Science of the Total Environment 756 (2021) 143553

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

An overview of methods of fine and ultrafine particle collection for physicochemical characterisation and toxicity assessments

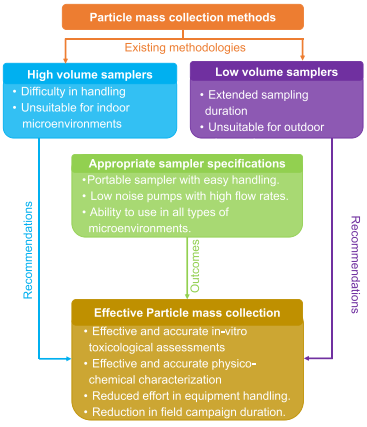
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HIGHLIGHTS

- PM mass collection for toxicological studies in various microenvironments is reviewed.
- Lower amounts of PM mass limit the workflows for toxicity assessments.
- Portable samplers with high flow rates are suitable for both indoor and outdoor measurement.
- Cyclone sampling technique effectively collects viruses in all environments.
- Predictive modelling aids in identifying hotspots for particle mass collection.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 August 2020
Received in revised form 8 October 2020
Accepted 2 November 2020
Available online 6 November 2020

Editor: Pavlos Kassomenos

Keywords:
Particulate matter

ABSTRACT

Particulate matter (PM) is a crucial health risk factor for respiratory and cardiovascular diseases. The smaller size fractions, ≤2.5 μm ($PM_{2.5}$; fine particles) and ≤0.1 μm ($PM_{0.1}$; ultrafine particles), show the highest bioactivity but acquiring sufficient mass for in vitro and in vivo toxicological studies is challenging. We review the suitability of available instrumentation to collect the PM mass required for these assessments. Five different microenvironments representing the diverse exposure conditions in urban environments are considered in order to establish the typical PM concentrations present. The highest concentrations of $PM_{2.5}$ and $PM_{0.1}$ were found near traffic (i.e. roadsides and traffic intersections), followed by indoor environments, parks and behind roadside vegetation. We identify key factors to consider when selecting sampling instrumentation. These include PM concentration on-site (low concentrations increase sampling time), nature of sampling

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https://doi.org/10.1016/j.scitotenv.2020.143553
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Ultrafine particles
Mass collection
Physicochemical characteristics
Toxicological assessments
Artificial intelligence

sites (e.g. indoors; noise and space will be an issue), equipment handling and power supply. Physicochemical characterisation requires micro- to milli-gram quantities of PM and it may increase according to the processing methods (e.g. digestion or sonication). Toxicological assessments of PM involve numerous mechanisms (e.g. inflammatory processes and oxidative stress) requiring significant amounts of PM to obtain accurate results. Optimising air sampling techniques are therefore important for the appropriate collection medium/filter which have innate physical properties and the potential to interact with samples. An evaluation of methods and instrumentation used for airborne virus collection concludes that samplers operating cyclone sampling techniques (using centrifugal forces) are effective in collecting airborne viruses. We highlight that predictive modelling can help to identify pollution hotspots in an urban environment for the efficient collection of PM mass. This review provides guidance to prepare and plan efficient sampling campaigns to collect sufficient PM mass for various purposes in a reasonable timeframe.

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1. Introduction

Particulate matter (PM) is of great concern due to its association with a variety of health impacts, such as respiratory and cardiovascular diseases (Dockery and Pope 3rd., 1994; Kappos et al., 2004; Heal et al., 2012). Numerous studies have demonstrated the association of airborne PM mass and respiratory admission in hospitals (Bell et al., 2014; Tecer et al., 2008). Other works have shown a strong association between the chemical composition and biological response, such as the influence of transition metals such as iron and manganese and their potential to cause oxidative stress by producing reactive oxygen species (Yuan et al., 2019; Charrier and Anastasio, 2015; Ghio and Devlin, 2001; Huang et al., 2003; Schlesinger et al., 2006). PM with an aerodynamic diameter of ≤10 μm (PM_{10}) can be inhaled by humans and deposited into the respiratory tract. Fine particles ≤2.5 μm ($PM_{2.5}$) - a subset of PM_{10} - show a much higher fraction of deposition in the alveoli (ICRP, 1994). Another subset, ultrafine particles (≤100 nm in diameter, also referred to as UFP or $PM_{0.1}$), contribute negligibly to the particle mass but significantly to particle number concentrations (PNC) and are thus measured by their counts (Kumar et al., 2010, 2014). UFPs are so small in their size that they can penetrate deep into the lungs and translocate to the other parts of the body (HEI, 2013). It is hypothesised that UFPs may have a greater potential for adverse health impacts compared with larger-sized particles (Kumar et al., 2014; WHO, 2013). Current ambient air quality regulations do not cover UFPs. They are also identified as one of the main contributing factors, after the fine PM fractions, responsible for adverse health effects observed at typical outdoor levels (Stone et al., 2017). UFPs, including the organic material attached to them, can cause acute respiratory and cardiovascular effects (Ahmed et al., 2020; Li et al., 2016), due to their large surface area, ability to generate reactive oxygen species (ROS), surface reactivity and their chemical composition of semi-volatile organic compounds. These effects induce potential cytotoxicity and inflammation in cells and need to be studied for their epidemiological effects on respiratory cells. Apart from UFPs, their larger counterparts such as $PM_{2.5}$ can also elicit varied biological responses, such as inflammatory responses causing COPD (chronic obstructive pulmonary disease) and other pulmonary infections. Furthermore, UFPs cause reactive oxidative stress on cells, effects on sensory receptors and neurons, and various other biological responses (Kreyling et al., 2006; Kodavanti and Watkinson, 2005; Gavett et al., 2003). Generally, PM toxicity refers to the absorption and distribution of chemical components of particles, causing adverse health effects throughout the body other than carcinogenicity (Rundell et al., 2007) and mutagenicity (Bennett and Brown, 2005). Table 1 illustrates the varied biological

Table 1
Summary of existing reviews on the physicochemical and toxicological studies on fine and ultrafine particles.

Study focus and topic areas covered	Author (year)
● Toxicological effects of $PM_{2.5}$ to understand the molecular pathways generating oxidative stress in particles and their transmission to the target organs.	Leni et al. (2020)
● Summarised the association of PM with pediatric asthma and reviewed their fundamental molecular mechanisms.	Xu et al. (2020)
● Toxicological studies of PM obtained from whole diesel exhaust and particle filtered exhaust involving animal and human exposure studies.	Weitekamp et al. (2020)
● The physico-chemical composition, exposure (in vivo) and toxicological (in vitro) studies on PM from underground railway emissions of PM and its effects on human health.	Loxham and Nieuwenhuijsen (2019)
● Summarised the time series and cohort study on $PM_{2.5}$ on their effects of cardiovascular and chronic respiratory effects and other health endpoints.	Yang et al. (2019)
● Summarised the in vivo/*vitro* studies on the immunotoxic effects of $PM_{2.5}$ exposure to pulmonary cellular effectors.	Wei and Tang (2018)
● Inflammatory health effects from various types of PM sources and different environments and their pathogenesis of diseases.	Wu et al. (2018)
● Collected data for studies on the divergent responses to PM exposure and the effects on different types of cell deaths and their assessment in PM-exposed models (in vivo).	Peixoto et al. (2017)
● Toxicological studies of $PM_{2.5}$ (both in vitro and in vivo) and its biological response in humans.	Mohammed et al. (2016)
● Toxicological effects of PM comparing reactive oxygen species (ROS) production, inflammation and oxidatively damaged DNA in different experimental systems.	Møller et al. (2014)
● Methods to evaluate the various toxicological effects of PM on the respiratory, cardiovascular, and nervous systems using in vivo and in vitro experimental models.	Nemmar et al. (2013)
● Toxicological effects of PM from various sources, especially their components and bioreactivity of PM from traffic emissions.	Kelly and Fussell (2012)
● Studies relating to different PM sources and their effects on human health.	Stanek et al. (2011)
● Studies on ambient PM and the effects of PM on oxidative stress, activation of antioxidant defences, inflammation and toxicity.	Li et al. (2008)
● Studies linking the in vitro toxicological effects and mechanisms of PM from traffic emissions, focusing on PM size fractions $< PM_{10}$.	de Kok et al. (2006)

responses that are reported in various review articles and a detailed summary of relevant in vitro and in vivo studies are provided in Supplementary Information (SI) Section S1. Studies reveal that the toxicological effects of the collected particles vary. The factors contributing to these varied effects are particle size distribution, particle shape and their specific chemical constituents and composition within the mixture (Mirowsky et al., 2013; Yuan et al., 2019; Lippmann and Chen, 2009; Ahmed et al., 2020; Min et al., 2011; Fuentes-Mattei et al., 2010; Ling and van Eeden, 2009). The studies reviewed in Table 1 suggest that PM cytotoxicity or genotoxicity depend on the heterogeneous chemical composition of PM and the toxicological outcomes are highly variable, which could be attributed to different source environments and emission sources (Gualtieri et al., 2011; Camatini et al., 2011).

Hence, there is an increasing interest in investigating the toxicity of these particles using in vitro cellular studies. Such a cellular analysis requires the collection of sufficient particle mass, which is challenging, especially for UFPs with negligible mass (Kumar et al., 2014). Hence, the focus of this review is on $PM_{2.5}$ and UFPs and the methods of collecting their mass in quantities that are sufficient for their physicochemical characterisation and testing in vitro cellular studies.

In order to assess the physico-chemical properties of PM for toxicological and epidemiological studies, various sampling methods have been developed. Generally, the collection of PM with a sufficient quantity for in vitro and in vivo toxicological studies can be impacted by various factors, including meteorological conditions, particle collection approaches and substrates used, to mention a few. There are numerous reviews on PM effects on cytotoxicity, mutagenicity, respiratory and other pulmonary disorders (Section S1); however, dedicated reviews of methods for collecting PM, especially fine and ultrafine particle sizes, for these toxicology studies, are few.

The review of past publications (Table 1) indicates a gap in PM collection strategies in a consolidated manner. This review aims to fill this gap by focusing on the methods used in PM collection for toxicological studies by exploring the various existing collection methods in indoor and outdoor environments. The main challenge is to recommend an efficient way - by reviewing the samplers and typical concentrations in different urban microenvironments - to collect enough mass of $PM_{2.5}$ and UFP particles for physico-chemical characterisation and toxicological assessments. Thus, the objective of this review is to: (i) provide a detailed quantification of particle concentrations in different microenvironments, (ii) review existing methods of particle collection and instrumentation, (iii) provide an effective way to collect sufficient amounts of particles, and (iv) make recommendations for PM collection methodologies for assaying toxicity.

2. Scope and outline

This article is based on the published scientific literature on PM collection methods used for physicochemical characterisation and toxicity assessments and related instrumentation for PM concentrations and mass collection on filters. The scope of this review is limited to stationary measurements of $PM_{2.5}$ and UFPs, methods of monitoring their concentrations and mass, as well as associated physicochemical and toxicity analyses. We also include a review of the collection of airborne viruses, given the ongoing SARS-CoV-2 pandemic. Other microorganisms such as bacteria's and pathogens are beyond the scope of the current paper. Also, mobile and personal sampling (particularly equipment used to evaluate occupational health hazards) and real-time monitoring for PM or associated human health effects are beyond the scope of this review.

We carried out a systematic literature search to include the most suitable scientific papers related to $PM_{2.5}$ and UFPs, urban microenvironments, toxicological studies, physicochemical characterisation, human exposure to PM, predictive modelling and certain other related keywords. The scientific databases such as Web of Science, Scopus, Science Direct, PubMed and Google Scholar were searched in different keyword combinations such as PNCs, mass concentrations of PM, high volume samplers, fine particulate samplers, gravimetric measurements, cell culture of PM, microenvironments, toxicity, cell models for PM exposure, ROS, oxidative stress, cytotoxicity, genotoxicity, gene expressions, lung epithelial cells, COPD, respiratory disorders, inflammatory responses, pulmonary fibrosis and A549 cells. The search procedure was repeated several times using Google and the relevant keywords. In all scientific databases, the search term combinations were modified depending on the success rate and precision of the outcome. This search resulted in over 1800 papers. The abstracts, experimental methodologies and conclusions of these collected papers were studied and approximately ten percent (~170) of them were found to be suitable for discussion in this review. The discussed articles also include relevant concepts and additional articles that have underpinned our previous research. The literature relating to $PM_{2.5}$ and UFPs toxicological and physicochemical assessments is voluminous and increasing rapidly. Hence, we considered English language articles published in peer-reviewed journals and reliable reports.

We start the review by providing a detailed synthesis of the typical concentrations in different micro-environments for the $PM_{2.5}$ and

ultrafine particle (UFPs) sizes (Section 3). The available particle collection methods and the instrumentation used for the toxicological studies of PM for both the particle sizes are discussed (Section 4), in addition to the need for the physicochemical characterisation (Section 5), considerations for $PM_{2.5}$ and $PM_{0.1}$ mass collection for in vitro toxicity studies (Section 6), and the ways to optimise the particle collection (Section 7). The article concludes with a final section summarising these results with concluding remarks, highlighting knowledge gaps and drawing future recommendations for obtaining sufficient amounts of PM for toxicity studies (Section 8).

3. Typical concentrations in different microenvironments

The stationary microenvironments, such as indoor, roadsides with/without green infrastructure, traffic intersections and parks broadly represent areas where urban populations spend most of their time. In general, the exposure levels in any microenvironment are indicated by the levels of PM mass concentrations for fine or coarse particles (e.g., Buonanno et al., 2011; Klepeis, 2006; Pacitto et al., 2018; Kumar et al., 2018) and particle number or surface area concentrations for UFPs (Franck et al., 2011; Giechaskiel et al., 2009; Knibbs et al., 2011). The concentration levels discussed in the review are obtained from published studies that have adopted filter, optical or sensor-based sampling approaches for estimating the concentration of $PM_{2.5}$ and UFPs in different urban microenvironments. Although the instruments discussed in Table S1 might employ different approaches to estimate concentration values, our focus here is mainly to understand the typical concentrations in these urban microenvironments and the instruments using varied measuring mechanisms are discussed in Section 4.2. Concentration levels in each microenvironment depend on factors such as the types of activities, including resuspension of dust, traffic density and vehicle idling activities in the outdoors (Pant and Harrison, 2013), cooking (Morawska et al., 2013), emissions from printers (Voliotis et al., 2017) or other activities in the indoors of homes and offices, site morphologies (Fujita et al., 2014) that could vary at different geographical locations. For instance, the pollution levels in countries such as China and India are different to those in the UK, Europe or USA, due to factors such as the type and usage of vehicles, road planning, traffic levels, fuel type and emission standards. Hence, we review the PM concentration levels in different countries and discuss the characteristics of different microenvironments in them. It allows us to understand the differences in mass and particle number concentrations of particles in fine and ultrafine particles and obtain typical average concentrations to aid the estimation of sufficient particle mass collection by an appropriate sampler in a reasonable time frame and enabling physicochemical and toxicity assessments.

The indoor microenvironment is of great importance since most of the population spend around 80% to 90% (Li et al., 2017; Delgado-Saborit et al., 2011; Leech et al., 2002) of their time indoors, such as in homes, offices, and automotive cabins. The factors such as built environment, ventilation, insulation and other indoor activities (Massey et al., 2016; Habil et al., 2015; Tan et al., 2013; Wayne and Roberts, 1998) affect the level of personal exposure in an indoor microenvironment. Hence, we consider indoor microenvironment as part of this review. Furthermore, microenvironments close to traffic emissions, such as traffic intersections and roadside areas are often covered, since these microenvironments are exposed to elevated levels of $PM_{2.5}$ and UFPs (Ö zkaynak et al., 2008; Fujita et al., 2007). The PM concentrations in traffic-related microenvironments were found to be higher, due to the contribution from the exhaust (tailpipe) and non-exhaust (e.g., tyre and brake wear, re-suspension of dust) emissions of vehicles; (Pant and Harrison, 2013; Kumar et al., 2013). The Non-exhaust emissions mainly generate coarse particles ($PM_{2.5–10}$) as opposed to the exhaust emissions which contribute largely to fine particles ($PM_{2.5}$) (Kam et al., 2012; Thorpe et al., 2007). Conversely, emissions arising from the tailpipe of on-road vehicles are the major contributors to UFP emissions in cities (Zhu et al., 2002; Fujitani et al., 2012; Kerminen et al., 2007; Wang et al., 2008; Weijers et al., 2004).

Parks have also been considered since urban vegetation reduces PM concentrations (Paoletti et al., 2011; Brack, 2002; Tiwari and Kumar, 2020), increases lung function (Sinharay et al., 2018) and benefits mental health and wellbeing during any physical activity (Kumar et al., 2019). This microenvironment is important for various community and physical activities such as running, walking and exercise (Cohen et al., 2007; Bedimo-Rung et al., 2005; Ulrich et al., 1991) and people of all ages spend considerable amounts of time in parks with the confidence of less air pollution (Lin et al., 2014). A recent review published on selecting appropriate green infrastructure (GI) to mitigate air pollution (Barwise and Kumar, 2020) suggests a predominant role of GIs in reducing roadside exposure to pollution. The magnitude of air pollution reduction is demonstrated by field investigations and computer simulations using airflow models (Tiwari and Kumar, 2020; Tiwari et al., 2019). Hence, PM concentration levels, size distribution and chemical composition in these microenvironments are of growing interest. We consider the roadside microenvironments adjacent to GI in this review since these potentially mitigate the impacts of air pollution exposure (Kumar et al., 2019; Abhijith and Kumar, 2019; Abhijith et al., 2017; Ottosen and Kumar, 2020).

3.1. Fine particles

Table S1 shows a summary of $PM_{2.5}$ concentrations measured in various studies in different microenvironments. The indoor environment represents different atmospheres, such as homes, office spaces, schools, local shops, malls and food courts (Tan et al., 2020; Kelly and Fussell, 2019; Rivas et al., 2014). In general, the source of indoor particulates is associated with anthropogenic activities, intended usage of the space, type of activity being carried out and their air-exchange rate by natural or mechanical ventilation via HVAC systems. Among these, indoor PM concentrations are governed primarily by factors such as cooking activities (especially involving charcoal) (Lim et al., 2012), smoking (Gurung et al., 2016; Lai et al., 2004), office laser printers (Tang et al., 2012), resuspension of particles following human use of the space (Nazaroff, 2004) and poor ventilation conditions (Matic et al., 2017; Chatzidiakou et al., 2015). The levels of PM concentration might increase or decrease according to the intensity of these activities. A detailed literature review suggests that indoor concentrations are mostly measured using cascade impactors and, in some studies, personal monitors, continuous monitoring devices such as Harvard compact cascade impactor, Sioutas personal samplers, GRIMM and any kind of wireless sensor system (Table S1) and passive sampling technique (Canha et al., 2014). The average level of indoor $PM_{2.5}$ concentrations, based on the studies summarised in Table S1, were found to be $18.1 \pm 6.9\ \mu g m^{-3}$ (Table S1). Contrarily, the concentrations of PM at traffic-related microenvironments, such as roadside and traffic intersections, depends on the density and the type of vehicles, time of sampling (i.e., peak versus off-peak hours) (Piotrowicz and Polednik, 2019; Shakya et al., 2017), vehicle idling times and traffic signals (Wang et al., 2008). The major contributors of PM from traffic-related microenvironments are primarily from passenger cars (Iijima et al., 2007), heavy-duty vehicles (HDVs), light-duty vehicles (LDVs) (Robert et al., 2007a, 2007b) and buses (Rivas et al., 2017). These traffic-related concentrations are measured by a range of instruments such as high-volume samplers (Cui et al., 2016; Lawrence et al., 2013), fine particle samplers (Jain et al., 2020) or mini volume samplers (Tobler et al., 2020). Based on the studies summarised in Table S1, their concentration levels can be $27.5 \pm 19.8\ \mu g m^{-3}$ and $33.1 \pm 5.3\ \mu g m^{-3}$ for roadside and traffic intersections, respectively (Fig. 1). Furthermore, park microenvironments located at a moderate distance from the urban activities are also impacted from local sources such as vehicular combustion, resuspension and various other cooking activities in an urban area (Harrison et al., 2001) and they were found to possess PM concentration

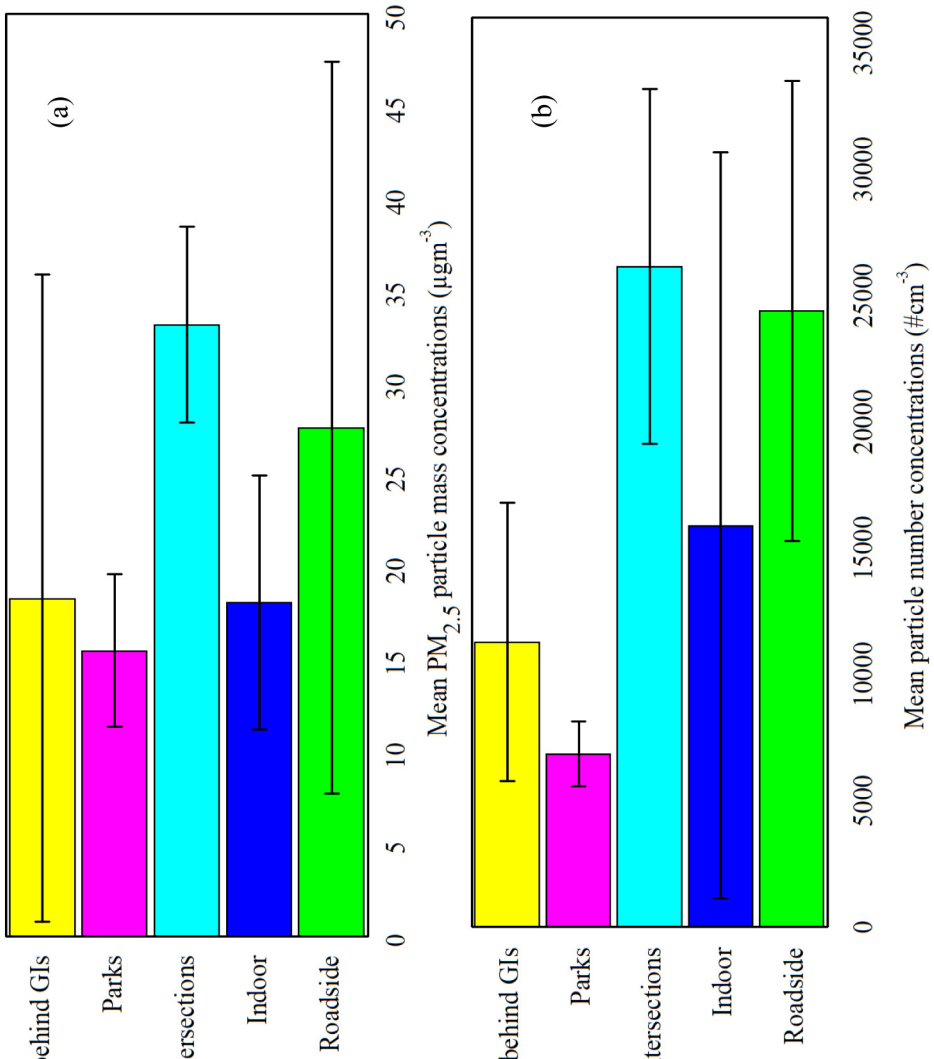


Fig. 1. Typical mean particle mass concentrations of (a) $PM_{2.5}$ and (b) UFPs at different microenvironments in an urban area. Error bars represent the standard deviation from Table S1 for (a) $PM_{2.5}$, showing a higher deviation in roadside concentrations (n =*11*), followed by traffic intersections (n = 10), indoor (n =9), roadside with GIs (n = 4) and parks (n = 5). (b) UFPs from Table S2, showing a higher deviation in traffic intersections (n = 7), followed by roadside (n = 8) indoor (n = 9), parks (n = 9) and roadside behind GIs (n = 5).

levels of 16 ± 4 μgm^{-3} (Fig. 1). These park microenvironments were found to be using dichotomous sequential samplers, TEOM based samplers and $PM_{2.5}$ cyclone samplers (Table S1). Finally, the GI (foliated tree line and roadside hedges) microenvironments, which are an emerging architecture to reduce air pollution, involved continuous monitoring devices, such as GRIMM aerosol mass spectrometers, Dust Trak monitors and other continuous monitoring devices (Table S1). These GIs were found to reduce air pollutant exposure by up to 63% and respiratory deposited dose by 36% (Abhijith and Kumar, 2019; Aldabbous and Kumar, 2014). Based on the studies summarised in Table S1, typical $PM_{2.5}$ concentrations around the GIs microenvironments were found to range from 18 ± 17 μgm^{-3} (Fig. 1). In general, the $PM_{2.5}$ concentrations are measured primarily by using PM samplers which are equipped with a filtering medium, which serves both for gravimetric and other related chemical analysis. Also, the aforementioned concentration values are average values and they may vary according to various factors with respect to different microenvironments. However, this detailed collection of average concentrations build an understanding of the $PM_{2.5}$ levels in the respective microenvironments to select the appropriate sampler (discussed in Section 4) and provide guidance for how long is needed to collect PM for toxicological studies (Section 7).

3.2. Ultrafine particles

UFPs originate mainly from combustion activities (Costabile et al., 2019; Kumar et al., 2010; Morawska et al., 2013; Settimo et al., 2020) and are measured by PNC due to their negligible mass compared with larger diameter particles (Heal et al., 2012; Kumar et al., 2014; Slezakova et al., 2019). Table S2 presents a review of UFP concentrations in the different microenvironments. The factors affecting the typical concentrations are nearly identical for UFPs in most microenvironments (Section 3). The indoor concentration of UFPs have been measured using instruments such as the condensation particle counters (CPCs; particle size range 0.006–3 μm). Based on the studies summarised in Table S2, their concentrations were found to be 1.6 ± 1.4 × 10^4 cm^{-3} (Fig. 1). The traffic-related emissions for roadside and traffic intersections use scanning mobility particle sizers (SMPS; 0.005–3 μm) and condensation particle counters (CPCs; 0.006–3 μm) for UFPs measurements (Table S2) with concentration levels of 2.4 ± 0.9 × 10^4 cm^{-3} and 2.6 ± 0.7 × 10^4 cm^{-3} (Fig. 1) for sites in and around London (Table S2). Finally, the park microenvironments were found to possess concentration levels of 6.8 ± 1.3 × 10^3 cm^{-3} (Fig. 1) which are measured mostly by using instruments such as SMPS (Table S2). The concentration levels prevailing in the roadside microenvironments with GIs use fast mobility particle sizer (FMPS;), differential mobility spectrometers (DMS; 0.005–2.5 μm), GRIMM (0.25–32 μm) and PTrak model instruments (0.02–1 μm) (Table S2) for UFP measurements and their mean concentrations levels are 1.1 ± 0.5 × 10^4 cm^{-3} (behind hedges) (Fig. 1). The UFP measurements were mostly carried out using instruments such as the SMPS in these different microenvironments. Toxicological study assays require reasonable quantities of particle mass (i.e., in milligrams; Section 6), which is remarkably lower for UFPs compared with $PM_{2.5}$. Thus the sampling time using air sampling equipment has to be planned appropriately, as discussed in the next section.

4. Methods of particle mass sampling

The PM collection strategies can vary according to the type of microenvironments. For instance, high volume samplers with heavier pumps are challenging to use indoors (due to the unsuitability of the pump noise in an office space or home environment). Similarly, the use of mini-volume samplers with lower pump capacities can have disadvantages in traffic-related or polluted outdoor environments due to the higher PM concentrations, potentially requiring more frequent change in filtering medium. Hence, the following section discusses the available methods and instrumentation for the collection of $PM_{2.5}$ and UFPs mass.

4.1. Fine particles

The mass of $PM_{2.5}$ particles for physicochemical and toxicity assessments is usually collected by three different types of air samplers, as summarised as in Table 2. They fall under the following categories: (i) high volume samplers, which mostly have a single impactor corresponding to the particle size of interest (e.g., $PM_{2.5}$ samplers, fine particulate samplers, and chemical speciation samplers); (ii) mini volume samplers (with two impactors of particle sizes PM_{10} and $PM_{2.5}$); and (iii) Cascade impactors, which have multiple impactors to cover varied particle sizes (e.g., Harvard compact cascade impactors, HCCI). These samplers, in general, collect the $PM_{2.5}$ particles in quartz (Islam et al., 2019; Hong et al., 2017) and PTFE filters (Jan et al., 2020), whereas the HCCIs use a polyurethane foam (PUF) impaction substrates and Teflon filters which can be subjected to chemical characterisation (Demokritou et al., 2004).

There are challenges in using impaction-based approaches for size-fractionated PM sampling. Conventional impaction-based samplers use impaction substrates coated with oil and other adhesives to minimise particle bouncing. However, it is not possible to use such PM samplers to collect mg-level PM for toxicological studies due to the minimal loading capacity of hard impaction substrates (Lee et al., 2005). Demokritou et al., 2002 developed a series of impaction-based samplers using a “dry” porous substrate (PUF) which enables the collection of huge quantities of particles with no particle bounce. Such PUF-based

Table 2
Summary of the type of instruments used for collecting $PM_{2.5}$ for cell/lung toxicity analysis.

Sampler type (purpose)	Nominal flow rate	Author (year)
Harvard impactors (Toxicological studies on structural and functional effects on the liver of mice exposed to $PM_{2.5}$)	30 LPM	Tavera Busso et al. (2020)
High-volume air samplers (Thermo Fisher Scientific, Waltham, MA, USA). ($PM_{2.5}$ exposure on cytotoxicity, oxidative stress, genotoxicity and inflammatory responses)	1050 LPM	Chen et al. (2020)
Fine particulate sampler (APM-550, Envirotech) (Cytotoxicity and genotoxicity assessments)	16.6 LPM	Islam et al. (2019)
Fine particulate sampler (Model: APM 550) (Cytotoxicity and reactive oxygen species (ROS) assessments)	16.6 LPM	Jan et al. (2020)
High-volume air samplers (Thermo Fisher Scientific, Waltham, MA, USA). ($PM_{2.5}$ varied dosages (ranging from 0 to 100 μg/mL) on human bronchial epithelial (HBE) cells expressing CYP1A1 (HBE-1A1))	1050 LPM	Chen et al. (2019)
Medium-volume air sampler (AMAE Co., Ltd., Shenzhen, China). (Effects of region- and season--dependent differences of $PM_{2.5}$ on cytotoxicity)	100 LPM	Song et al. (2020)
High-volume sampler (TH1000H, Wuhan Tianhong Intelligence Instrumentation Facility, China). (Metabolic responses of HepG2 cells to $PM_{2.5}$ exposure)	16.6 LPM	Ye et al. (2019)
Tianhong TH-150C $PM_{2.5}$ sampler (Cardiac developmental toxicity assessments of $PM_{2.5}$ exposure due to excessive ROS)	100 LPM	Ren et al. (2019)
High volume sampler (TH-1000CII, Wuhan Tianhong, China), (Short-term exposure of $PM_{2.5}$ developing pulmonary fibrosis)	1050 LPM	Sun et al. (2020)
High-volume impactor sampler (HVIS). (Inflammatory responses of human bronchial epithelial (DHBE) to $PM_{2.5}$ exposure)	400 LPM	Sotty et al. (2019)
High-volume cascade impactor (Staplex TFIA-2). (Toxicological studies on BEAS-2B cells for varied durations).	700 LPM	Borgie et al. (2015)

impaction samplers have varied flow rates from 5 to 1000 LPM (Chang et al., 2013; Sarnat et al., 2003; Demokritou et al., 2002). Another challenge associated with impaction-based PM samplers is the efficient extraction from filters of particles for toxicological studies without altering their physico-chemical properties. Pal et al. (2015) developed a method which enables the extraction of PM from substrates without any such alteration.

Among these different types of samplers, high volume samplers are the most commonly used samplers in $PM_{2.5}$ collection for toxicological studies (Table 2). These are categorised as high-volume samplers due to their high flow rates, ranging from 16.7 LPM to 30 LPM that is usually denoted as 16.7 to 30 LPM, respectively (Jan et al., 2020; Deng et al., 2013; Fuentes-Mattei et al., 2010; Oh et al., 2011; Billet et al., 2007; Deng et al., 2006; Dagher et al., 2005; Demokritou et al., 2004; Hsiao et al., 2000). These samplers are highly efficient in collecting large quantities of particles in a short period of time with similar power requirements as domestic appliances. The high volume samplers do not employ multiple stages of impactors, as cascade impactors do, rather, they possess a single impactor with the particle size of interest ($PM_{2.5}$ impactor) which retains particles of ≤2.5 μm diameter on a filter holder equipped with either Teflon or quartz filters (Islam et al., 2019). These types of impactors are generally used in outdoor environments, usually for collecting particles from traffic-related (such as roadside, traffic intersections) microenvironments (Table 2). Certain other toxicological studies have used the cascade impactor methodology, especially the HCCI with four impactor stages corresponding to the PM_{10}, $PM_{2.5}$, $PM_{1.0}$, and $PM_{0.1}$. This impactor can fractionate by size and collect large amounts of particles since this sampler uses the inert PUF substrates (without adhesions) in each stage with a backup filter (Teflon) at the end (Bello et al., 2009). These types of samplers can collect both $PM_{2.5}$ and $PM_{0.1}$ (UFPs) effectively (Pal et al., 2015). Additionally, these samplers can be used both in indoor and outdoor environments due to their dry vacuum pump which generates less noise, while sampling a high-volume of air (900 LPM). Apart from the high-volume samplers and cascade impactors, certain studies use mini volume or low volume samplers with a flow rate of 5 LPM. These types of samplers are used in sites with very high concentrations of particles, where a study needs to collect particles continuously for a limited period (for months' time) during any form of traditional activity such as a winter heating period (Niu et al., 2017). Also, these mini volume samplers are well suited for an indoor environment, since they produce less noise in an office space and the sampling can be carried out without creating undue disruption to the office environment. However, due to the low flow rate, they tend to increase the sampling duration excessively.

Hence, for the collection of $PM_{2.5}$ particles, the selection of samplers plays a key role in managing the PM collection duration, with due consideration given to noise levels and concentration levels. For instance, a high volume sampler will be disruptive to use in an indoor environment to reduce the sampling time, whereas a mini volume sampler is ineffective in an outdoor environment with lower concentration levels. Thus, an informed selection of sampler is required, which satisfies criteria such as lower noise when used indoors and high particle collection efficiency with an adequate flow rate to collect the required amount of particles for physico-chemical and toxicological studies.

4.2. Ultrafine particles

Toxicological studies assaying UFPs could require a large amount of particle mass, usually up to milligram (mg) quantities (Demokritou et al., 2002). In general, UFPs for chemical characterisation and toxicological studies are collected using cascade impactors, with varied flow rates (Table 3). The samplers involving the collection of UFPs generally operate at a flow rate of 9 to 30 LPM (Gao and Sang, 2019; Weggeberg et al., 2019). However, there are high volume impactors such as high volume five stages plus back-up cascade impactor (HVFCI) (Badran et al., 2020) with a flow rate of 1100 LPM and the high-volume impactor sampler (HVIS) with a flow rate of 400 LPM (Sotty et al., 2019). These high volume impactors come with varied stages and predominantly use filter sizes of 8″ × 10″, (Badran et al., 2020; Borgie et al., 2016; Sotty et al., 2019). However, these impactors do not use collection substrates at each stage, instead, they use filters in the first stage to retain particles with a higher diameter and a backup filter at the last stage which collect particles of UFP sizes. The filters used may also vary, for instance, Teflon (PTFE), quartz fibre filters (Badran et al., 2020), polycarbonate filters (Sotty et al., 2019) and glass-fibre filters (Borgie et al., 2016) are used for toxicology and physicochemical characterisation studies. The HCCI plays a prime role in UFP collection for toxicological studies since this sampler, unlike the high-volume samplers, collects particles at all the impaction stages (Setyawati et al., 2020; Pennanen et al., 2007; Demokritou et al., 2002). Apart from the PUFs, the sampler is equipped with a final back up filter (which is usually Teflon) for collecting the particles ≤0.1 μm diameter. The HCCI is most suitable to collect particles in all microenvironments due to its high flow rate (30 LPM), easy installation, more compact design and comparatively low noise levels due to the dry vacuum pump. In addition, impactors such as Micro-Orifice Uniform Deposit Impactors (MOUDI; MSP corporation), are used extensively to collect PM mass for toxicological studies

Table 3
Summary of the type of instruments used for collecting UFP for in vitro and in vivo analysis of lung toxicity.

Sampler type (purpose)	Nominal flow rate (diameter range)	Author (year)
Portable current air sampler (AP BUCK LP-12) with Sioutas cascade impactor. (Physicochemical characterisation, lung cancer cell adhesion and metastasis)	9 LPM (0.25 μm–2.5 μm)	Gao and Sang (2019)
High volume five stages plus back-up cascade impactor (model 235, 68 m^3/h, Tisch, USA) (Toxicity effects of chemical determinants on cells)	1100 LPM (0.3 μm–2.5 μm)	Badran et al. (2020)
Cascade impactors (Moudi model 100-S4 Specials from MSP/Copley Scientific) (Chemical characterisation, source estimations and bioaccessibility investigations)	30 LPM (0.18 μm–18 μm)	Weggeberg et al. (2019)
High-volume impactor sampler (HVIS). (Inflammatory responses of human bronchial epithelial (DHBE) to $PM_{2.5}$ exposure)	400 LPM (0.18 μm–2.5 μm)	Sotty et al. (2019)
Micro-orifice uniform deposit impactor (MOUDI™; MSP Inc., Minneapolis, MN, USA). (Cardiopulmonary health response with a 6.3% increase in systolic blood pressure and diastolic blood pressure).	30 LPM (0.056 μm–18 μm)	Liu et al. (2018)
Dekati gravimetric impactor (DGI, DGI-1570, Dekati Ltd.). (Quasi-UFPs emitted from pure ULSD for cytotoxicity and gene expression studies).	70 LPM (0.2 μm–2.5 μm)	Zhang and Balasubramanian, 2018
MOUDIs (Model 110, MSP Corp., MN, USA). (UFPs exposure on oxidative stress, cytotoxicity and genotoxicity were investigated in A549 cells).	30 LPM (0.056 μm–18 μm)	Corsini et al. (2017)
MOUDIs (Model 110, MSP Corp., MN, USA). (genotoxic evaluation was performed on A549 cells (human lung carcinoma cells)	30 LPM (0.056 μm–18 μm)	Marabini et al. (2017)
High volume cascade impactor (Staplex TFIA-2).(Toxicological studies on BEAS-2B cells for varied durations),	700 LPM (0.3 μm–5 μm)	Borgie et al. (2015)
High-volume ultrafine particle (HVUP) sampler. (UFP exposure studies on Reactive Oxygen Species (ROS) formation).	400 LPM (0.18 μm–2.5 μm)	Campbell et al. (2014)

of UFPs (Table 3). These impactors generate a sampling flow rate of 10 to 30 LPM (Wang et al., 2013) and they use both Teflon and Quartz filters according to the focus of their studies on PM (either physicochemical characterisation or toxicological studies) (Shirmohammadi et al., 2016). Further, electrical low-pressure impactors (ELPI+) are being used to analyse the UFP size distribution and to measure/collect the mass of UFPs. ELPI+ makes real-time measurements of the particles in the 0.006–10 μm size range. It possesses a unipolar corona charger and a cascade impactor, in which the particles are classified into 14 size range fractions (Kuuluvainen et al., 2016; Järvinen et al., 2014). The ELPI+ also uses a vacuum pump and conducts particle sampling at a flow rate of 10 LPM. This is a high-resolution instrument which records data in the frequency of 1 Hz. The ELPI+ has two collection substrate types, they are (i) aluminium foils (used for gravimetric measurements) and (ii) polycarbonate filters (used for chemical analysis) (Dekati Ltd., 2011). Furthermore, personal samplers such as Sioutas cascade impactors (SCI) are used for collecting bulk mass of particles in five cut-sizes: 2.5 μm, 1.0 μm, 0.5 μm and 0.25 μm (Das et al., 2020). These impactors are generally used for collecting particles to conduct personal and occupational exposure and associated toxicological assessments (Das et al., 2020; Ramdhan et al., 2020). The above discussion suggests that the UFPs were sampled extensively using the cascade impactors mechanisms and the MOUDI impactors for many toxicological studies. However, the MOUDI impactor possesses a limited holding capacity when collecting particles in mg or gr levels. Certain cascade impactors, such as Berner low-pressure impactor (BLPI) and nano-BLPI with flow rates 24.8 LPM and 17.2 LPM, are usually unsuitable for collecting particles <0.32 μm. This is due to differences in impactor design (such as volumetric flow, vapour pressure etc.), particle bounce and evaporation (Fonseca et al., 2016). However, the HCCI demonstrated effective collection of UFP particles in both indoor and outdoor microenvironments (Setyawati et al., 2020; Demokritou et al., 2004). Thus, the HCCI can be favoured for collection of UFPs for use in toxicological studies. The ELPI+, on the other hand, provides high-resolution particle size distributions. However, the UFP mass collection by ELPI+ has not been reported extensively in the literature. Thus, an effective sampling design can be made by considering the concentration levels in the targeted microenvironment and an estimate of the equipment's ability to collect sufficient quantities of PM within a reasonable time duration.

4.3. Quality assurance

The quality assurance of the instruments used and the samples collected (PTFE and Quartz filters are most often used for collection of $PM_{2.5}$ and UFPs) should be examined to ensure the credibility of the sample collected. In the case of PTFE and Quartz filter samples, filters should be weighed using standard reference methods (US-EPA, 2017) before and after use for sampling. Field blanks can also be processed as a base reference with an acceptance range of ±30 μg (US-EPA, 2017), in addition, travelling blanks and laboratory blanks should be taken to evaluate any contamination of the filters during the study. Filters should be transported using petri dishes inside a cool box to prevent loss of volatile substances (Lagler et al., 2011). At the same time, the samplers should undergo a leak test and be verified for their volumetric flow rate in the field using traceable flow meters, before starting each study. Additionally, the temperature and pressure sensors should be checked to ensure that they remain within their variation limits (mostly ±1 K, ±10 hPa) (Lagler et al., 2011). The sampler inlets should be cleaned before any campaign and extra cleaning is required when the sampling is on the kerbside. Furthermore, quality assurance methods, such as equivalence tests (US-EPA, 2017; Lagler et al., 2011) can be performed by comparing the reference method samplers with candidate methods to ensure the credibility and acceptance of the sampler results during each study.

5. Physio-chemical characterisation of fine and ultrafine particulate matter

Physicochemical characterisation of PM is essential since the elemental composition, ionic composition and organic matter content will play a vital role in influencing the toxicological responses. In addition, the PM composition and hence bio reactivity will vary according to the type of the microenvironment they are collected from.

Although initial measurements of weight, number, concentration and size distribution of particles are carried out during sample collection (Sections 4.1 and 4.2), the measurements are often insufficient to characterise the full complexity of PM that is a mixture of various organic and inorganic compounds, which contribute different ways to the overall toxicity (Park et al., 2018). The constituents exhibit a wide range of sizes, morphologies and chemistries (bulk and surface), which are averaged with bulk methods.

To fully characterise these complex environments, a combination of complementary experimental techniques are needed. The concentration of ions, elements, and carbonaceous species (typically organic and elemental OC and EC, respectively) in PM are measured using ion chromatography (IC), graphite furnace atomic absorption spectroscopy (GF-AAS), inductively coupled plasma spectroscopy methods (ICP-OES/MS) and an OC-EC carbon analyzer, the thermal-optical transmittance (TOT) or thermal/optical reflectance (TOR), respectively (Liang et al., 2016; Qi et al., 2018). Together with TOT and TOR, thermogravimetric analysis (TGA) can be used to investigate the PM's oxidation and volatility characteristics. During TGA, samples are analyzed for changes in mass as a function of temperature, and this analysis can be performed under an inert and/or oxidizing atmosphere (Klingshirn et al., 2019). The thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) method has also been developed for the analysis of the organic fraction of $PM_{2.5}$ (Ding et al., 2009). The elemental composition of PM can also be examined via X-ray fluorescence (XRF) (Hamdan et al., 2018) and the identification of the crystalline phases by X-ray diffraction (XRD) (Satsangi and Yadav, 2014). These two methods are often used on fractionated samples to measure the size-dependent variations in PM.

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy compositional analysis (EDXS) can provide spatially resolved information about the morphology, diameter and diameter distribution, agglomeration state, elemental composition and crystallinity (via electron diffraction) of PM. The advantages of these techniques are that spatially resolved images and chemical maps can be acquired at the length scale of individual particles to measure variability in the chemistry and size distribution between particles and within individual particles in the PM mixture (Liati et al., 2016). Electron energy loss spectroscopy in the transmission electron microscope can also be used to map the valence of metals within PM to predict their oxidative potential.

5.1. Destructive versus non-destructive

Several techniques used to characterise the physical and chemical characteristics of PM require an amount of material for testing that can be difficult to collect practically. The minimal amount of material required and the information generated by a given method is often set by the physical principles of the instrumentation (e.g. the diffraction limit) or engineering constraints such as the detector sensitivity. Some methods are intrinsically destructive, e.g. ICP-MS and TGA and other theoretically non-destructive methods may cause some mass loss due to the sample preparation required or limit further applications, e.g. samples for TEM must be mounted on grids. Therefore, the order in which the PM is characterised must start with the non-destructive methods first before proceeding with destructive techniques such as ICP-MS for bulk analysis of trace metal content. In Table 4, a subset of commonly used characterisation techniques and required PM amounts are listed.

Table 4
Estimated PM mass requirements for selected experimental techniques.

Technique	Destructive (Yes/No)	PM	Approximate Detection limit	Approximate PM mass for single replicate
ICP-MS (elements)	Y	Processed	1 $ngmL^{-1}$ (Prudnikov and Barnes, 1998)	<100 μg
IC (ions)	Y	Processed	>50 $ngmL^{-1}$ (Creatchman, 1999)	<100 μg
TOR (carbon analysis)	Y	On filter	1 μg carbon/cm^2 (Chow et al., 1993)	10 μg/cm^2 Chow et al. (1993)
TGA (volatile fractions)	Y	On filter, processed	N/A	>2.5 mg Lapuerta et al. (2007)
TD-GC/MS (VOCs)	Y	Processed	50 ng (Chow et al., 2007)	>1 μg Chow et al. (2007)
XRF (elements)	N	On filter, processed	<10 ng mL^{-1} (Klockenkämper and von Bohlen, 1996)	100 μg Klockenkämper and von Bohlen (1996)
XRD (minerals)	N	On filter, processed	>40 μg (Sturges and Harrison, 1989)	>2 mg Sturges and Harrison (1989)
SEM (imaging)	N	On filter, processed	N/A – on principle individual particles can be examined	
TEM (imaging)	N	Processed	N/A – individual particles can be examined	

Note: ICP-MS (Inductively coupled plasma mass spectrometry); IC (Ion chromatography); TOR (Thermal/optical reflectance); TGA (Thermogravimetric analyzer); TD-GC/MS (Thermal desorber combined with gas chromatography coupled to mass spectrometry); XRF (X-ray fluorescence); XRD (X-ray powder diffraction); SEM (Scanning electron microscope); TEM (Transmission electron microscope).

5.2. Selection of characterisation techniques

The techniques selected for analysis depend on the mass of PM collected and the composition and mass of each component of PM within the mixture. For example, the low detection limit of techniques such as ICP-MS, TOR and TD-GC/MS analysis makes it feasible to characterise very small amounts of trace metals or gases in PM. It is, however, difficult to estimate the total mass needed for each technique, as PM contains a mixture of elements. In practice, 1 cm^2 punch-outs of a filter could be used for these analyses. However, these methods are destructive and provide information averaged from the whole sample. On the other hand, XRF offers the benefits of being both highly sensitive and non-destructive (Klockenkämper and von Bohlen, 1996) but does not have the sensitivity or detection limits of ICP-MS. Other non-destructive bulk methods, such as XRD can require milligrams of PM for analysis although techniques such as microXRD can overcome these stringent requirements (Sturges and Harrison, 1989). Finally, methods such as thermal gravimetric analysis (TGA) for measuring the pyrolysis properties of PM and their gaseous decomposition products, e.g. to give information about the % of organic and metals in a sample, are both destructive and require high amounts of PM (Lapuerta et al., 2007). In contrast to the methods described above, electron microscopy needs very small mass, has high spatial and energy resolutions for chemical analysis and mapping and provides information at the length scale of the individual particles within the mixture (Liati et al., 2016). However, the sampling volumes are very small, so these methods need to be correlated with bulk techniques to ensure that the collected data are representative of the whole sample collected.

5.3. Analysis of PM on, and extracted from, the filter to more accurately understand the cellular response

Some techniques such as SEM, Raman spectroscopy and XRF (Satsangi and Yadav, 2014; Hamdan et al., 2018) can characterise PM directly on the collection filters, so the PM is in its near-native state. Other on-filter methods include SEM-EDX, XRF, micro-XRD, which are used to image the size distribution, chemistry and phase of the particles. However, in practice, the filter will also influence the PM, principally by altering its aggregation state, so that it is no longer truly representative of ambient PM. The use of impactors for PM collection could also change the morphology of the PM during the high-speed collision processes. Many techniques, e.g. ICP-MS requires that the PM is removed from the collection filter, often using sonication, and is suspended in a liquid such as methanol mounted on a substrate such as a TEM grid. Extraction by sonication into organic solvents will change the properties of the PM, i.e. the original size distribution of the sampled PM and its chemistry. Extraction into aqueous solvents could also dissolve some of the inorganic fraction due to ion redistribution. Other issues with particle extraction include contamination from the filters, i.e. generation of broken PTFE fibres during sonication, preferential extraction of certain components of the PM (depending on the extraction medium is used), and contamination from glassware used for the extractions which will also alter the outcomes of cell viability assays. When the particles are transferred to cell culture media, they also need to be freeze-dried by lyophilization, and transferred into cell culture media which can also change the chemistry and aggregation state of the particles, so the PM must also be characterised in this media (Chen et al., 2013).

Depending on the separation method, filter type used and the properties of collected material, recovered amounts of PM can vary significantly. Some constituents might be lost or retained within the filter during separation. Gravimetric analysis of total $PM_{2.5}$ mass has shown

Table 5
Summary of common techniques/assays used to evaluate the cytotoxicity of $PM_{2.5}$ and $PM_{0.1}$.

Technique	Cell assessment	References
Light, electron or confocal microscopy	Morphological characteristics	Mukhopadhyay et al. (2007)
Trypan blue	Apoptosis	Strober (2015)
MTT/XTT assay	Necrosis	Fotakis and Timbrell (2006)
LDH assay	Morphological characteristics such as cell membrane damage	Van Meerloo et al. (2011)
Annexin-V-FITC propidium iodide	General toxicity	Rieger et al. (2011)
OP_{DTT} chemical assay	ROS	Park et al. (2018)
OP_{ESR} spectrometry		
DCFDA assay		
Ames test	Genotoxicity	
SCGE Comet assay	DNA damage	

Note: MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; XTT = 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilide; LDH = Lactate dehydrogenase; FITC = fluorescein isothiocyanate; OP_{DTT} = Oxidative potential Dithiothreitol; OP_{ESR} = Oxidative potential Electron Spin Resonance; DCFDA = 2′,7′-dichlorofluorescein diacetate; SCGE = Single Cell Gel Electrophoresis.

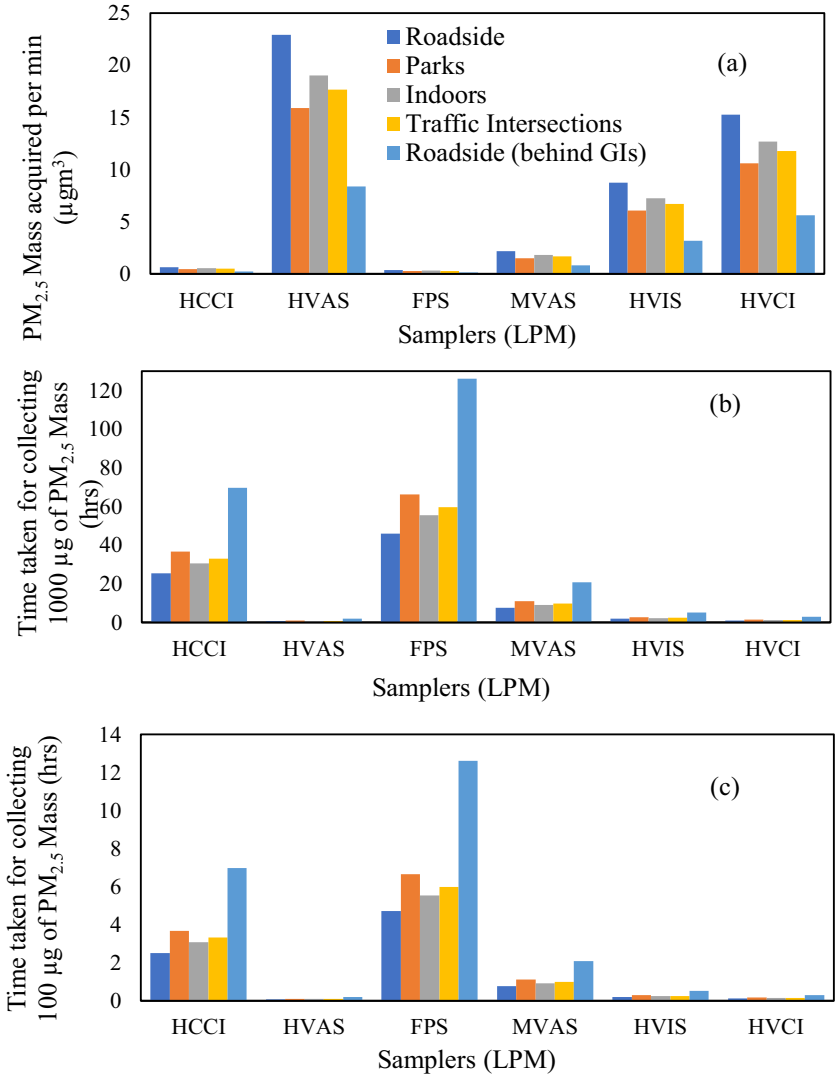


Fig. 2. Schematic representation of the various samplers (x-axis in all the figures) used widely to obtain (a) time required for collecting $PM_{2.5}$ particle mass per minute (y-axis). (b) To obtain 1000 μg of $PM_{2.5}$ particle mass for conducting toxicological studies and the time required for collecting them (y-axis). (c) To obtain 100 μg of $PM_{2.5}$ particle mass for conducting physicochemical studies and the time required for collecting them (y-axis). The values in the y-axis are calculated using the typical concentration in each microenvironment. The x-axis represents the various samplers used (from top) HVCI (high volume cascade impactor, 700 LPM), HVIS (high volume impactor sampler, 400 LPM), MVAS (medium volume air sampler, 100 LPM), FPS (fine particulate sampler, 16.6 LPM), HVAS (High volume air sampler, 1050 LPM), HCCI (Harvard compact cascade impactors, 30LPM).

that extractions are around 80% efficient. However, specific PM constituents like metals and organics have much lower extraction efficiencies (on average, 47 ± 22% and 25 ± 14%, respectively) (Roper et al., 2015). There is no perfect method for PM extraction, and available techniques need to be adjusted for the optimal extraction of constituents of interest.

These artefacts generated during the extraction of PM from filters make it challenging to relate the toxicological outcomes of cell culture experiments to the health effects of exposure to aerosolised PM direct from the air. Another issue is that collection/extraction-induced aggregation of the PM will change sedimentation and diffusion of the PM in the cell culture media as they settle on the cells and also cellular uptake which will alter the dose of PM delivered extracellularly and to the inside of the cells affecting their bioreactivity (Hinderliter et al., 2010). Electrostatic filters have also been used to collect PM, but these also induce aggregation, and there is evidence that these filters produce ozone as a by-product due to corona discharge (Rim et al., 2013), which is damaging to cellular health (Wiegman et al., 2014). In practice, it is not possible to fully overcome these issues to yield PM which are fully representative of ambient PM, but by fully characterising the PM at each stage of collection/extraction into the in vitro system, more accurate relationships can be drawn between PM properties and the cellular response.

6. In vitro toxicity of $PM_{2.5}$ and $PM_{0.1}$: considerations for particle collection

Numerous factors influence the mass of PM required for toxicological assays. These include the number of toxicological endpoints assays, number of cell donors (for primary cells) and cell repeats required to produce statistically significant results (which can range typically from $n = 3$ to $n = 6$). PM cytotoxicity and genotoxicity are correlated with PM concentration and exposure time. These studies must be carried out at increasing doses of the PM from 50 to 100 μgmL^{-1} up to 5 mgmL^{-1} and different time points (e.g. from 3 h up to 7 days), to measure the dose and time-dependent responses of the cells to the PM Islam et al. (2019); Jing et al. (2019). Thus, toxicological studies can require substantial amounts of mass (up to 10 mg) to assay the effects of the PM on cellular health, including assays of oxidative stress, inflammation, cell death and mitochondrial activity. Another issue, discussed in the previous section (Section 5), is that the PM has to be extracted off the filters and dispersed into solvents such as methanol, which could

Table 6
Time required to collect the required amount of $PM_{2.5}$ particles for toxicity and physicochemical assessments using the different types of air samplers utilized in the previous studies.

[a]Type of microenvironment (concentration; μgm^{-3})	[b]Instrument (Flow rate in LPM)	[c]Mass ($\mu gmin^{-1}$) (c) = (b × (60/1000) × a/(60))	[d]Time for physicochemical assessment (hrs) (100 μg of mass needed) (d) = 100/(a × b × (60/1000))	[e]Time for toxicity assessment (hrs) (1000 μg of mass needed) (e) = 1000/(a × b × (60/1000))
Roadside (28 ± 20)	Harvard impactors. (30 LPM)	0.7	2.5	25.5
	High-Volume air samplers (Thermo Fisher Scientific, Waltham, MA, USA). (1050 LPM)	22.9	0.07	0.8
	Fine particulate sampler (APM-550, Envirotech). (16.6 LPM)	0.4	4.7	46
	Medium-volume air sampler (AMAE Co., Ltd., Shenzhen, China). (100 LPM)	2.2	0.8	7.7
	High-volume impactor Sampler (HVIS). (400 LPM)	8.7	0.2	2
	High volume cascade impactor (Staplex TFIA-2). (700 LPM)	15.3	0.1	1.1
Parks (16 ± 4)	Harvard impactors. (30 LPM)	0.5	3.7	36.7
	High-Volume air samplers (Thermo Fisher Scientific, Waltham, MA, USA). (1050 LPM)	15.9	0.1	1.0
	Fine particulate sampler (APM-550, Envirotech). (16.6 LPM)	0.3	6.6	66.4
	Medium-volume air sampler (AMAE Co., Ltd., Shenzhen, China). (100 LPM)	1.5	1.1	11.0
	High-volume impactor Sampler (HVIS). (400 LPM)	6.0	0.3	2.8
	High volume cascade impactor (Staplex TFIA-2). (700 LPM)	10.6	0.2	1.6
Indoors (18 ± 7)	Harvard impactors. (30 LPM)	0.5	3.1	30.7
	High-Volume air samplers (Thermo Fisher Scientific, Waltham, MA, USA). (1050 LPM)	19.0	0.1	0.9
	Fine particulate sampler (APM-550, Envirotech). (16.6 LPM)	0.3	5.5	55.4
	Medium-volume air sampler (AMAE Co., Ltd., Shenzhen, China). (100 LPM)	1.8	0.9	9.2
	High-volume impactor Sampler (HVIS). (400 LPM)	7.2	0.2	2.3
	High volume cascade impactor (Staplex TFIA-2). (700 LPM)	12.7	0.1	1.3
Traffic Intersections (33 ± 5)	Harvard impactors. (30 LPM)	0.5	3.3	33.1
	High-Volume air samplers (Thermo Fisher Scientific, Waltham, MA, USA). (1050 LPM)	17.6	0.1	0.9
	Fine particulate sampler (APM-550, Envirotech). (16.6 LPM)	0.3	5.9	59.7
	Medium-volume air sampler (AMAE Co., Ltd., Shenzhen, China). (100 LPM)	1.7	0.9	9.9
	High-volume impactor Sampler (HVIS). (400 LPM)	6.7	0.3	2.4
	High volume cascade impactor (Staplex TFIA-2). (700 LPM)	11.7	0.1	1.4
Green infrastructure roadside (behind vegetation) (18 ± 17)	Harvard impactors. (30 LPM)	0.2	6.9	69.8
	High-Volume air samplers (Thermo Fisher Scientific, Waltham, MA, USA). (1050 LPM)	8.3	0.2	2
	Fine particulate sampler (APM-550, Envirotech). (16.6 LPM)	0.1	12.6	126.1
	Medium-volume air sampler (AMAE Co., Ltd., Shenzhen, China). (100 LPM)	0.8	2.1	20.9
	High-volume impactor Sampler (HVIS). (400 LPM)	3.1	0.5	5.2
	High volume cascade impactor (Staplex TFIA-2). (700 LPM)	5.6	0.3	3.0

[a] The typical concentration values from different microenvironments and their respective;
[b] Instrument flow rates used to calculate the required;
[c] μg of samples collected min^{-1}, 100 μg and 1000 μg for [d]physicochemical and [e]toxicological assessments are obtained from Table S1.

alter their physico-chemistry. Although alternative solvent-free methods, such as electrostatic collectors can be used to overcome this issue (Ning et al., 2008; Sillanpää et al., 2008). The identification of cellular modifications caused by PM is essential to understand its harmful effects and related respiratory and cardiovascular diseases. In this section, we describe and list the assays and techniques used to evaluate the toxicity of $PM_{2.5}$ and $PM_{0.1}$ to illustrate how the selection of toxicological endpoints being assayed determine the mass of PM needed.

Table 5 describes the different techniques that have been used to assess the cellular toxicity of $PM_{2.5}$ and $PM_{0.1}$ and mechanisms of cytotoxicity. Different assays can measure a number of endpoints including nuclear damage with DNA breaks, chromatin de-condensation, and organelle damage such as lysosome, endoplasmic reticulum and mitochondrial damage, leading cell to apoptosis and death. The ability of PM to generate reactive oxygen species (ROS) can be estimated using Dithiothreitol (DTT) chemical assay (OP_DTT) and Electron Spin Resonance Spectrometry (OP_ESR). Genotoxicity and nuclear damage

caused by PM can be measured using the Ames test and the Single Cell Gel Electrophoresis (SCGE) COMET assay (Park et al., 2018). In-depth mechanistic studies of which cell signalling pathways are affected by exposure to PM can be assayed using techniques such as polymerase chain reaction (PCR). Light histology, confocal and electron microscopies visually distinguish features of cellular damage, such as membrane blebbing and nuclear fragmentation. Confocal microscopy can be used to screen for mechanisms of cell damage such as oxidative stress and reactive oxygen species (ROS) generation inside fixed or live cells following exposure to PM. For example, fluorescent dyes such as Annexin-V-FITC and propidium iodide indicate cell death by apoptosis or necrosis (Peixoto et al., 2017) and cellular probes CellROX are activated in the presence of ROS to assess the formation of free radicals and Mitosox, to assess mitochondrial ROS. High-resolution chemical electron microscopy and super-resolution microscopy techniques can also identify which PM sizes and chemistries are internalised, the location and chemistry of the $PM_{2.5}$ and $PM_{0.1}$ inside the cells and subcellular effects after exposure to PM. These complementary approaches

Table 7
The time required to collect the required amount of UFP particles for toxicity and physicochemical assessments using the different types of air samplers utilized in the previous studies.

[a]Type of microenvironment (concentration; μgm^{-3})	[b]Instrument (Flow rate in LPM)	[c]Mass ($\mu gmin^{-1}$) (c) = (b × (60/1000) × a/(60))	[d]Time for physicochemical assessment (hrs) (100 μg of mass needed) (d) = 100/(a × b × (60/1000))	[e]Time for toxicity assessment (hrs) (1000 μg of mass needed) (e) = 1000/(a × b × (60/1000))
Roadside	Sioutas cascade impactor (9 LPM)	0.1	57.8	578.7
(3.2 ± 2.2)	High volume five stages plus back-up cascade impactor (Tisch, USA) (1100 LPM)	3.6	0.5	4.7
	Micro-orifice uniform deposit impactor (MOUDI; MSP Inc., Minneapolis, MN, USA) (30 LPM)	0.1	17.4	173.6
	High-volume impactor sampler (HVIS) (400 LPM)	1.3	1.3	13.0
	Dekati gravimetric impactor (DGI, DGI-1570, Dekati Ltd.) (70 LPM)	0.2	7.4	74.4
	High volume cascade impactor (Staplex TFIA-2) (700 LPM)	2.2	0.7	7.4
	High-volume ultrafine particle (HVUP) sampler (400 LPM)	1.3	1.3	13.0
Parks	Sioutas cascade impactor (9 LPM)	0.1	115.7	1157.4
(1.6 ± 0.9)	High volume five stages plus back-up cascade impactor (Tisch, USA) (1100LPM)	1.8	0.9	9.5
	Micro-orifice uniform deposit impactor (MOUD; MSP Inc., Minneapolis, MN, USIA) (30 LPM)	0.1	34.7	347.2
	High-volume impactor sampler (HVIS) (400 LPM)	0.6	2.6	26.0
	Dekati gravimetric impactor (DGI, DGI-1570, Dekati Ltd.) (70 LPM)	0.1	14.9	148.8
	High volume cascade impactor (Staplex TFIA-2)	1.1	1.5	14.9
	High-volume ultrafine particle (HVUP) sampler (400 LPM)	0.6	2.6	26.0
Indoors	Sioutas cascade impactor (9 LPM)	0.1	45.2	451.7
(4.1 ± 3.5)	High volume five stages plus back-up cascade impactor (Tisch, USA) (1100LPM)	4.6	0.4	3.7
	Micro-orifice uniform deposit impactor (MOUDI; MSP Inc., Minneapolis, MN, USA) (30 LPM)	0.1	13.5	135.5
	High-volume impactor sampler (HVIS) (400 LPM)	1.6	1.02	10.1
	Dekati gravimetric impactor (DGI, DGI-1570, Dekati Ltd.) (70 LPM)	0.3	5.8	58.0
	High volume cascade impactor (Staplex TFIA-2)	2.9	0.6	5.8
	High-volume ultrafine particle (HVUP) sampler (400 LPM)	1.6	1.0	10.2
Traffic intersections	Sioutas cascade impactor (9 LPM)	0.1	33.0	330.7
(5.6 ± 4.1)	High volume five stages plus back-up cascade impactor (Tisch, USA) (1100LPM)	6.3	0.3	2.7
	Micro-orifice uniform deposit impactor (MOUDI; MSP Inc., Minneapolis, MN, USA) (30 LPM)	0.2	9.9	99.2
	High-volume impactor sampler (HVIS) (400 LPM)	0.2	0.7	7.4
	Dekati gravimetric impactor (DGI, DGI-1570, Dekati Ltd.) (70 LPM)	0.4	4.2	42.5
	High volume cascade impactor (Staplex TFIA-2)	3.9	0.4	4.2
	High-volume ultrafine particle (HVUP) sampler (400 LPM)	0.2	0.7	7.4
Green infrastructure roadside (behind vegetation)	Sioutas cascade impactor (9 LPM)	0.1	185.2	1851.8
(1.0 ± 0.4)	High volume five stages plus back-up cascade impactor (Tisch, USA) (1100LPM)	1.1	1.5	15.1
	Micro-orifice uniform deposit impactor (MOUDI; MSP Inc., Minneapolis, MN, USA) (30 LPM)	0.1	55.5	555.5
	High-volume impactor sampler (HVIS) (400 LPM)	0.4	4.1	41.6
	Dekati gravimetric impactor (DGI, DGI-1570, Dekati Ltd.) (70 LPM)	0.1	23.8	238.1
	High volume cascade impactor (Staplex TFIA-2)	0.7	2.4	23.8
	High-volume ultrafine particle (HVUP) sampler (400 LPM)	0.4	4.2	41.6

improve understanding of the mechanisms, time-scales of PM-mediated potential toxicity and could identify which PM sizes and chemistries are most damaging.

7. Optimisation in particle collection

The knowledge of the average concentrations of the particles in the different microenvironments (Section 4) can enable the researchers to plan the sampling duration to collect the required amount of particle mass (Section 5) and their subsequent toxicological assessments (Section 6). These studies involving particle mass collection for toxicity and physico-chemical characterisation assessments demonstrate that the particles collection rate highly depends on the instrument's flow rate, site morphologies and the prevailing atmospheric conditions. The following section discusses, firstly, the necessary ways to collect the particles effectively. Secondly, it provides specific recommendations to increase the likelihood of obtaining the required quantities of particles.

7.1. Fine particles

The toxicological studies of $PM_{2.5}$ discussed in the previous sections reveal that these studies require particles in mg quantities. In general, the $PM_{2.5}$ particles were collected mainly using instruments with a medium volume flow rate of 16.7 LPM (Table 2). However, some studies use instruments with a high volume flow rate ranging from 100 to 400 LPM (Table 2). The average concentrations extracted from the previous literature can be combined with the instrument flow rate to plan the field campaign.

Furthermore, the maximum sample mass which can be collected on a single filter paper (be it PTFE or glass fibre or quartz or polycarbonate) should also be calculated; this data can work as a guide in changing the filter paper effectively, especially for continuous outdoor measurements. However, preliminary monitoring on the particle mass concentration has to be carried out (using optical instruments) as this helps in planning the duration to change the collecting medium (filter paper) which is highly dependent on the concentration in respective microenvironments.

The toxicological assessments are carried out in different doses such as 5, 10, 20, 50, 100 and 200 μgmL^{-1} (Islam et al., 2019) for estimating the exposure to different compounds and also for multiple experiments. These dosage proportions may vary according to the interest of the researcher and their objectives. However, commonly the dosage levels start from 1 to 5 μgmL^{-1} and extend up to 200 to 300 μgmL^{-1}. Hence, as noted above, there is a need to obtain particle mass in mg quantities for these assessments. Therefore, by considering the dosage levels mentioned in a wide variety of literature, it is evident that an average of 1000 μg (or 1 mg) of particle mass is at least required for conducting a toxicological study. The time required for collecting the mentioned 1000 and 100 μg for each microenvironment has been calculated using the typical concentrations obtained (Fig. 2) from various sources and have been tabulated in Table 6.

The schematic representation of the time taken by various samplers (Fig. 2) demonstrates that the samplers with a high flow rate ranging from 400 to 1050 LPM require the least time to collect the targeted $PM_{2.5}$ particle mass. In contrast, the fine particulate sampler (16.6 LPM) and Harvard impactors (30 LPM) require longer to collect the targeted mass. This is because the samplers with higher flow rate possess a larger pump capacity resulting in reduced sampling duration. However, these samplers with larger pump capacity are not suited for all types of microenvironments. For instance, samplers with larger pumps are usually not suitable for an indoor environment due to factors such as increased noise levels (noise pollution) creating an unpleasant ambience, vibrations on the floor, potential deterioration of air quality (if oil pumps are used), usage of space, and difficulty in transportation, etc. However, these factors need not be considered when high volume samplers are used in a roadside or traffic intersections microenvironment (generally outdoors). The fine particulate samplers and Harvard impactors possessing a lower flow rate compared with high volume samplers are much better suited for indoor conditions, especially the Harvard impactors since these impactors were mounted on a tripod and possess a higher pump capacity with less noise compared to fine particulate samplers. Also, the Harvard impactors collect a significant amount of particle mass when used outdoors (traffic intersections or roadside). However, the high-volume samplers will collect more mass, compared to these samplers.

7.2. Ultrafine particles

The mass concentrations of UFPs are significantly low when compared with $PM_{2.5}$. However, the mass of UFPs is collected using methodologies similar to $PM_{2.5}$ mass collection. The UFPs are generally collected using impaction sampling techniques coupled with high volume samplers ranging from 400 to 1100 LPM. However, samplers with flow rates ranging from 9 to 70 LPM are used, but mostly in indoor environments. The sampling time will generally be higher for UFPs due to their lower concentration, and high-volume samplers are usually preferred to reduce the sampling duration while collecting the required amount of particle mass. In general, the toxicological assessments require samples of large quantities (in mg), for multiple experimental investigations to study their exposure efficiently. Similarly, the physico-chemical characterisation of UFPs requires an average of at least 100 μg of particle mass for estimating elements, ions and carbon content present. Therefore, it can be said that an average of at least 1000 μg and 100 μg of particle mass will be required to conduct toxicological and physicochemical assessments, respectively. This particle mass quantity might increase or decrease according to the research requirements and study objectives.

The particle mass collection can be optimised by first calculating the sampling time required to collect the required particle mass. This can be calculated by conducting preliminary measurements using handheld optical instruments showing the mass concentrations of the UFPs. These mass concentrations can be used in calculating the sampling time for each of the commonly used samplers (Table 3). The obtained sampling time (Table 7) will provide an insight into the sampling duration for collecting the required mass of particles.

The schematic representation (Fig. 3) of the sampling time required by each sampler shows that SCI with a low flow rate of 9 LPM (Das et al., 2020; Ramdhan et al., 2020) requires a significantly longer sampling time than the HVFCI with its high flow rate of 1100 LPM (Badran et al., 2020). Both of these instruments employ a similar sampling technique but have a significant difference in air flow rate. The SCI is personal sampling equipment while the HVFCI is high-volume equipment, and they have their own merits and demerits in operation and handling. Likewise, the Harvard impactor with a flow rate of 30 LPM exhibits higher collection efficiency on PTFE filters and can efficiently collect the mass of UFPs. Hence, a sampler should be selected with a considerable flow rate as well as being suitable for all types of microenvironments for obtaining the particle mass in a shorter duration of time.

7.3. Particle mass collection with viruses

Microorganisms such as bacteria, fungi and viruses associated with atmospheric PM are referred to as bioaerosols (Chen et al., 2020), and they are transported in the air by means of dust or droplet transmission (Rahmani et al., 2020; Pan et al., 2019; Patterson et al., 2017). These bioaerosols can originate from human activities such as coughing or sneezing (Asadi et al., 2020; Dhand and Li, 2020; Jones et al., 2020; Lotfi et al., 2020), singing (Gregson et al., 2020; Hamner et al., 2020), talking or breathing (Asadi et al., 2019; Yan et al., 2018) or by specific mechanical processes (Sopeyin et al., 2020; Schünemann et al., 2020; Verreault et al., 2008). Airborne transmission of infectious bioaerosols

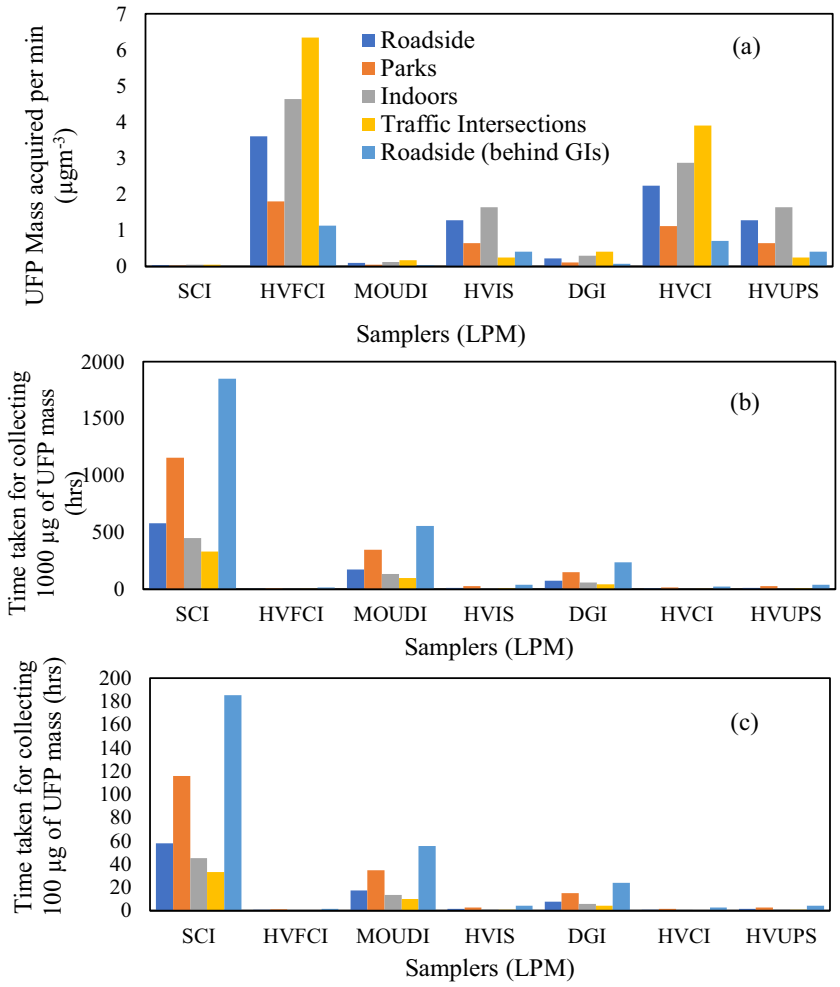


Fig. 3. Schematic representation of the various samplers (y-axis in all the figures) used widely to obtain (a) time required for collecting UFP particle mass per minute (y-axis); (b) to obtain 1000 µg of UFP particle mass for conducting toxicological studies and the time required for collecting them (y-axis). (c) To obtain 100 µg of UFP particle mass for conducting physicochemical studies and the time required for collecting them (y-axis). The values in the y-axis are calculated using the typical concentration in each microenvironment. The x-axis represents the various samplers used for particle mass collection (from top) HVUPS (high volume ultrafine particle sampler, 400 LPM), HVCI (high volume cascade impactor, 700 LPM), DGI (Dekati gravimetric impactor, 70 LPM), HVIS (high volume impactor sampler, 400 LPM), MOUDI (micro-orifice uniform deposit impactor, 30 LPM), HVFCI (high volume five stages plus backup cascade impactor, 1100LPM), SCI (Sioutas cascade impactor, 9 LPM).

comprising viruses, bacteria or fungi (de Sousa et al., 2020) can increase appreciably in enclosed environments that have a high density of people and limited ventilation (Kumar and Morawska, 2019). The airborne respiratory pathogen can pose serious health risks when transported through air from one place to another (Pan et al., 2019; Tang, 2009) such as in hospitals from patients exhaling infectious airborne pathogens (Chow and Mermel, 2017) or in restaurants (Kim and Lee, 2020; Li et al., 2020). The infection and spread of these airborne pathogens will be rapid in spaces with enclosed settings (combined with crowd) such as refugee camps, homeless shelters, slums and prisons. For example, WHO reported that the spread and infection of M. tuberculosis was found to be significantly higher in these similar settings (WHO, 2009). The recent coronavirus pandemic (or SARS-CoV-2 virus) is a severe concern. The infection risk of such outbreaks can be partly controlled by measures such as increased hand hygiene, indoor ventilation and disinfecting surfaces and 'cleansing' indoor air (Rahmani et al., 2020; Morawska et al., 2020). Coronaviruses are a group of related RNA viruses with large genomes which came to notice as causative agents of severe acute respiratory syndrome (SARS) in 2003. These respiratory viruses can be spread through respiratory droplets as seen with flu viruses (Drosten et al., 2003). Unlike other microorganisms such as bacteria, viruses cannot be collected on a general sampling media, since they have DNA or RNA genomes encased in a fatty liquid membrane and require a host cell in order to reproduce and cause infection (Asadi et al., 2019). The sampling of airborne viruses in a suitable sampling media still poses a challenge for effective sampling. Hence, in the subsequent text, we review the current methods and the samplers available (Table 8) for sampling viruses, especially SARS-CoV-2 (given the importance of the ongoing pandemic) and to recommend an appropriate sampler to be utilized for effective collection of viruses.

Airborne PM and airborne viruses are collected by similar methodologies. However, the fundamental difference in particle collection for airborne viruses is the collection medium. It has been demonstrated that airborne viruses can be collected efficiently in a liquid collection medium such as agars (which can be used for culturing the airborne viruses), mineral oil (used in liquid impingers) and centrifugal tubes (by suspending a suitable liquid medium) (Pan et al., 2019). These types of collection medium (liquid and agar) are used for sampling airborne viruses, due to efficient and easy recovery of the samples, and for processing the aliquots of collected samples for virus detection and isolation. Whereas the virus extraction from the filtration process using respective filters (when used in a similar way as used in airborne PM sampling) will dehydrate viruses (Verreault et al., 2008) and deteriorate their infectivity.

Table 8
List of samplers used for collecting airborne pathogens (viruses, bacteria and fungi) and their detection limit by previous studies.

Type of sampler used	Type of airborne virus	Collection medium	Detection technique	Reference
Coriolis μ air sampler	SARS-CoV-2 (COVID-19)	5 mL conical vials with collection medium (DMEM)	PCR Positive Culture negative	Zhou et al. (2020a, 2020b)
NIOSH BC 251 bioaerosol samplers (3.5 LPM)	SARS-CoV-2 (COVID-19)	Centrifugal tubes and 0.3 μm PTFE Filters	PCR Positive Culture negative	Chia et al. (2020)
High-resolution slit sampler system (30 LPM)	SARS coronavirus	Liquid layer with viral culture medium	PCR Positive Culture negative	Booth et al. (2005)
Andersen cascade impactors (28.3 LPM)	Swine viruses	Culture medium (Agar)	PCR Positive	Alonso et al. (2015)

Note: the presence of SARS-CoV-2 in the air is possibly highest in the first week of illness (Chia et al., 2020). Hence, it is suggested to collect particles, for example, in a hospital environment near patients during the first week of illness.

The sample handling is also found to be different for airborne PM and pathogens (viruses, bacteria, fungi and other microbes). The PM samples collected in filters need to be desiccated, whereas the samples containing viruses should not be desiccated, since the samples might dehydrate and cause inactivation of the viruses (Lindsley et al., 2010; Fabian et al., 2009). Additionally, the samples collected for airborne viruses should be taken for further analysis (such as polymerase chain reaction (PCR) for the detection or any other analytical methods) in a short duration of time, compared to PM samples which can be stored for a longer period.

The airborne viruses are sampled using similar kinds of sampling techniques that are used for airborne PMs, but with some modifications on certain factors such as collection medium, flow rates and sampling duration. The different types of sampling techniques used for collecting airborne viruses (Fig. S1) fall under the following categories, such as (i) Solid impactors (cyclone samplers); (ii) Liquid impingers (All Gas Impingers, AGIs, and AGI-like samplers); (iii) Filters; (iv) Electrostatic precipitators; (v) Water-based condensation samplers; and (vi) Other samplers (Pan et al., 2019; Verreault et al., 2008).

Among these sampling techniques, impactors, filters, impingers and cyclone samplers, aid in collecting airborne viruses effectively (Haig et al., 2016; Reponen et al., 2011). The impactor sampling technique uses Andersen samplers, which works on the principle of inertial impaction and traps particles of specific cut off diameter from 0.65 to 7 μm (Tseng and Li, 2006), with air passing through the six stages of impactor at a flow rate of 28.3 LPM on to a petri dish consisting of liquid agar medium (White et al., 2020). The slit samplers draw particles through narrow holes or slits onto a petri dish which holds a culture medium (Booth et al., 2005). In the case of sampling by liquid impactors, all-glass impinger (AGI) samplers are used, where the sampler draws the air stream inside the liquid collection medium through diffusion (Hermann et al., 2006).

The filter sampling mechanisms are used widely when the particle sizes of airborne viruses are <500 nm. Filters used include PTFE, gelatin, glass fibre filters and cellulose filters. However, the filtering processes cause structural damage to the virus and might dehydrate the virus significantly (Verreault et al., 2008). Among these filters, gelatin is highly efficient in collecting airborne viruses without affecting the viability of viruses significantly (Zhao et al., 2014). However, gelatin filters are relative humidity (RH) dependent; they desiccate the viruses when used in low RH and get dissolved when used in high RH conditions (Verreault et al., 2008).

Cyclone sampling techniques have been used in Coriolis μ air sampler (Bertin instruments) and NIOSH BC 251 bioaerosol samplers. These samplers exert centrifugal forces on particles, causing them to deviate from the airflow and get impacted on the collection wall and so these samplers are not highly efficient, especially for particles with 100 nm (most free virus particles fall under this particle size) (Cooper and Alley, 2010). However, improvements have been made to these conventional cyclone samplers to increase their efficiency. For instance, a sampler developed by the National Institute for Occupational Safety and Health (NIOSH) possesses a multi-stage cyclone operation, whereby the liquid medium discharges and collects the particles against the cyclone walls (Kenny et al., 2017; Alonso et al., 2015). Likewise, the Coriolis μ air sampler provides efficient particle collection in a short duration of time (10 min although the airborne viruses obtained depends on their concentration in air) due to its high flow rate (from 100 to 300 LPM) and the airborne viruses are collected directly onto a collection medium such as Dulbecco's minimal essential medium (DMEM), which is particularly suitable for genetic analyses such as PCR, since the collected samples can directly be introduced with nucleic acid extraction kits (which is the preliminary step in PCR analysis). Currently, this type of sampler is being used by Centres for Disease Control and Prevention (CDC) of Shenzhen and Guangzhou, China as well as in Europe to collect coronavirus SARS-CoV-2 and evaluate the risk of aerosol contamination in hot spots of the cities (Zhou et al., 2020a, 2020b). On the other hand, bio-samplers such as the MD8 airscan sampling device have been used for sampling viruses such as the MERS-coronavirus (MERS-CoV) inside hospitals (Kim et al., 2016). These samplers work on a filtration principle; by drawing air through gelatin filters of diameter 80 mm and 3 μm pore diameter. The virus collection efficiency is found to be higher for RNA viruses (Zhao et al., 2014). However, these samplers use gelatin filters which, as described earlier, are highly RH dependent; when exposed to higher HR it dissolves the sample but dessicates the sample when exposed to lower RH (Verreault et al., 2008). Thus, at higher RH, the gelatin dissolves the sample and at lower RH it desiccates the sample. Further information on a wide array of biosamplers for the sampling and detection of viruses, especially coronaviruses, can be found elsewhere (Rahmani et al., 2020; Pan et al., 2019; Verreault et al., 2008). Among all the mentioned sampling techniques, it is found that the airborne viruses collected through cyclone separators and impactor type of sampling techniques are viable in collecting particles for virus detection with an efficiency of 39% for Coriolis μ air sampler (in a short duration of time, such as 10 min) (Zhou et al., 2020a, 2020b), 67% for NIOSH BC251 samplers (Chia et al., 2020), 20% in high-resolution slit samplers (Booth et al., 2005) and 40% detection in Andersen cascade impactors (Table 6). Hence, it is evident that the cyclone separator sampling technique is found to be highly efficient for collected airborne viruses. For instance, the recent pandemic SARS-CoV-2 airborne viruses (1–4 μm) have been detected with a higher efficiency (Chia et al., 2020) using cyclone sampling techniques incorporated in Coriolis μ air sampler and NIOSH BC 251 bioaerosol samplers. Therefore, the samplers using cyclone (using centrifugal forces) sampling mechanisms (Coriolis μ air sampler and NIOSH BC 251 bioaerosol sampler) can be used for effective virus detection and recovery for genetic analyses such as PCR.

Airborne virus collection largely depends on the efficiency of viral recovery compared to airborne PM collection, where the capture efficiency is found to be vital (Verreault et al., 2008). Other factors include RH, temperature, light, irradiation, suspension medium and collection medium (Pan et al., 2019). Additionally, virus morphology, virus type and characteristics such as a hydrophilic or hydrophobic nature should

also be considered when carrying out sampling for airborne viruses (Tseng and Li, 2006). Among these, RH is the most common factor which is studied for biological collection efficiency, since it plays a significant part in virus collection. For instance, non-lipid enveloped viruses become unstable below 70% RH, whereas lipid enveloped viruses become unstable above 70% (Tellier, 2006).

Similarly, in samplers using filtration, RH plays an important role, since the desiccation poses a problem for viruses collected on filters (Lindsley et al., 2010; Fabian et al., 2009). Hence, RH should be maintained at an optimum rate of around 30–70% (Verreault et al., 2008). In addition, temperature and the choice of collection medium are important factors which should be considered for the successful collection of effective airborne viruses. These will affect the efficiency of the biological collection of airborne viruses during the sampling process. The virus's infectivity and detection are highly dependent on RH, and viruses such as SARS-CoV, human coronavirus 229E (predecessors of the latest SARS-CoV-2) remains infectious in a moderate RH (around 30–70%) environment (Warnes et al., 2015; Casanova et al., 2010; Verreault et al., 2008).

Airborne pathogen (especially viruses) collection is found to be efficient when a liquid medium is used with a high flow rate of air (ranging from 100 to 300 LPM). A collection medium using filters is unfeasible because of filter desiccation (in case of PTFE, polycarbonate and glass fibre filters) whereby the microorganisms get dehydrated during this process and cause a greater loss in recovering the viruses. Gelatin filters are, however, efficient in collecting these viruses provided the measurements are taken in an RH controlled (which is mostly indoors) environment. The impaction and impinger sampling technique is found to be less efficient compared to the cyclone sampling technique (using centrifugal forces), where the airborne particles are collected on the sides of the centrifugal tube with the collection medium inside. These types of samplers operate at a high flow rate and the collection medium used in most of these samplers can be readily used for further gene analysis processes such as PCR for virus detection. Hence, the samplers with cyclone sampling techniques (Coriolis μ air sampler and NIOSH BC 251 samplers) using centrifugal forces are effective in collecting airborne viruses in any kind of environment (both indoors and outdoors).

7.4. Optimisation of particle mass sampling via modelling

The strategy of particle mass sampling can be optimised by predicting the air pollution concentrations by predictive models, which can enable to identify the pollution hotspots (places with increased pollution levels). These hotspots can be used as suitable sampling locations since there is a higher probability to obtain large amounts of particles in a significant time frame. These predictive models use different forms of mathematical modelling techniques such as artificial neural networks (ANN) (Maleki et al., 2019), adaptive-network fuzzy inference systems (ANFIS) (Prasad et al., 2016), statistical models (Lei et al., 2019) and Data Assimilation (DA) techniques (Arcucci et al., 2019a; Arcucci et al., 2017).

The statistical models are limited to utilize the standard classification or existing regression models (Zhu et al., 2018) and possess difficulties in modelling complex non-linear relationships (Prasad et al., 2016). On the other hand, self-learning modelling techniques such as ANN (Kurt and Oktay, 2010; Nagendra and Khare, 2005) and ANFIS (Prasad et al., 2016) are found to increase accuracy in forecasting compared to statistical models. Apart from these techniques, there exist several new modelling techniques being invented by the machine learning communities to refine the modelling to focus specifically on a defined problem. These ML techniques, when coupled with DA, are found to increase the accuracy and sensitivity of the model outcomes (Arcucci et al., 2017). For example, if incompressible Navier-Stokes equations are being used to describe a turbulent flow of particles, where the concentration of the particle mass is seen as a passive scalar (Song et al., 2018). These techniques will implement mutual information methods which measure the effect of selected data or selected placement on a posterior uncertainty of a Gaussian Process. The output simulations of ML techniques (coupled with DA) can be used to train Artificial Intelligence (AI) technologies, which in turn will increase the accuracy of the wind flow patterns and determine the emission strategies in the respective microenvironments. It will facilitate the model to predict the pollution levels precisely and identify the potential hotspots, thus exhibiting the optimal locations for effective particle collection. Hence, when ML trains a function, the optimal locations of pollution levels can be computed with minimised effort, which can subsequently allow identifying the areas of high pollution level (Arcucci et al., 2019b). The effectiveness of these techniques has been mathematically proven and tested for some test cases (e.g. outdoor measurements of CO_2). For instance, Dur et al. (2020) developed a modified version of the algorithm from Krause et al. (2008) and Arcucci et al. (2019b) using a Computational Fluid Dynamic (CFD) simulation of outdoor air pollution propagation in a vast domain in London, UK (Song et al., 2018). The number of nodes (which defines the sampling area) in the mesh used in this case is 767,559. The domain has been decomposed into 32 subdomains with an average of 24,000 starting locations per subdomain. Two optimal locations are identified to be the potential locations for optimal sensor placement at this site (Dur et al., 2020). Thus, a similar strategy could be applied for PM mass sampler placements.

In terms of accuracy computed as a mean squared error with respect to a control variable, this approach shows a reduction of the error which goes from 8.90×10^{-1} for locations randomly chosen to 3.35×10^{-02} for the optimal locations. A similar approach has been implemented in Tajnafoi et al. (2020) where an ML model is trained using data from a CFD simulation of air pollution in a room within the Clarence Centre building located in Elephant and Castle in London, UK. The simulated training dataset consists of 10,000 grid points, which represent potential instrument locations distributed in a 3D space. The number of selected locations, in this case, is 7. In terms of accuracy, this approach showed a reduction of the error, which goes from 1.70×10^{-1} for the seven random locations to 5.00×10^{-4} for the optimal locations. The accuracy of a modelling technique is vital since it provides an estimation of the misfit between the measures collected from the selected location and the estimates provided by a forecasting model. Reductions of the errors are mandatory for effective prediction.

In the last decade, these methods have been accepted as powerful tools in forecasting the outcomes. However, these DA methods quantify certain uncertainties, and these can be custom-developed to minimise the discrepancy between numerical results and observations, where forecast and observations are assumed as the sources of information having errors that will be described by error covariance matrices (Arcucci et al., 2019a). A Variational DA (VarDA) model by Arcucci et al. (2019a) demonstrated that this model could reduce the error in the forecasting model and adapted to decrease the error at simulating the location of the sensor and the rest of the domain. Hence, the DA models used for the sensor placement can be adapted to estimate the hotspots for particle mass collection by customising the data assimilations to reduce the errors in estimating those hotspots. In the case of particle mass collection, the number of placement could be reduced to a few locations. These technologies provide a list of the coordinates of the best locations in decreasing order with respect to the impact they have in terms of mutual information. In case the possible location is only one, the best location to consider is the first in the list provided by the technologies. At the moment, these approaches have not been used for selecting optimal sites for mass collections. However, these technologies are general and can be used if a predictive model can be implemented to simulate the particle mass dispersion.

The modelling techniques and overall approach described above have not been used for the purpose of an effective mass collection yet. This is primarily due to the lack of access to model results that require specific expertise, resources and knowledge base. However, it can be a novel approach for selecting effective locations to collect particle mass on filters when relevant information is available.

8. Summary, conclusions and future work

This article focused on the methodologies involved in $PM_{2.5}$ and UFP mass collection to study their physicochemical characterisation and toxicological effects. We provided a critical analysis of on (i) the typical concentrations of $PM_{2.5}$ and UFP mass in different urban microenvironments; (ii) the different types of samplers and which sampler will serve as a viable option depending on the microenvironment; (iii) the average sampling time for $PM_{2.5}$ and UFP particle mass in each microenvironment according to the sampler and typical concentrations; (iv) physico-chemical characterisation techniques for $PM_{2.5}$ and UFPs (both destructive and non-destructive) and the types of analytical techniques and instruments used; (v) various toxicological analysis methods of $PM_{2.5}$ and UFPs and the instruments used; (vi) particle mass collection optimization for $PM_{2.5}$ and UFPs; (vii) airborne virus sampling and evaluating the efficient sampling technique with efficient recovery of pathogens; and (viii) role of predictive modelling to identify the hotspots for efficient particle collection in a reduced sampling duration.

The key conclusions drawn from this study are as follows:

- Over the last two decades, it has become increasingly necessary to perform toxicological assessments of $PM_{2.5}$ and UFP particles. Airborne PM exposure to these two size fractions has been demonstrated to significantly correlate with the occurrence of adverse cellular effects, cardiovascular and chronic respiratory diseases. The toxicity of PM is generally higher in small size fractions (such as $PM_{2.5}$ and UFPs), and a strong association exists between the physico-chemical characterisation and their toxicity effects. These studies require a relatively large amount of particle mass to analyse these biological mechanisms, and it is often difficult to obtain the targeted particle mass in a reasonable period. Limited studies have discussed the particle mass (which is an important aspect) collection effectively by selecting an appropriate type of instrument according to the characteristics of each microenvironment in urban areas; this review provided viable suggestions to use the appropriate type of instrument for the respective microenvironments.
- We reviewed the typical concentrations in five different urban microenvironments (roadside; traffic intersections; indoors; parks; roadside behind GIs). As expected, the mass concentrations near to traffic-related activities such as roadsides and traffic intersections were found to be higher compared than those indoors where elevated mass concentrations were observed at study sites with poor ventilation and cooking activities. The park and roadside sites behind GI showed a lower mass concentration, due to the presence of urban vegetation and roadside GI. These typical concentrations served as a viable input in calculating the sampling time required by each instrument for obtaining the targeted particle mass.
- The sampling instrument and its volumetric flow rate play an essential part in the sampling duration for collecting the targeted mass of particles for the analysis. The volumetric flow rate on these samplers can range from 16 to 1100 LPM for both $PM_{2.5}$ and UFP, and these samplers largely use either impactor or cyclone sampling techniques. The particles collected in indoor environments generally use impaction sampling techniques (with flow rates 16 to 30 LPM) in a medium volumetric flow rate for collecting particles effectively without causing any increased noise levels or unpleasant conditions during sampling (in indoor environments). The impaction sampling technique, coupled with high volume suction pumps ranging from 400 to 1100 LPM, can be deployed in most of the outdoor microenvironments. Certain samplers, such as Harvard impactors, can be used for both indoor and outdoor environments since they have a significant flow rate and can collect particles in both fine and ultrafine size range. These types of samplers are as efficient as high-volume samplers and easy to operate and handle compared to high volume samplers.
- Though there are many methods available for physicochemical examination of PM, their use can be restricted by numerous factors. In particular, the amount of PM collected may limit viable options. When only limited amounts of PM are available, the experimental workflows to characterise the PM needs to be carefully planned. The PM samples need to be cycled through non-destructive experiments before attempting destructive approaches. On-filter experiments might be favoured over experiments requiring PM extraction. In general, if more mass of PM can be collected, more comprehensive and accurate analysis can be performed.
- Different mechanisms such as inflammatory processes, oxidative stress and genotoxicity are involved in PM cytotoxicity. Fine and UFPs exhibit significant toxicological effects due to their particle diameter, which aids their entry into the airways and bloodstream and causing cellular effects. The cellular toxicity of PMs and their subsequent damaging effects in different organs is attributed to the type of cell line, culture conditions, and the particle size, composition and concentration. In short, higher the PM concentration and exposure time lead to highest toxicity, and it strictly depends on the absorbed toxic pollutants. The oxidative stress seems to be generated mostly by the metal fraction while the genotoxicity by the organic fraction. Higher levels of PM and different cell lines are used in cell toxicity studies compared to a real-life situation. Methodological differences hamper direct comparison of results between different studies. The $PM_{0.1}$ toxicity grade is correlated not only with the relative mass, as for bigger PM, but also with particle number and surface area. Due to the higher capacity to absorb organic pollutants, $PM_{0.1}$ result in more toxic effects compared to $PM_{2.5}$, due to their small size and ability to produce damaging effects in the lung and the brain. More studies about its neurotoxicity and correlation with neurodegenerative diseases are needed. The identification of cellular modification caused by PM is essential to understand its harmful effects and correlated diseases. Bioassay and imaging techniques are essential tools employed to understand the cytotoxicity mechanism of PM.
- Toxicological assessments are carried out in different doses. Hence, they require an average of 1000 μg of particle mass in both the size fractions. Similarly, it is possible to say that the physico-chemical characterisation needs an average of 100 μg of particle mass (for elements, ions and carbon analysis). $PM_{2.5}$ mass was largely collected by six different types of samplers and the UFP mass by seven different samplers. The samplers with higher volumetric flow rates (1100 LPM) require very short duration compared to samplers with low volumetric flow rates (9 LPM). However, the high volume samplers cannot be used in all the urban microenvironments, due to difficulties in handling and operation. The Harvard impactors are easy to handle, equipped with a quieter pump and have a higher collection efficiency, demonstrating that it is a viable option to collect PM particles effectively.
- With respect to airborne pathogens, in particular viruses, collection conducted using samplers with cyclone (centrifugal forces) sampling techniques, has been proven to be effective. The samplers with cyclone sampling techniques use centrifugal forces, which cause a cyclone, by collecting the particles on the surface of the collection vessel in a medium such as DMEM. Thus, the cyclone sampling technique (Coriolis μ air samplers and NIOSH BC 251 samplers) with a suitable medium is viable for virus collection.
- Predictive modelling approaches using AI can be used in identifying locations for effective measurements of the particle mass. In particular, data assimilation and machine learning techniques can help to identify the potential hotspots for effective particle mass collection. The effectiveness of these technologies has been mathematically proven and tested for some test cases (e.g. measurements of CO_2). These technologies have not been used for effective mass collection yet. However, there is potential for these technologies to aid particle mass collection.

This review identified the different PM collection methodologies and recommended appropriate sampling strategies for various

microenvironments for effective particle collection. These microenvironments will not possess similar characteristics since some will have higher concentration due to proximity to emission sources as opposed to others with very low concentrations. The morphology of certain environments facilitates the use of samplers with high flow rate and certain indoor microenvironments do not favour using these types of instruments; hence a low flow rate instrument is used, and the sampling duration extends significantly, not to mention the particle mass collection will also be affected. Also, monitoring stations, sporadic unattended measurements are not feasible in all the microenvironments due to vandalism or any other local difficulties. Hence, there is a need for a sampler with high flow rate (as high volume samplers) as well as portable (as low volume samplers) with easy handling and good collection efficiency, which will facilitate the user to utilize in all kinds of microenvironments. Usage of these kinds of appropriate samplers supports in conducting a more comprehensive and accurate analysis of physico-chemical characterisation and toxicological effects of PM. Prior to conducting any sampling activity, the morphology of sampling environments and their prevailing average ambient concentration can be studied by pilot investigations using handheld monitors. The duration for collecting the targeted particle mass can be calculated using these pilot investigations, which in turn will help in finding the appropriate sampler to complete the sampling in a significant time frame. Future investigations should focus on using predictive modelling techniques such as data assimilation and machine learning, whose effectiveness has been proved mathematically and can be used for identifying the potential hotspots for effective particle mass collection.

Author credits

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Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This work has been supported by the EPSRC funded project, Health assessment across biological length scales for personal pollution exposure and its mitigation (INHALE; Grant No. EP/T003189/1).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143553.

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