inoculation technique in which humidified materials were exposed to the ambient air of a residential house for 10 days [36].Their method is based on the use of an environmental chamber, in which the samples are inoculated not directly by the experimenter but through spore production by the microorganisms present in a potting soil deposited in the bottom of the chamber. In this type of experiment, guidelines [112,113] recommend carrying out a virulence test, which usually involves placing agar plates in the chamber and checking the time required for microbial growth to cover the whole surface of the agar plates. This virulence test ensures the airborne contamination of samples.

4.4. Incubation conditions

The incubation period is the test period during which microorganisms are in contact with the material. Incubation conditions (humidity, temperature and nutrient supply) have a direct influence on the microbial growth. Table 4 summarises the various incubation conditions found in the literature on testing microbial growth on building materials.

High relative humidity of the air enhances microbial growth during experiments. For example, all the standards for microbial investigation on materials recommend that the relative humidity of the incubation chamber should be between 70% and 97% depending on the test [110,111,114–117]. During short-term testing

Table 2
Microorganisms identified most frequently on surfaces in indoor environment. N.I. = Not identified. '+' = genus found on the corresponding material.

Genera									Species	Materials	Identification	Ref.
Cladosporium	*Ulocladium*	*Alternaria*	*Aspergillus*	*Penicillium*	*Stachybotrys*	*Chaetomium*	*Acremonium*	Bacteria				
	+			+	+		+		*P. chrysogenum*, *Stachybotrys* spp., *Ulocladium* spp.	Gypsum board, wallpaper	Cultures and Observations	[28]
			+			+			*A. fumigatus*, *A. melleus*, *A. niger*, *A. ochraceus*	Concrete, floor		
<10%	<10%		<10%	+	<10%	<10%	+		*A. versicolor*. *Actinobacteria*	Wood	Cultures and	[58]
+	<10%		<10%	+	+	<10%	+			Wallpaper	Observations	
<10%	<10%		<10%	+	+		+			Gypsum board		
+	<10%		<10%	+	<10%	<10%	+	+		Mortar, concrete, bricks		
+	+	+	+	+	+				*A. niger*, *A. versicolor*, *P. expansum*, *P. brevicompactum*, *P. chrysogenum*, *C. cladosporoide*, *S. chartarum*, *U. chartarum*, *A. alternata*	Paint, gypsum board, wallpaper, wood, etc.	Cultures and Observations	[62]
+			+	+	+	+	+		sp.	Wood, chipboard, cement, wallpaper, bricks, etc.	Cultures and Observations	[30]
			+	+	+			+	*S. chartarum*, *P. aurantiogriseum*, *A. versicolor*. Gram−	Gypsum board, dusts	Molecular, chemical	[11]
			+	+	+			+	*Streptomycetes* spp.	Painted plaster	Molecular	[59]
+	+	+	+	+					sp.	N.I.	Cultures and Observations	[6]
+		+	+	+	+				*C. sphaerospermum*, *P. chrysogenum*, *A. versicolor*, *A. alternata*, *S. chartarum*	N.I.	Observations, molecular	[10]
+	+	<10%	+	+			<10%		*P. chrysogenum*, *P. olsonii*, *C. sphaerospermum*, *C. cladosporiorides*, *A. versicolor*, *A. fumigatus*, *A. niger*	N.I.	Observations, molecular	[45]

Table 3
Non-exhaustive list of standards and impacting parameters for laboratory testing: exposure of materials to microorganisms.

Standards	Type of test	Materials	Microorganisms	Inoculation	Specific conditions	T °C, %HR	Duration
JIS Z 2801 [110]	Antibacterial activity	Antibacterial products (plastics, metals, ceramics, etc.)	*E. coli*, *S. aureus*	Droplet	Contaminated surface control (film)	35 °C, 90%	24 h
ISO 27447 [111]	Antibacterial activity	Ceramics: semiconducting photocatalytic	*E. coli*, *S. aureus*, *Klebsiella pneumoniae*	Droplet	Contaminated surface control (film)	–	4 h–8 h
NF EN ISO 846 [114]	Biodeterioration, fungistatic activity	Plastics	*A. niger*, *A. terreus*, *P. funiculosu*, *P. ochroloron*, *Paecitomyces variotii*, *Gliocladium virens*, *C. globosu*, *Aureobasidium pullulans*, *Scopulariopsis brevicaulis*. *Pseudomonas aeruginosa* (bacteria)	Droplet or spraying	Nutrient medium incomplete/complete	20–35 °C, 95%	≈4 weeks
ASTM D 3273 [112]	Fungistatic activity	Interior coatings	soil contaminated with: *A. pullulans*, *A. niger*, *Penicillium* sp.	Aerial (environmental chamber)	–	32 °C, 95%	4 weeks
ASTM D 6329 [115]	Biodeterioration, fungistatic activity	Building materials	Soil contaminated with: *Aspergillus* spp., *Stachybotrys chartarum*, *Fusarium moniliforme*, *Penicillium* spp., *Cladosporium* spp.	Aerial (environmental chamber)	–	32 °C, 95%	4 weeks
EUROCAE ED-14E [116]	Fungus resistance	Airborne equipment	*A. niger*, *A. flavus*, *A. versicolor*, *Penicillium funiculosum*, *Chaetomium globosum*	Spraying	Contaminated surface control	30°, 97%	4 weeks
XP ENV 807 [113]	Resistance against microorganisms from soil	Wood preservative products (paint, stain, etc.)	Natural soil	Burying in contaminated soil	–	27 °C, 70%	8, 16, 24, 32 weeks
XP ENV 12404 [117]	Fungicidal-fungistatic activity	Mortar-masonry preservative products (paint, stain, etc.)	*Serpula lacrymans* (or other dry rot fungus depending on region)	Contact with contaminated medium	Complete nutrient medium	22 °C, 70%	12 weeks

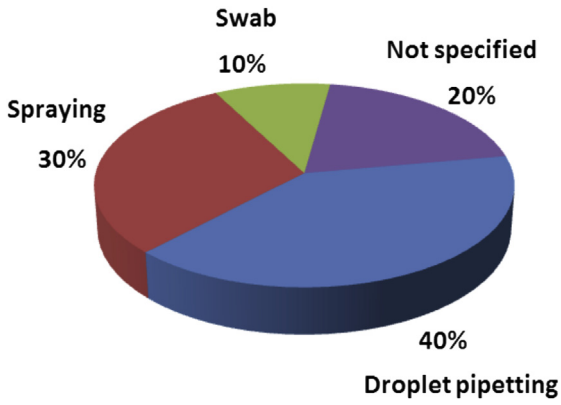


Fig. 4. Inoculation techniques used in laboratory experiments on building materials. Frequency of use estimated from 20 papers.

(typically a few hours), inoculation is usually performed with an aqueous medium, by depositing drops or spraying. In this case, high relative humidity prevents drying of the inoculum. In other cases, high relative humidity helps to maintain optimal growth conditions for microorganisms in order to reduce the test duration to a minimum. The main devices used for controlling the relative humidity of the air are: saturated salt solutions [102], vermiculite [66], water-filled container [46], and air-flow controlled systems [7]. If the aim is to use optimal growth conditions to reduce testing times, it is sometimes necessary to prepare the samples beforehand. Standards on fungal proliferation recommend placing samples in a controlled atmosphere with a relative humidity higher than 50% for several days. Various studies have used such conditioning [5,7,36,68,102]. This preparation may be a key step in the assessment of microbial contamination on building materials.

Microbial growth is also strongly dependent on cardinal temperatures [85]. Cardinal temperatures are the minimum, maximum and optimum growth temperatures and are specific to the selected species. Standards recommend choosing the temperature for incubation with respect to the species Table 3. The testing process is therefore carried out in an incubator at a temperature generally higher than 25 °C. Tests are also performed at room temperature when the aim is to use field conditions.

The addition of a nutrient source (agar, broth, etc.) to a substrate provides sustainable and accelerated microbial growth but is far from representing actual growth conditions. However, in the absence of a nutrient source, microbial growth is uncertain and takes much longer. In addition, the results obtained in experiments with or without nutrient supply do not provide the same information. NF EN ISO 846 describes two different tests in particular. In one case, an incomplete nutrient media (without carbon source) enables the inherent resistance of the substrate to microbial growth to be observed: microorganisms can grow only at the expense of the material. In the other case, growth is promoted by providing a complete nutrient medium: any growth inhibition shows a fungistatic effect of the material [114].

4.5. Materials

Table 4 shows the different types of materials used in studies on artificial contamination. Some studies focus on concrete and mortar, wallpaper or ceiling tiles, but plaster-based or gypsum-based materials are most frequently tested. It is important to note that very few studies undertake physicochemical and surface characterisation of the materials to establish relationships between adhesion or proliferation mechanisms on surfaces and the chemical/mineralogical nature of the material. Generally, the materials tested are those found in the indoor environment. These materials are either collected on site or purchased from a supplier. In addition, most trials focus primarily on microbial growth in terms of the toxicity, resistance to growth or antimicrobial effect of the materials; they are rarely conducted to describe and explain the substrate–organism interactions during the microbial growth process.

5. Bio-receptivity of materials – determining factors

In this part, various factors highlighted by authors in laboratory studies are discussed. The results point out, in particular, the major influence of water and of the chemical composition and pH of materials.

Table 4
Incubation conditions for microbial growth testing on building materials. NS = Not specified.

Ref.	Materials	T °C	%HR	%HR Control	Nutrient input	Testing duration
[34]	Wood, gypsum board, wallpaper, …	20–3	75, 80, 95	Airtight chamber + saturated salt solutions	No	31 and 55 days
[87]	Concrete	30	High	Continuous air flow	Yes (spraying)	174 days
[102]	Ceiling tiles	21 ± 3	NS	Saturated salt solution	No	28 days
[103]	Natural gypsum, phosphogypsum	32	95–100	NS	No	4 weeks
		25	100	NS	Yes/No	14 days
[105]	cellulose-containing and inorganic ceiling tiles	25	80	Filtered air flow, standing water	Yes/No	10 days
[5]	Cellular concrete, gypsum-carton board, paint gypsum-carton board	22–25	70–80	NS	Yes (spraying)	2 years
[68]	Mortar plastering	25	75, 85, 100	Saturated salt solution	Yes (flooding)	30 days
[27]	Wood, gypsum board, ceiling tile	–	75, 85, 95	Climatic chamber + saturated salt solutions	No	5 weeks
[86]	Plasterboard	20–23	NS	Standing water + filtered air flow (Once a day, 10 min, 400 ml/min)	No	Until growth stabilisation
[7]	Gypsum board, concrete, mortar, wallpaper, etc.	25	69, 78, 86	43.5 cm Controlled air flow system	4*No	7 months
		20	76, 86, 90	With waterbath		4 months
		10	80, 90, 95			4 months
		5	79, 87, 91			4 months
[118]	Wood frame wall assemblies	20–35	70–95	Climatic chamber	No	19, 18, 16 weeks
[107]	Concrete, mortar	25	95–100	NS	Yes (spraying)	7 days
[66]	Cement paste	26	NS	Moistened vermiculite	No	4 weeks
[36]	Green material (sunflower board, bamboo flooring, etc.)	30	90–95	Saturated salt solution (K_2SO_4)	Yes (various)	3 to 8 weeks
[104]	Plasterboards and aluminium	28	95	Climatic chamber	No	45 days
[109]	Wood, gypsum, cement-based board, etc.	10, 22	75–95	Climatic chamber + controlled air flow	No	12 weeks

5.1. Water activity – equilibrium relative humidity

The major role of water on microbial growth is widely reported by the literature. According to the International Energy Agency, the susceptibility of substrates in dwellings to mould largely depends on the water activity [73]. The water activity, a_w, of a solid (or a liquid) is directly linked to the water potential that affects the pressures on the cell wall of a microorganism [119,120]. When water balance is reached in a system, water activity is defined as the ratio of partial vapour pressure to the pressure of pure water (saturated vapour pressure), i.e. 1/100 of the equilibrium relative humidity (ERH) for a defined temperature. The term "water activity" is widely used, the activity is easy to measure, and its major impact on microbial growth has been studied for many years [32,73,121–126]. Microbial growth is no longer limited by water activity for values greater than 0.7 (up to approximately 1, which is the maximum value of a_w) for most microorganisms [73,120,127]. It should be noted that water availability and temperature are interdependent and, for example, increasing temperature has been found to lead to a reduction in the a_w requirement of the moulds [32,33].

Actually, microbial investigations on building materials tend to reason in terms of ERH [54,7,65,109,128]. It appears that construction materials become the target of microbial growth when the ERH reaches a value greater than 70% for wooden materials, 85% for gypsum-board and around 90–95% for cementitious and concrete materials [7,35]. The works of Johansson et al. provide different ranges of critical %RH (yielding values enabling microbial growth after 12 weeks of incubation) according to the nature of material [109].They also highlight the influence of the temperature, the incubation time and the assessment criteria for mould growth on the results of such testing. Adan reported a significant increase of the rate of development of *P. chrysogenum* during testing on gypsum substrates when raising RH from 86 to 97% [88].In addition, various authors have suggested that fungal growth is minimal under non-wetting conditions at 85–95% RH and have pointed out that wetting events favour the germination, the proliferation, and the diversity of mould on building materials [27,34,38,118,129].

Several studies also show that ERH measurements could be used as a microbial contamination indicator for construction materials in water-damaged buildings [54,65]. Pasanen et al. stated that ERH of a material describes the water availability for microorganisms better than the moisture content does [54]. Some authors have even developed mathematical models for predicting contamination by moulds, which use RH as a major parameter [88,130–132].

5.2. Chemical composition

The components of colonised materials are a potential nutrient source that can favour the development of microorganisms [6,88,104,133]. The works of Hoang et al. and Gutarowska indicate that cellulose-based materials are more sensitive to contamination than inorganic materials (gypsum, mortar, concrete, etc.) [36,37] because cellulose can be metabolised by the microorganisms. Moreover, Hoang et al. state that the intake of dust, organic compounds, etc. from outside can also be a nutrient source on a wall and this is a factor that increases the risk of colonisation, even on materials that are not naturally sensitive, such as plasterboard [36]. Besides, the addition of a carbon source (carboxylmethyl cellulose) or emulsion paint, for example, can furnish nutrients that also induce a reduction of the a_w requirement of moulds [32,33].

5.3. pH

Most bacteria prefer neutral pH. Thus, building materials with pH levels between 6 and 8 are more sensitive to microbial colonisation than cementitious materials, which are alkaline (pH around 12–13) and therefore relatively insensitive to colonisation at early ages. However, over time, the carbonation process reduces the pH of these materials to values around 9, which allows microbial growth. Some studies deal with the contamination of mortars that have undergone accelerated carbonation and show that their bio-receptivity is considerably increased [66,134,135]. These materials thus become the target of significant contamination. A study by Tran et al. also confirms the crucial influence of pH on the colonisation of mortars by phototrophic algae. In this work, the colonisation of carbonated mortars occurs earlier (15–20 days) and spreads faster: the contamination of whole surface (100%) is reached after around 90 days on healthy mortar and after only 30 days on carbonated ones [40].

5.4. Physical properties of surface

It is widely agreed that the proliferation and growth of microorganisms on building materials are conditioned by the presence of nutrients and sufficient available water. It should be noted that most building materials are characterised by high porosity and surface roughness. The high porosity gives them particular behaviour regarding water absorption. When the environment provides high relative humidity or moisture events, porous materials can become supplies of water for microorganisms and offer them a larger growth subsurface [36]. In addition, surface roughness and porosity could favour the attachment of nutrient components carried by dust resulting from the activity in buildings. It was also shown that treating cement mortars with water repellent compounds decreased the rate of algal fouling at their surface [136].

The study by Tran et al. [40] also demonstrates the influence of roughness on the colonisation of mortars by algae. They observe that the samples with rough surfaces are covered much faster than smoother samples. Asperities on surfaces promote the attachment of algae and then favour colonisation [39–41]. On the other hand, Adan observed that decreasing the surface roughness of gypsum-based finishes accelerated fungal growth, with a more pronounced effect for low values of roughness [88]. He suggested that the interface areas of fungal structures were then enlarged, which promoted interactive processes. Nevertheless, he noted a slight delay in fungal growth for gypsum with low porosity (water/binder ratio <0.6), which would be explained by a probably decreased availability of nutrients [88].

These works appear to support the hypothesis by Coppock and Cookson that mould growth could be related to the porosity and possibly the pore-size distribution of substrates, even though no clear correlations have been established yet [137].

Various studies have already focused on the phenomena involved in the adhesion of microorganisms to non-porous materials, such as metals, glasses, plastics, etc. [84,138,139] and the mechanisms of bacterial adhesion to biomaterials and bacteria–material interactions [140]. These studies highlight the important role of contact angle, and physicochemical and electrochemical reactions that can occur between the substrate and adherent organisms. However, this kind of investigation has hardly been conducted, if at all, on building materials since their porous nature and their behaviour towards water make this analysis even more complex. This hinders the understanding of mechanisms of microbial growth on these materials, making interpretations and predictions of proliferation relatively difficult.

6. Conclusion

In the framework of indoor air quality degradation caused by microorganisms, the study of microbial proliferation on building

materials is often suggested. *In-situ* microbial investigations on building materials have been carried out to give a better picture of the indoor microflora and identify potential contaminants, which could be connected with health hazards for the occupants. Various sampling and analysis methods have been tested in investigations dealing with the microbial contamination of building materials. Swab sampling, adhesive tape sampling and contact plate sampling are methods initially developed in microbiology for smooth surfaces and so are not very suitable for this kind of rough, porous, dusty materials. The few studies focusing on their efficiency on concrete show very low values compared to glass or steel. There is a clear need to adapt and standardise methods or to diversify the techniques used to be able to report the microbial populations actually present on a building material surface as accurately as possible. In addition, cultures, observations, and chemical and molecular analyses provide a wide range of methods for microbial investigations depending on the purpose of the study. Standardisation would be helpful in the choice of a methodology, by considering different parameters such as the relevant species (if known), the aim of the study, the limits of detection, etc. The presented studies list the different organisms, some potentially toxic and allergenic, that colonise surfaces depending on several factors such as material type and moisture. The fungal genera most frequently found in indoor environments, all techniques taken together, are *Cladosporium*, *Penicillium*, *Aspergillus* and *Stachybotrys*; the bacteria are Gram negative bacteria and mycobacteria.

Laboratory testing gives information for a better understanding of the phenomena governing microbial development on building materials. Standards have been developed to assess the proliferation resistance and antimicrobial activity of some materials. Few standards are suitable for building materials such as gypsum or cementitious materials, which are generally highly porous and have specific chemical compositions. These standards usually recommend specific testing conditions to enhance microbial growth and reduce the durations of tests, i.e. high temperature (20 °C), high humidity (<70%) and a nutrient intake. Although these conditions provide sustainable and accelerated microbial growth, they are very different from actual conditions on building material in an indoor environment. The contamination is generally estimated by surface observations or CFU counting. It should be noted that very few correlations between the intensity/nature of proliferation and the chemical/mineralogical nature of the material are reported in the literature. There is wide agreement on the major action of water on microbial growth: growth on building materials is favoured for equilibrium relative humidity values higher than 70%. The chemical composition and the pH of materials also influence microbial growth. The characterisation of the physicochemical interactions between substrates and microorganisms and the adhesive properties of the microorganisms themselves have not yet been studied for building materials exposed to indoor conditions. This lack of information significantly hinders the understanding and prediction of microbial growth on building materials.

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