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A Methodological Review of Tools That Assess Dust Microbiomes, Metatranscriptomes and the Particulate Chemistry of Indoor Dust

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Review

A Methodological Review of Tools That Assess Dust Microbiomes, Metatranscriptomes and the Particulate Chemistry of Indoor Dust

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Abstract: Indoor house dust is a blend of organic and inorganic materials, upon which diverse microbial communities such as viruses, bacteria and fungi reside. Adequate moisture in the indoor environment helps microbial communities multiply fast. The outdoor air and materials that are brought into the buildings by airflow, sandstorms, animals pets and house occupants endow the indoor dust particles with extra features that impact human health. Assessment of the health effects of indoor dust particles, the type of indoor microbial inoculants and the secreted enzymes by indoor insects as allergens merit detailed investigation. Here, we discuss the applications of next generation sequencing (NGS) technology which is used to assess microbial diversity and abundance of the indoor dust environments. Likewise, the applications of NGS are discussed to monitor the gene expression profiles of indoor human occupants or their surrogate cellular models when exposed to aqueous solution of collected indoor dust samples. We also highlight the detection methods of dust allergens and analytical procedures that quantify the chemical nature of indoor particulate matter with a potential impact on human health. Our review is thus unique in advocating the applications of interdisciplinary approaches that comprehensively assess the health effects due to bad air quality in built environments.

Keywords: indoor dust; allergens; metagenomics; particulate matter; microbiomes; transcriptomes; health effects

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1. Introduction

1.1. Overview of Indoor Dust

Dust is a collective term to describe the large diversity of organic and inorganic particles of various size and composition that are found in the atmosphere either in suspended or settled forms. Indoor dust (ID) is a type of captured dust which is a complex mixture of particulate matter (PM), plant materials (fragments and pollen), fibers from construction materials, furniture and textile residues, skin flakes and the hair of animals, insect parts and microbes, as well as atmospheric dust and combustion particles [1].

Besides indoor emissions from activities such as cooking and cleaning, a predominant portion of the air pollution in homes is by virtue of the influx of outdoor pollutants in the air. In addition to human subjects, indoor environments are also inhabited by pets, plants and insects with their peculiar microbiomes, skin cells, fur, feathers and hair follicles,

as well as natural exudates and secretory glands that add biological complexity to the indoor air. For instance, an average person sheds enough skin, sweat and hair each day to feed a substantial number of dust mites [2]. Likewise, human and animal activities such as breathing, sneezing and coughing cause the discharge of a plethora of microbial communities in indoor environments [3]. Infiltration of air through intentional (ventilators, windows and doors) and unintentional ventilation pollute the indoor environment with outdoor pollution [4]. During warm and humid conditions dust mite communities get increased, and they thrive in a temperature range of 20–25 °C and humidity levels of 70–80% [5]. Likewise, the growth of fungi (molds) on walls, edible material and fabrics, as well as their secreted enzymes and toxins (allergens), further aggravate the quality of indoor air. Depending upon the duration of indoor human occupancy, the lifestyle of the inhabitants, sociodemographic conditions and many more factors, the indoor air is manifold more polluted and biologically more complex than the outdoor air [6,7] (Figure 1).

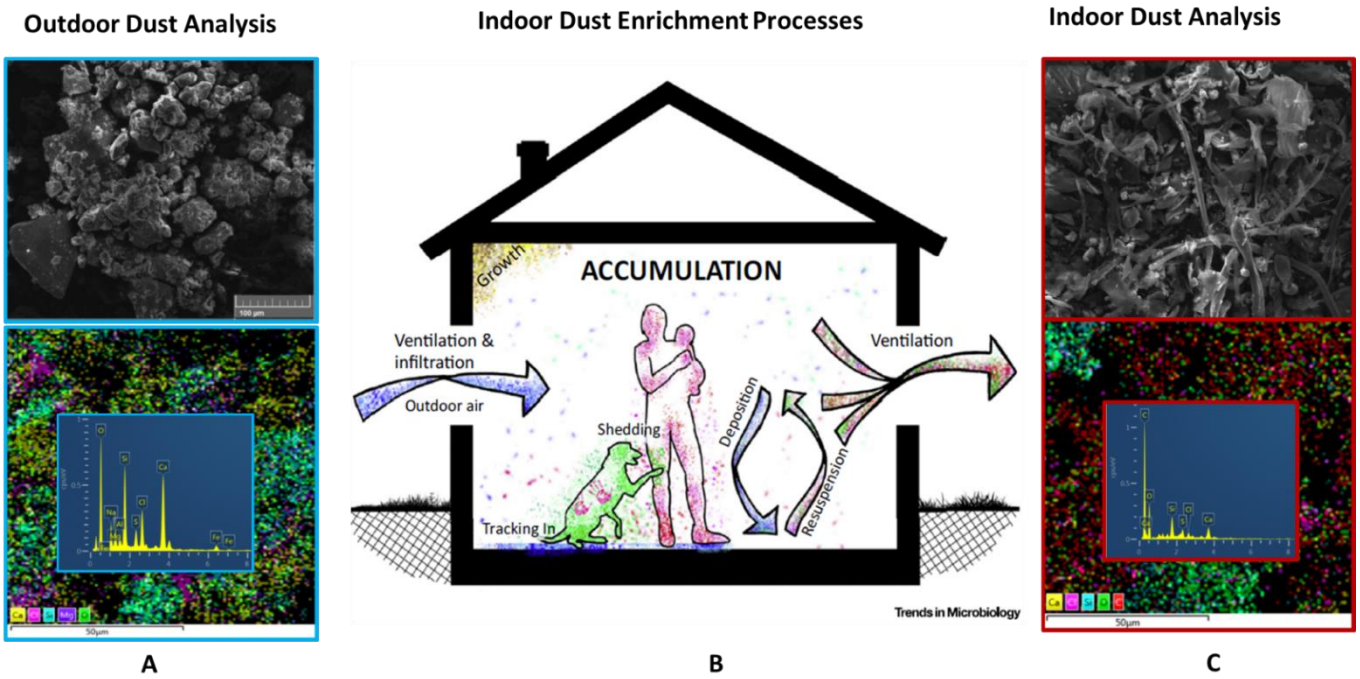

Figure 1. The outdoor dust particles after entering the built environments (BEs) turn more polluted owning to human-mediated activities. Figure Legend: Influx of dust particles (**A**) that interact with human subjects, and human- and animal-mediated microbial communities inside built environments (**B**). Food particles, released human skin, hair and many indoor activities cause the loading of captured dust with diverse entities of a chemical and biological nature (**B**): adapted from Trends in Microbiology [7]). Scanning electron microscopic (SEM) images of indoor dust with weird structures such as skin pigment, human and animal hair, fabric material, food particles, heavy metals, as well as microbes (**C**). Scanning electron microscopy (SEM, TESCAN VEGA, LMU) coupled with energy-dispersive X-Ray spectroscopy (EDX, INCAx-act, Oxford Instruments, Abingdon-on-Thames, UK) was used as a case study example to analyze the surface morphology and elemental composition of the dust samples (**A**,**C**). EDX analysis showed typical inorganic elements, mainly O, Si, Ca, Al and Fe (**A**,**B**: lower panels). Panels A and C are a courtesy of Dr. Cijo Xavier, Newcastle University, while Panel B is adopted from [7].

1.2. Biogeological Processes That Cause the Loading of Outdoor Dust Particles with Biotic and Abiotic Factors That Impact Human Health

In geological terms, the process of weathering and variations in day and night temperatures exert a strain on rocks, which breaks them into pieces and small particles. The finest particles in the atmosphere (that originate from various sources such as eroded soils moved by the wind as a consequence of aeolian processes, volcanic eruptions, as well as

anthropogenic and natural pollution) culminate in outdoor dust sources and thus generate aerosol in the atmosphere [7,8]. The composition of desert dust may include aeolian prokaryotic communities (APC), including aeolian microorganisms along with mineral species that travel across a large geographical area [8]. The "Global Dust Belt" from the western coastal areas of North Africa, crossing the Middle East and entering Central Asia (10 to 30° N roughly) is known to have the highest atmospheric dust load [9] in the form of aeolian microbiomes [10]. Thus, the aeolian particles are already loaded with biological inoculants even before their entry into the indoor atmospheres [11]. It is therefore argued that airborne microorganisms have long been considered to influence the health of humans and ecosystems in general [12,13].

2. The Genesis and Health Effects of Indoor Dust

The global trend towards urbanization and harsh outdoor conditions owing to climate change have compelled an ever-growing number of people to live and work in indoor conditions. This unnatural and built environment (BE: indoor) now represents a modern ecological habitat for human beings as well as for human-associated pets and other domestic animals. The indoor environment contains novel habitats for microbial life that have overlapping as well as distinct chemical and physical properties than the outdoor world (Figure 1). Humans carry a large number of the colonizing microbes into these niches by shedding them from their bodies or shifting them by means of their bodywear and shoes, whereas the rest come from air and water sources [14,15]. Microbes are also brought indoors by pets, cats, dogs and birds [16,17]. The increased rate of urbanization, rapid industrialization and heavy construction activities, as well as the generation of mega structures, highly particulate indoor environments in the big cities of the world [18]. The uninterrupted use of air-conditioning in the indoor environment keeps the air in circulation as well as the captured dust. This circularity keeps the indoor particulate matter in contact with multiple human subjects and each contact further enriches indoor dust with conserved as well as peculiar microbial consortia in indoor environments [19]. Likewise, prolonged summers with high humidity encourage microbes to easily propagate and constant air circulation makes them further amenable to interacting more frequently with humans and home surfaces [20]. Therefore, a routine analysis of the microbial communities and quality assessment of the indoor air is worth attempting for the health of prolonged indoor occupants.

The air quality index (AQI) [21] is a descriptive gauge used to map air pollution, and AQI values at/below 100 are generally considered healthy and safe; values exceeding this range are presumed as unhealthy. It is noteworthy to mention that AQI values cannot be extrapolated to indoor air quality, and factors such as carbon dioxide (CO_2), indoor air temperature and humidity, as well as volatile organic compounds (VOCs) and microbial activity, diversity and abundance should be optimized to approximate a modified indoor air quality index (IAQI). In this regard, two main factors contribute to the quality of air in a home: particulate matter and gaseous pollutants such as VOCs [22,23]. The former is made up of microscopic particles, liquid droplets, or a combination of both. The particulate matter in the air is comprised of acids such as nitric and sulfuric acids, organic chemicals, metals, dust and soil particles, as well as tiny biological residues [24] including microbes such as viruses, bacteria and molds, as well as plant pollens, dust mites and the remains of cockroaches and their droppings, and animal dander [1].

Approximately two-thirds of the dust in homes is brought in from the outside [25] (Figure 1). In addition, dust can contain some of the toxic compounds such as rodent waste, paint particles, pollen, bacteria, viruses, insect parts and heavy metals [26] (Figure 1). Occupants show mild to harsh symptoms of allergy to dust mites such as congestion, a runny nose, watery eyes, itching and sneezing [27]. Likewise, humans are more exposed to fungal and bacterial infection when a caregiver or infected symptomatic or asymptomatic individuals are among the occupants [28]. Not all bacterial and fungal communities of the indoor airspace are equally infective; many among the microbial communities are not

even pathogenic in nature [29]. There are reports that some of the pathogenic bacteria in indoor environments are resistant to antibiotics such as some of the Staphylococcus aureus strains [30]. Poor indoor air quality and a dusty atmosphere also invoke asthma [31] and occupants with previous morbidities, and young children as well as elderly populations are more vulnerable to the ill effects of indoor dust [32,33].

An important factor associated with the study of indoor dust chemistry is the allergic condition that occurs due to increased immunoglobulin E (IgE)-mediated sensitization to dust mite allergens, as exemplified in the case of asthma. Fu et al. [34] reported that Izhakiella and Robinsoniella (bacterial families) were associated with the occurrence of severity of asthma in a study of classroom floor dust in Malaysia [35]. Dust mite allergens are some of the common indoor allergens that lead to perennial allergic rhinitis, occurring throughout the year. The etiology associated with dust mites is that their guts contain a powerful digestive enzyme, peptidase 1, that causes allergy in humans [36]. When inhaled, they cause a sensitization reaction as they leak through epithelium into the respiratory system by cleavage of the tight junctions in between the epithelial cells. This leads to epithelial hyperpermeability, which passes on house dust mite allergens to dendritic antigen-presenting cells (APC) that trigger an immune response. There are many studies suggesting that disruption of the airway epithelial barrier is caused by these allergens. Allergens such as house dust mites (HDMs), including allergen Der p1, with proteolytic activity, can directly impact tight junctions and act indirectly via the activation of protease-activated receptor-2. This effect, when combined with biological agents such as rhinoviruses/coronaviruses or physical agents such as cigarette smoke, can promote increased permeability of the airway epithelium facilitating inflammation [37]. The role of type 2 cytokines such as IL-4 and IL-13 in disrupting the epithelial barrier integrity of the nasal epithelial cells of patients suffering from allergic rhinitis been reported. The most notable among house dust mites is Dermatophagoides pteronyssinus, D. farinae, which is commonly found in dry areas. In areas with tropical and subtropical climates, the glycyphagid mite Blomia tropicalis (storage mite), which coexists with D. pteronyssinus, is a dominant allergen source. Dust mite allergy causes perennial allergic rhinitis where allergic symptoms occur throughout the year [38]. In addition to dust mites, house dust allergy can also be caused by mold, cockroaches, pollen and pets with natural stimulants that elicit immunogenic responses in human beings. Minimizing the indoor air contamination by cleansing or avoiding dust mite allergens leads to fewer allergic reactions in indoor occupants. Persistent exposure to dust mite allergens for a sensitized individual can lead to sinusitis, atopic dermatitis and the precipitation of acute asthmatic attacks. It is noteworthy to mention that dust mite allergy is a case of frequently underdiagnosed, even misdiagnosed, and mistreated health condition. Although a vast array of in vivo and in vitro diagnostic tests are available for detecting dust mite allergy, the role of laboratory facilities and the training of laboratory personnel play an important role in the process of diagnosis. Therefore, the outcome of dust mite allergy diagnostics depends on the ability to identify dust mites as the source of the allergic manifestation in human subjects. However, to improve outcomes further, in vitro and in vivo investigations to determine the specific etiology of specific dust microbes have to be conducted.

COVID-19 has caused tremendous misery in the modern period, forcing the human species to adapt to a new normal in terms of social connections [39]. The involvement of atmospheric dust (specifically aerosol) in human-to-human transmission of the viral infection had never been discussed so intensively before this pandemic [40,41]. As viruses are most abundantly prevalent in the environment in the form of pathogenic or non-pathogenic microparasites [42], their survival and interaction with living species has been the subject of tremendous interest. How do viruses survive in different dusty settings? This is a pressing question related to their transmission pattern in the indoor built environments. Although some viruses are blood-borne, several of them are transmitted by the fecal–oral route (HAV) [43]. Aerosol transmission of viruses has also been well documented (REFF), and currently, indoor settings and aerosol transmission have been assessed to be the major

factor contributing to the spread of SARS-CoV-2 [44,45]. Virome analysis of the dust samples collected from indoor settings thus makes a good case for the transmission of viruses in dusty ambiences.

3. Toolkit to Assess the Complexity of Indoor Dust Environments

3.1. Overview on the Meta-Analysis of Indoor Dust Assessments

As discussed, indoor dust is a complex particulate matter decorated with microbial communities, fortified by home-based organic and inorganic compounds with diverse eliciting potencies to evoke physiological responses in human tissues such as the skin, nasal epithelium or bronchial lining upon exposure. Various studies have been conducted that assessed the health effects of indoor dust on human subjects; however, we advocate for a comprehensive integrated meta-analysis that dissects the chemical nature of indoor dust particles by applying analytical and surface analysis techniques with invasive and non-invasive approaches. Likewise, the application of modern high-throughput genomics tools can be used to decipher the microbial consortia of indoor dust particles, and modern transcriptomics and metabolomics approaches will unleash gene-expression and the metabolic profiles of human cells that encounter the dusty surroundings (Figure 2). In the following, we elaborate on pertinent tools (Figure 2) in order to dissect the complexity of dust-mediated health effects in occupants of indoor environments.

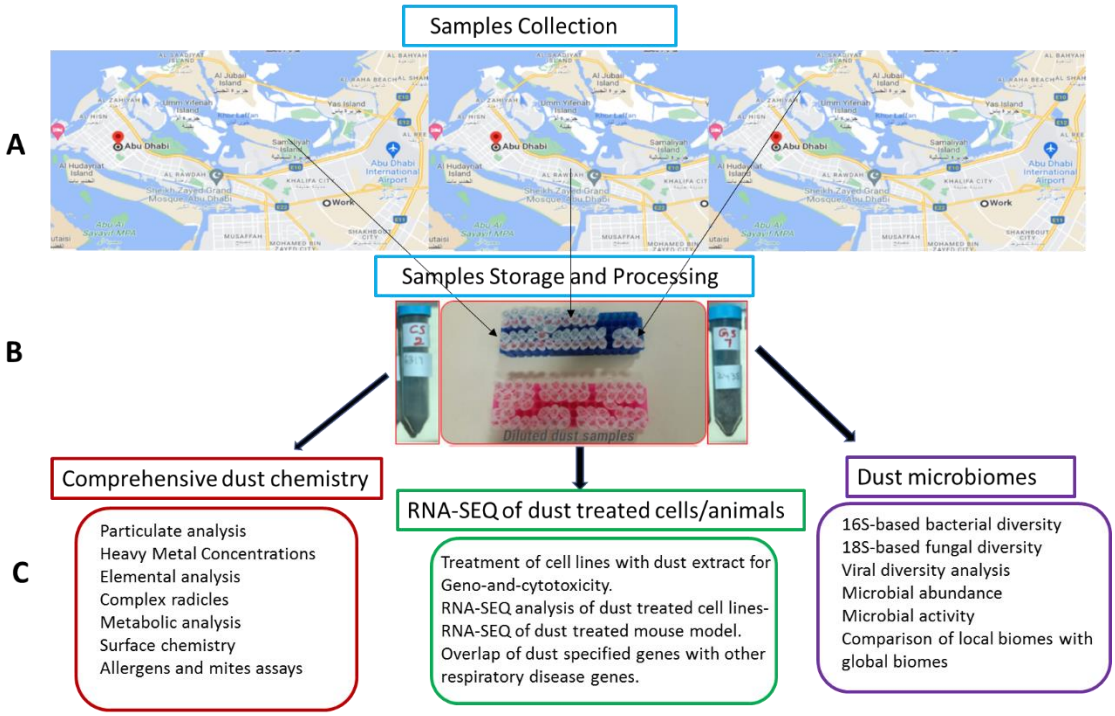

Figure 2. A unified framework to assess the health effects of indoor dust by applying an integrated meta-analysis. Figure legend: schematic representation of the meta-analysis of indoor dust effects on occupants. Inclusive sampling across multiple locations with accurate location and coordinates in the study zone (**A**). The collected samples are properly archived and stored to avoid degradation and contamination (**B**). Analytical and microscopic techniques are applied to study the chemical and particulate nature of indoor dust. Microbial diversity, abundance and activity of dust is studied by applying metagenomic analysis following next generation sequencing (NGS) approaches (**C**).

3.2. Representative Sampling of Indoor Dust Environments for Genomics/Transcriptomics and Chemical Analysis

Sampling of the dusty material (meta-sample) from the site plays a pivotal role in the overall indoor dust health impact assessment program (Figure 2). Prior to the sampling procedure, the environmental conditions of the site such as humidity, temperature and

precipitation are given close attention [46]. Distinct climatic characteristics are due to geographical location, the topography of the area, the nature of water cover, pressure distribution points, and air masses that prevail in a geographical area [47]. Therefore, complete geo-climatic data such as GPS-based coordinates, humidity, temperature and other relevant information is recorded with each sampling attempt. A representative sampling strategy can be adopted to sample multiple times from the given indoor environment for a valid statistical inference later during the phase of data analysis. A vacuum-based sample collection device is more appropriate to obtain samples from various locations such as households, car cleaning shops, vehicles, companies, etc. Buildings are varied in multiple characteristics; they vary for instance in location, building materials, purpose and maintenance. Furthermore, collection of samples within the built environment may rely on distinct collection matrices (dust, surface, air) and materials (wipes, filters, swabs, etc.) [48]. It is noteworthy to mention that sampling techniques are modified and adapted to the purpose of study and research focus. Sampling with vacuum filtration techniques are considered more appropriate for suspended dust than settled dust to obtain time-resolved insight into the indoor air quality effects. Approximately 50–100 g of the settled dust is a reasonable amount to perform various analyses related to the assessment of dust health effects. The samples are normally collected in sterile polythene sampling bags and stored at 4 °C for future processing.

3.3. Comprehensive Assessment of the Chemical Basis of Dust Particles and Surface Chemistry

Assessment of the chemical composition of particulate matter collected from indoor environments will help us to understand the underlying health effects on the occupants. Likewise, determining the chemical composition of contaminants also assists with decontamination efforts, as well as improving the traceability of irritants in the indoor environment. Understanding and identifying the chemical nature of dust particles helps to trace the origin and aids in the cleansing or treatment of the source of the dust particles in an indoor environment. Besides chemical composition, the size and volume of the dust particles is another important consideration for indoor dust elimination (cleansing). The collected samples can be screened for the presence of PM 2.5 to PM 10, as particulate matter. This will help to characterize specific macroscopic and microscopic contaminants that can then be selectively controlled or removed from a dusty environment. Analytical procedures are applied to determine the concentration of heavy metals, ions and radicles. Moreover, a scanning electron microscope (SEM) will be used to physically observe the complexity of the dust and associated pollutants and microbes. The metabolic nature of the particles can be assessed as dust particles meet food materials which provides ideal growth conditions for the microbes during high humidity, high temperature and indoor air circulation. To get a metabolic insight into the dust, high-performance liquid chromatography (HPLC) can be applied for common metabolites that may meet dust from food material and human subjects. However, to get a comprehensive impression of the dust metabolites, gas chromatography–mass spectrometry (GC-MS) can be applied to map the metabolic profiles of the dust in various built environments. Optical microscopy at high magnification can also be used to assess the composition of the dust sample. SEM coupled with energy dispersive X-ray spectroscopy (EDX) can be instrumental in identifying dust and debris particle shape, size and surface elemental composition. X-ray fluorescence (XRF), atomic absorption spectroscopy (AAS) and ICP-MS determine dust composition and levels of trace metals down to parts per billion (ppb) (Figure 1). Likewise, ion chromatography can be deployed to measure the concentration of major anions such as fluoride, chloride, nitrate, nitrite and sulfate, as well as important cations such as lithium, sodium, ammonium, potassium, calcium and magnesium in the aqueous phase of the dust particles in the range of parts per million (ppm). The identification of individual components in dust is carried out with SEM in order to obtain high-resolution imagery of the dusty indoor atmosphere by applying image analysis (Figures 1 and 2). Likewise, determination of fiber types in dust can be determined by applying optical microscopy and SEM (Figure 1).

3.4. Determination of Dust Allergens in Indoor Dust Samples and Assessment of Allergic Responses in Occupants Exposed to Dust Mite Allergens

Currently, clinicians diagnose allergies based on their patients' symptoms, medical history of allergic reactions, IgE blood test, basophil activation test (BAT), atopic patch tests and skin prick test [49]. In vitro techniques such as ELISA is a method of choice employed to detect these allergens from dust samples. It can detect both total and selective IgE levels. The principle of ELISA is that it uses pairs of monoclonal antibodies (mAbs) directed against non-overlapping epitopes on the allergen molecule or it binds mAb and polyclonal rabbit antibodies in order to facilitate the detection process. Though ELISA offers quantitative assessment of indoor air quality, its application in large epidemiological studies is limited owing to the fact that the protocol is time-consuming, as separate tests are required for each allergen tested. Moreover, applying this method to large-scale environmental population studies where multiple allergens are tested is costly and involves many working hands, and the chances of technical errors are also high. ELISA is a better choice than the radio allergen sorbent test (RAST), an in vitro test for the detection of the serum IgE of the patient bound to the allergen and detected using radiolabeled anti-IgE antibodies [50]. Another option is the employment of a fluorescent multiplex array. In this method, beads with unique ratios of internal fluorescent dyes coupled to allergen-specific antibodies are thoroughly mixed with a sample that binds the allergen (in the dust) of interest. A biotinylated detection antibody is added, followed by a streptavidin-conjugated fluorophore. The beads are read by an instrument equipped with lasers to identify each bead set and quantify the fluorescent intensity arising from the bound allergen. In order to determine the concentration of allergens in the sample, the fluorescence intensity for each allergen is compared to a newly established standard curve. Multiple antigens can be detected on a single slide using microarrays such as single-plex (ImmunoCAP) and multiplex (ImmunoCAP ISAC) assays. ImmunoCAP is a commercially available immunosorbent allergen chip (ISAC) that can determine the complete allergen sensitivity profile of an individual. The MeDALL chip microarray can be used to monitor IgE and IgG reactivity profiles to detect and quantify more than 170 allergens [51]. The cellular response induced by dust allergens can be studied by employing a mouse model of chronic allergy. Regulation of gene expression has been shown to play a pivotal role in the modulation of toxic pathways, and in this regard, gene expressions levels have the potential to act as biomarkers. Therefore, studying the modulation of a gene expression profile identified using whole genome expression techniques, such as microarrays or NGS-based expression analysis using blood RNA, and the analysis of gene expression networks by ingenuity pathway analysis (IPA) needs to be validated. Different cell lines such as the A549 alveolar epithelial cell line, lymphocytes, swine kidney cells and human bronchial epithelial (NHBE or BEAS-2B) cells can be used to monitor the effects of dust allergens in indoor and outdoor environments [37]. It is noteworthy to mention that nine out of 13 reported cytotoxic effects, with a reduction in cell viability, inflammation (one), and oxidative stress (one) being some of the complications identified. Pro-inflammatory responses were also recurrent (five out of 13), with a singular study revealing pro-inflammatory responses in airway epithelial cells and others revealing the production of TNFα. Genotoxicity was also observed along with DNA damage in lymphocytes. Another study reported the buccal micronucleus cytometry (BMCyt) assay as a good, non-invasive biomarker of cyto-genotoxicity in target organs impacted by dusty environments. Therefore, single assays are not predictive or reliable when it comes to assessing the health effects of dust allergens. The development and assessment of multiple assays would be desirable for a comprehensive understanding of dust allergens [52].

3.5. Assessing the Microbial (Bacterial and Fungal) Communities Confined to the Indoor Environment

As discussed, indoor dust is a habitat for many fungal and bacterial communities. Some of these microbial communities can be cultured under standard laboratory conditions on appropriate culture media, whereas many of the microbial communities cannot be

cultured per se. Culture-independent techniques are more inclusive in mapping diversity, abundance and functions of microbial communities, while assessing their role in indoor environments. The application of high-throughput sequencing technology has increased the amount of metagenomics data pertinent to environments sites [53]. However, studies pertaining to microbial diversity and abundance in built environments are still not widely attempted. Continued technological advancement in high-throughput approaches (such as, new sequencing technologies, lengthening reads, increased sequencing depth) with frequently updated techniques, practices and analysis protocols (e.g., the publication of novel bioinformatic workflows) [54] have provided ample opportunities to dissect the abundance and diversity of environmental microbiomes. Yet, the perpetual developments in such technologies have further complicated meta-analyses procedures for indoor environment research. Complex building microbiomes limit the possibilities toward standardized methods owing to variations in the lifestyles of the occupants. We infer that meta-analyses within the built environment could be even more difficult than for other microbial studies in soil or human bodies. Understanding the degree of influence of factors such as the ventilation system, biogeography, building function and occupants priorities would limit any generalization made for the impact of microbiomes on human health in built environments [55]. Despite these impediments, meta-analyses involving next generation sequencing approaches are the most powerful tools to study indoor dust microbiomes.

The dust particles are loaded with various type of microorganisms and chemical compounds. [56]. Different techniques have been developed to purify the total DNA from environmental samples [57–59]. There are two types of approaches used to lyse the cells: through chemical and/or enzymatic and/or mechanical lysis [59,60]. DNA yield of those methods has its own disadvantages in the lysis of certain microbial populations in different types of samples [60–62]. DNA extraction from dust samples can be followed by soil and sediment extraction methodologies [62]. DNA-based molecular biology tools have been widely applied thus far to microorganisms or microorganisms inhabiting the interface of soil and plants. It is noteworthy to mention that it is not only unculturable microbes that are probed in term of diversity while using DNA-based diversity assessments, but the molecular characterization of microorganisms cultured in vitro offers unprecedented opportunities [63]. In this regard, the first NGS-based study report on the assessment of soil biodiversity was published in 2006. A metagenomics analysis approach to dust samples was conducted to elaborate on the microbial population in Korea by using pyrosequencing by Roche (NGS) [62]. Dust samples from the International Space Station were reported and the microbial 16S rRNA gene and archaeal 16S rRNA gene were sequenced by using an Illumina Miseq device. Current NGS platforms sequence millions of reads in a relatively short period of time, and the sequencing capability in term of depth and accuracy improves every year. NGS technologies have been widely applied to conduct metabarcoding surveys on soil biodiversity conducted in diverse environmental contexts such as grasslands, agricultural fields and forests, but also deserts, and Arctic and Antarctic) habitats have been targeted [64,65].

Metabarcoding is a molecular approach based on the assumption that each OTU (operational taxonomical unit) can be unambiguously identified through an explicit DNA-sequence (barcode). The general strategy for dust microbial diversity consists of: (i) the isolation of DNA either from dust or dust-based microbes; (ii) the amplification of a specific DNA sequence selected for its taxonomic value; (iii) sequencing the relevant DNA stretch as amplicons; and (iv) execution of quality control to obtain the high-quality clean datasets, after which clean reads that can overlap with each other are assembled to tags and further aligned and clustered to OTU. Taxonomic classifications are assigned to an OTU representative sequence using the Ribosomal Database Project database. Analysis such as alpha diversity, beta diversity, differential species analysis, network and model prediction are carried out based on OTU profile table and taxonomic annotation results. [66]. The sequenced communities can then be subjected to various analyses. For instance, richness of samples (species number) and diversity (Shannon index), ordination of similarity (NMDS

with Bray-Curtis) and barplots of community composition. These, and similar analyses, give a clear picture of the microbial diversity of dust samples collected from various sites in a given geographical location. These diversity patterns can be compared with other regions to see if there is any significant overlap among the microbial consortia. Besides regional diversity, such analysis also sheds light on social stratification, community hygiene and the load of harmful microbes in the indoor air.

3.6. Sequencing of the Dust Virome for Viral Detection, Quantification and Diversity

Recently, state of the art techniques have been developed for the qualitative detection and characterization of previously unexplored or genetically variant viruses [67,68]. Such approaches have led to the detection, characterization and assembly of de novo full-length genome sequences from biological samples; therefore, the identification of new genetically distinct viruses as variants have been materialized [69]. These newer tools are now revealing the biological existence and genetic composition of the human virome and elaborating its intrinsic complexity and inter-individual variability in different contexts. The adult virome contains RNA and DNA viruses with a majority of bacteriophages [70], which affect human health by impacting the human microbiota [71]. Phages are the most abundant infectious agents on earth in most habitats [72] and due to their potential to kill bacteria, they play a key role in shaping the structure and function of a bacterial community [73–75] such as facilitation of gene transfer between species, production of toxin and virulence factors and encoding genes that may provide metabolic flexibility [74–77]. Due to a lack of proper tools in the past, virologists speculate about the existence of many viral sequences in bacterial genomes; however, the development of more robust methods has instigated endeavors to explore the virome in terms of health and diseases [67]. Some studies have developed efficient extraction procedures for viral nucleic acids from soil samples using a combination of approaches including ultrafiltration, ultracentrifugation, etc., for isolation of virus-like particles (VLPs) followed by shotgun sequencing [68]. Such modern approaches such as VLP isolation coupled with the traditional filtration and elimination of bacteria from VLPs has enabled us to use relatively simple viral backgrounds for metagenomic analysis, thus increasing the probabilities of miscalculations and increasing the chances of thorough characterization and novel discovery. In principle, the soil virus detection and characterization protocols can be applied to dust-bearing viruses with great implications for many human-infecting viruses such as COVID-19.

3.7. Natural Exposures to Indoor Dust or In Vitro Interaction between Dust and Human Cells/Animal Models and the Change in the Cellular Transcriptome

House dust is a complex mixture of regulatory compounds with signaling potential coupled with loaded microbes. Exposure to a dusty atmosphere in built environments invokes physiological responses in the human occupants. Inhalation or ectopic exposure to dust particles have increased the risk of multiple health problems such as respiratory diseases, asthma and cancer. Since dust is a highly complex entity with a plethora of elicitors (chemical and microbials), comprehensive human transcriptomics can be performed and compared among occupants of high indoor air quality vs. low indoor air quality. It is worth mentioning that there are several factors that may also differ when it comes to comparing the transcriptomic profiles of occupants based on their natural exposure to differential indoor air quality environments. Another possibility to assess the health impacts of indoor dust is to use an animal model in the study of dust effects. For this purpose, a mouse model can be investigated upon exposure to indoor dust samples. Female BALB/c mice (8–10 weeks) can be obtained and kept under aseptic conditions. To establish a model to investigate the effect of exposure of mice with chronic allergy to dust can be achieved by exposing the control mice to the sampled dust. In addition, the cellular response induced by dust allergens can be studied employing a mouse model of chronic allergy. Regulation of gene expression plays a pivotal role in the activation of toxic pathways, and in this regard, gene expression patterns have the potential to act as biomarkers. Furthermore,

comprehensive RNA-SeQ analysis can be performed upon exposing these animals to collected dust, in order to assess gene expression changes with better resolution. The third possibility is the treatment of dedicated cell cultures pertinent to respiratory pathways with collected dust samples. Human nasal mucosal cells, preferably HNEpC, keratinocytes, A549 alveolar epithelial cell lines, lymphocytes, swine kidney cells, human bronchial epithelial (NHBE or BEAS-2B) cells, T cells and B cells can be co-cultured with dust aqueous solutions. RNA isolation and subsequent sequencing of the dust-treated samples of these cells lines will generate a comprehensive dataset that can be further compared with the gene expression profiles available in public databases (such as GEO) to investigate diseases of a respiratory nature, and allergies or dermatitis of various types.

4. Conclusions

Unnatural built environments such as homes, offices and workplaces represent a modern ecological habitat for the species Homo sapiens. The indoor environment contains special niches for microbial life that have different physical and chemical features compared to the outdoor environment. Human subjects bring a great variety of the colonizing microbes, such as viruses, bacteria and fungi into these indoor habitats by shedding them from their bodies along with skin, hair follicles, nasal and oral discharges, or moving them indoors by means of their clothes and shoes or pets. Environmental factors of a biotic and abiotic nature are given special attention when it comes to sampling indoor dust for the assessment of its health impacts. Dust samples can be screened for the presence of PM 2.5 to PM 10 and analytical procedures can be applied to determine the concentrations of heavy metals, ions and radicles. Moreover, scanning microscopy can be used to physically observe the complexity of the dust and asso Unnatural ciated pollutants as well as the microbes. The metabolic nature of the particles will be assessed as dust particles meet food materials, which provides ideal growth conditions for the microbes during high humidity, high temperature and indoor air circulation. Antibody-based approaches are used to screen samples for allergens that may come from indoor insects and pets, and their secreted enzymes, in the dust, in order to assess the quality of indoor air.

Despite the advent of modern culture-independent molecular techniques such as NGS-based analysis of the 16S and 18S ribosomal RNA genes, and even sequencing of the whole genome, knowledge about indoor microbial communities, and their interactions with humans and indoor surfaces is still very sparse. To assess microbial communities confined to the indoor environment, microbial DNA can be isolated from the collected samples and can be subject to sequencing to better comprehend the level of microbial diversity and abundance in the collected samples. The identified microbial consortia can then be matched to human microbiomes and many other publicly available data sources that deal with indoor surface microbiota in various geographical locations. Lastly, the effect of indoor dust on human genes can be determined by treating relevant cell-lines with indoor dust samples for detailed RNA-SEQ analysis. This will catalogue the type of genes that are regulated upon exposure to dust. The expressed genes can be compared with various publicly available datasets pertaining to allergic responses, asthma and related skin complications.

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