



Atmospheric particulate matter adhesion onto pollen: a review

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Abstract From anther dehiscence to its atmospheric journey, airborne pollen grains can come into contact with other particulate matter (PM) and the external wall of pollen grains can be polluted by adhered atmospheric particles. Pollen and PM are some of the most common asthma triggers. The aim of this review was to discuss literature reporting pollen–particle complexes in the outdoor environment so their potential role in enhancing the risk of allergy could be examined. Twenty-five studies report pollen–particle complexes observed in outdoor conditions. PM adhesion onto the pollen surface was investigated in the literature by three different experimental approaches: direct collection from anthers, bulk exposure of pollen to PM in polluted areas, and direct sampling of

airborne pollen during pollination. Four processes are proposed to explain deposition of PM on the surface of pollen grains: sticking of particles to anthers and pollen during dehiscence, airborne pollen–particles coagulation, wet and dry scavenging of pollen and particles, and co-deposition of PM and pollen in the respiratory system. Despite potential adverse health effects, the magnitude of pollution of pollen by PM is poorly understood. To address this issue, we suggest the following guidelines for research: (1) Possible artifacts during sampling and handling of anthers must be carefully avoided; (2) Control pollen grains (i.e. non-polluted) should be carefully selected; (3) A better knowledge of pollen atmospheric deposition and resuspension is needed; (4) The health effects of pollen–particles complexes need more attention regarding symptoms and allergic sensitization; (5) Use of individual pollen samplers could give a

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depiction of the degree of pollution of pollen that is actually inhaled.

Keywords Airborne pollen · Atmospheric pollution · Pollen sampling · Pollen allergy

1 Introduction

Atmospheric particulate matter (PM) is harmful to health (Davidson et al. 2005; Kim et al. 2015), and it affects more people than any other pollutant (World Health Organization 2018). The link between PM and pollen allergy or asthma is widely documented in the literature (González-Díaz et al. 2017; Kim et al. 2013, 2015; Schiavoni et al. 2017). For example, on days with high levels of PM, the number of medical consultations and hospital admissions due to pollinosis rises (Phosri et al. 2017). PM has also been shown to exacerbate asthma (Polosa 2001). Indeed, the number of emergency room admissions due to asthma often increases with higher PM concentrations (Kim et al. 2015). In particular, diesel exhaust particles are able to enhance the development of allergic immune responses (Riedl and Diaz-Sanchez 2005; Saxon and Diaz-Sanchez 2005). The sharp rise in particulate air pollution from motor vehicles is even suspected to cause increases in type I allergies (Polosa 2001). PM can also affect allergy and asthma by adhesion onto pollen grains (coagulation processes) and formation of pollen–particle complexes (Schiavoni et al. 2017). After adhesion on pollen, particles could be loaded with allergens by diffusion of proteins in the presence of water. For example, purified pollen allergens from grass (*Lol p 1*) were shown to bind to diesel exhaust particles during in vitro experiments (Knox et al. 1997). In addition, by using immunogold labeling technique, Ormstad (2000) found that soot particles collected in indoor air carried birch allergens on their surface (Ormstad 2000). Pollen allergens were also shown to be bound to combustion particles in urban air (Namork et al. 2006). Moreover, the release of proteins by pollen grains has been shown to be enhanced by the presence of particles on their surface (Behrendt et al. 1991, 1997). Particles adhered on pollen grains could interact with allergens and uptake proteins from pollen grains, thus favoring their

dispersal in the respirable fraction of atmospheric aerosols (Solomon 2002).

The sampling of pollen on plants or in the atmosphere has shown that pollen grains from polluted areas are more prone to get contaminated by PM than pollen from less-polluted areas (Amjad and Shafighi 2012; Azzazy 2016; Behrendt et al. 1991, 1992; Bianchimano et al. 2014; Cerceau-Larival et al. 1990, 1991, 1996; Duque et al. 2012, 2013; Guedes et al. 2009; Kalkar Surekha and Renu 2014; Majd et al. 2004; Nilsson 1988; Okuyama et al. 2007; Peltre 1998; Rezanejad 2007; Rezanejad et al. 2003; Ribeiro et al. 2014, 2015; Schäppi et al. 1996; Sedghy et al. 2016; Shahali et al. 2009; Wang et al. 2012). Although the effects of gaseous pollutants on pollen grains have been widely studied and reviewed (Frank and Ernst 2016; Sénéchal et al. 2015), the interaction between pollen and PM has been much less studied. The reasons for this discrepancy include: (1) the need for a systematic single pollen grain analysis to detect, count and chemically characterize adhered particles and (2) the difficulty to reproduce realistic exposure of pollen to PM pollution in the laboratory.

Besides health effects, the adhesion of particles on pollen grains may induce effects on pollen germination. Pollutants are well known to interfere with pollen germination, generally provoking a decrease in germination capability of pollen (Sénéchal et al. 2015; Wolters and Martens 1987). Germination of *Camellia japonica* is inhibited by the adhesion of fullerene nanoparticles but in very elevated concentrations of particles (Aoyagi and Ugwu 2011). The examination of floral stigmas of chicory (*Cichorium intybus* L.) polluted by PM near roadsides found that there was not a significant relationship between total particulate levels found on stigmas and pollen germination (Jaconis et al. 2017). *Pinus nigra* and *Corylus avellana* pollen samples were harvested from three areas with different levels of air pollution and were tested for reproductive capability; pollen viability of both species was higher in areas with low road traffic, and pollen viability of *P. nigra* was negatively correlated with PM₁₀ concentrations (Gottardini et al. 2004).

The aim of this review is to examine the literature reports on the occurrence of pollen–particle complexes in outdoor environment (i.e., PM adhered to pollen grains) so that their potential role for a heightened risk of allergy after inhalation could be examined. The processes that explain how pollen is

polluted by particle adhesion are discussed. The literature is reviewed on the basis of the sampling strategies used to study pollen exposure to PM: pollen sampled on the plants, pollen exposed to outdoor pollution, and sampling of airborne pollen directly in the atmosphere. We point out two main gaps: (i) only a very limited number of papers provide quantitative assessments of pollen pollution by particles in the atmosphere and (ii) sampling artifacts were not systematically checked and may lead to mis-estimation of PM pollution of the pollen grains. Finally, guidelines for future research are proposed.

1.1 Processes of PM pollution of the pollen surface

Four processes are proposed to account for the outdoor deposition of PM on the surface of pollen grains from anemophilous plant species (Fig. 1: processes labeled a–d).

Pollen may be polluted by particles before pollination directly on plants during dehiscence (a). During pollination, airborne pollen grains and PM may collide in the aerosol phase leading to an adhesion of particles on the surface of pollen (b). These two processes (a and b) are documented in the literature and articles are reviewed here. Once deposited on soil or leaves (c), pollen might be polluted by particles deposited directly on pollen or in its vicinity during its immobile stay. To our knowledge, those processes of contact of pollen with particles on the ground are not documented in the literature. To be inhaled, deposited pollen should then be resuspended. Little is known about the processes of pollen resuspension, but relative humidity and surface wind speed are relevant parameters (Aylor 1976; Helbig et al. 2004). Upon inhalation (d), pollen and PM could land on the same spot in human upper airways. PM could first initiate inflammatory reactions on the pulmonary epithelium creating a chemical microenvironment with adjuvant properties to the allergenic reaction consecutively initiated by the deposition of a pollen grain (Saxon and Diaz-Sanchez 2005; Taylor et al. 2007). Whatever the nature of the interaction between pollen and PM is, i.e., specific binding or co-deposition, the pathophysiological consequences will result from the effects of both PM and allergens. Nasal deposition is, however, out of the scope of this article. We suggest the readers

to refer to recent reviews (Kim et al. 2013, 2015; Sénéchal et al. 2015).

During pollen sampling (e), particles may be deposited onto the surface of previously impacted pollen. This analytical artifact may hamper a correct assessment of the occurrence of PM adhesion on pollen while airborne.

The processes of PM pollution of the pollen surface are driven by several physical processes: gravitational coagulation, Brownian collision, and electrostatic interaction. Discussion about the relative importance of those physical processes is beyond the scope of this review (readers should refer to chapters related to coagulation in Seinfeld and Pandis (2012) (Seinfeld and Pandis 2012). Adhesion of particles also depends on physical, morphological, and chemical properties of the pollen surface. The pollen species different average electrostatic charges may influence pollen and PM interaction in the atmosphere (Bowker and Crenshaw 2007a, b). Moreover, the pollen wall ornamentation as well as the volume of extracellular lipids constitutive, with proteins, of the pollen coat may influence the ability of PM to stick to the pollen grain surface. Indeed, the pollen coat has been shown to significantly enhance pollen adhesion to solid surfaces by means of formation of capillary bridges (Lin et al. 2013).

1.2 Pollution of pollen by PM was studied by three types of experiments

For this review, we gathered 25 articles in which pollen grains were sampled and observed with PM on their surface (Tables 1 and 2). Most of the articles only provide a few examples of microscopic images of pollen, typically for high and low exposures to particulate pollutants (Fig. 2).

Most articles did not provide any statistical counting of particles on individual pollen grains (Table 1). To the best of our knowledge, only four studies provided valuable data on either the total number of pollen grains carrying PM on their surface (Behrendt et al. 1991; Sedghy et al. 2016) or the number of particles deposited on single pollen grains (Ribeiro et al. 2014, 2015) (Table 2).

PM adhesion onto the pollen surface was investigated by three different experimental approaches: (i) Pollen was collected from anthers in polluted areas (12 studies) (Amjad and Shafighi 2012; Azzazy 2016;

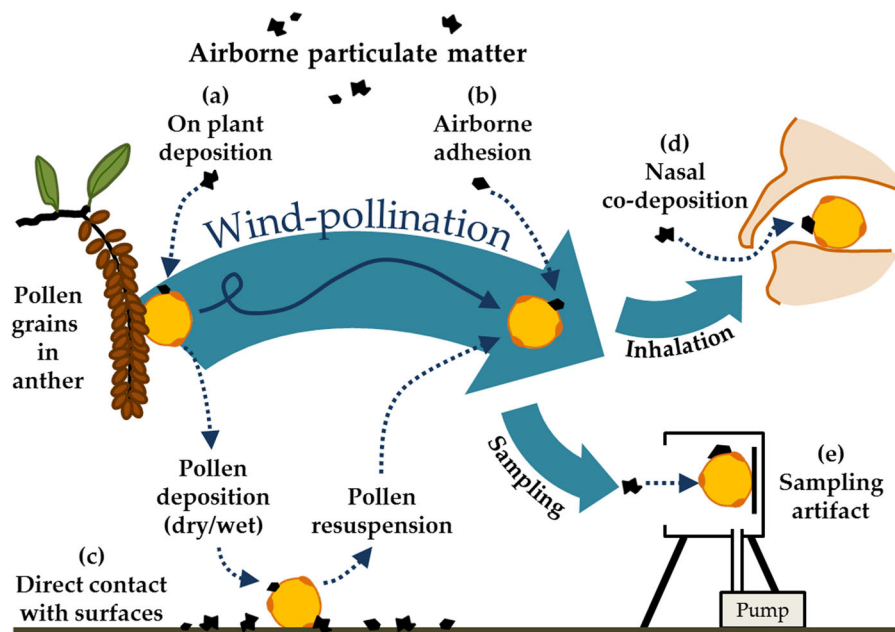


Fig. 1 Schematic view of the processes that may induce the sticking of airborne particulate matter (PM) on the surface of pollen grains. Before pollination (a), pollen might be polluted directly on plants during dehiscence. During pollination (b), airborne pollen grains and PM may collide causing adhesion of particles on the surface of pollen. Once deposited on soil or

vegetation (c), pollen may be polluted by particles deposited on it and in its vicinity. Upon inhalation (d), pollen and PM may land on the same spot in human upper airways. During sampling (e), particles may be deposited onto the surface of previously impacted pollen

Behrendt et al. 1992; Bianchimano et al. 2014; Chehregani et al. 2004; Guedes et al. 2009; Kalkar Surekha and Renu 2014; Majd et al. 2004; Rezanejad 2007; Rezanejad et al. 2003; Sedghy et al. 2016; Shahali et al. 2009); (ii) Pollen was collected from anthers in low polluted areas and then exposed to PM in highly polluted areas (six studies) (Cerceanu-Larival et al. 1990, 1991, 1996; Chehregani et al. 2004; Peltre 1998; Wang et al. 2012); (iii) Pollen was sampled in the atmosphere during pollination (eight studies) (Behrendt et al. 1991; Duque et al. 2012, 2013; Nilsson 1988; Okuyama et al. 2007; Ribeiro et al. 2014, 2015; Schäppi et al. 1996). The experimental approach (i) allows to investigate pollution on plant (process (a) on Fig. 1). The experimental approach (ii) allows to investigate airborne interactions between pollen and PM (process (b) on Fig. 1). The airborne sampling approach (iii) allows to investigate the average processes of PM pollution on pollen (processes (a), (b), (c), and (e) on Fig. 1). All three experimental approaches rely on microscopic examination of single pollen grains to assess the presence of particulate pollution. Spectroscopic analyses were

sometimes performed to obtain the chemical composition of polluting particles. Below is a summary of the main findings reported for each of the three experimental approaches.

1.2.1 Pollen directly sampled on plants

Anthers were harvested from plants in areas with different levels of pollution (i.e., rural, forest, urban, industrial, traffic). Pollen was extracted from anthers by sieving, sometimes after crushing mature catkins or cones or even with solvent washing. PM pollution on pollen grains was observed for several plant species and different cities (Tables 1 and 2). It must be mentioned that three studies were performed with pollen from entomophilous plant species (Chehregani et al. 2004; Rezanejad 2007; Rezanejad et al. 2003). Higher PM pollution of pollen collected from the anthers could be expected with entomophilous plant species for the following reasons: (i) The pollen is more prone to be in contact with air pollutants in open flowers, and (ii) the abundant and sticky pollenkit of

Table 1 Summary of the literature on pollution of pollen by PM

Azzazy (2016)	Industrial and non-polluted areas (<i>Sadat, Egypt</i>)	Pollen collected from trees
<i>Eucalyptus globulus</i>		SEM + LM
Adhesion of particles onto the surface of PGs collected in industrial area		
Shrinkage and abnormal shape of PGs from trees grown in polluted area		
Bianchimano et al. (2014)	3 zones in urban areas with different vehicle traffic (<i>Bahia Blanca, Argentina</i>)	Pollen collected from anthers
<i>Chenopodium album</i> L.*		SEM + LM
Numerous particles attached to the surface of PGs from all 3 areas		
Surekha and Renu (2014)	Industrial and control areas (<i>Nagpur, India</i>)	Pollen collected from anthers
<i>Cassia siamiae</i> /* <i>fistula</i> L.		SEM
PM was attached to the surface of PGs collected in industrial area		
Duque et al. (2013)	Urban and control from public parks (<i>Porto, Portugal</i>)	Pollen collected from trees for control
<i>Acer</i> spp./ <i>Platanus</i> spp./ <i>Pinus</i> spp.		Airborne PGs collected with HTS
		SEM + EPMA
Most of the airborne PGs had adsorbed particles on their surface		
Major influence of oceanic aerosols revealed by an increase in Cl, Na, and Mg		
Mineral dust contributes to modify the surface of PGs (detection of Si)		
Amjad and Shafighi (2012)	City center with heavy traffic and less-polluted areas around the city (<i>Tehran, Iran</i>)	Pollen collected from anthers
<i>Chenopodium album</i> L.		SEM + LM
PM accumulated on the surface of PGs from polluted area		
Duque et al. (2012)	Urban and control from public parks (<i>Porto, Portugal</i>)	Pollen collected from trees for control
<i>Platanus</i> spp.*		Airborne PGs collected with HTS
		SEM/EPMA
Observation of particles adhered on the surface of PGs		
Wang et al. (2012)	Roadside area (<i>Saitama, Japan</i>)	Pollen deposited onto carbon tape and exposed to polluted urban atmosphere for 9 h
<i>Cryptomeria japonica</i> *		SEM
Adsorption of particles on the surface of PGs		
The adsorbed particles are crystalloid (possibly from secondary particle formation)		
Guedes et al. (2009)	Rural and urban areas (<i>Porto, Portugal</i>)	Pollen collected from anthers
<i>Chenopodium alba</i>		LM + SEM + RMS
No mineral dust was observed (neither in rural area nor in urban area)		
Observation of carbon-containing particles with size varying from 10 to 50 µm adsorbed on the surface of PGs from urban area		
Adhered carbon particles have spectral characteristics similar to DEPs		
Shahali et al. (2009)	Urban area with heavy traffic (<i>Tehran, Iran</i>)	Pollen collected from cones at different time intervals
<i>Cupressus arizonica</i> *		SEM/EDX
Observation of cracks and collapses in the surface of PGs and presence of adsorbed particles		
Increase in sulfur, copper, nickel and iron content in PGs		
Okuyama et al. (2007)	Urban and mountainous sites (<i>Yokohama, Mt. Oyama, Japan</i>)	Control PGs sampled on trees, polluted PGs collected airborne (gravimetric collector)
<i>Cryptomeria japonica</i> *		SEM

Table 1 continued

Particles of different shapes adhered to the surface of PGs		
Elements of soil origin (Al, Fe, and Si) were detected		
Elements of anthropogenic origin (S, Zn) were detected		
Rezanejad (2007)	City center and control area 30 km from the city	Pollen collected from plants in both areas
<i>Spartium junceum</i> L.*	(Tehran, Iran)	SEM
Agglomeration of atmospheric particles on the surface of PGs		
Chehregani et al. (2004)	City center and control area outside the city (Tehran, Iran)	PGs collected from plants and then exposed to urban pollution 10–20 days
<i>Zinnia elegans</i> *		SEM
In polluted areas, airborne particles accumulate on the surface of PGs and change the shape and tectum of pollen		
Majd et al. (2004)	City center and control area 30 km from the city	Pollen collected from plants
<i>Canna indica</i> *	Tehran, Iran)	SEM
Observation of color change, folded shape and particles on the surface of PGs from polluted area		
Rezanejad et al. (2003)	Polluted and non-polluted areas (Tehran, Iran)	Pollen collected from plants
<i>Lagerstroemia indica</i> L.		LM + SEM
Observation of agglomeration of PM, cellular material release and damages on the surface of PGs from polluted areas		
Peltre (1998)	Polluted and non-polluted areas (Mulhouse, France)	PGs exposed to polluted air
<i>Betula verrucosa</i> / <i>Dactylis glomerata</i>		
Pollution creates a coating on PGs surfaces		
Particles observed on the surface of PGs		
Morphological changes of PGs and loss of allergens		
Schäppi et al. (1996)	Sampling areas with different levels of pollution (Zürich, Payerne, and Davos, Switzerland)	Airborne pollen collected with Andersen impactors
<i>Betula verrucosa</i> / <i>Phleum pratense</i>		SEM
Observation of interactions between aerosols and PGs surfaces excepted in the area with the lowest pollution		
Because of the small scale of the analysis, conclusive quantitative evidence for differences between samples from variously polluted regions could not be obtained		
Cerceau-Larrival et al. (1996)	Urban exposure (Stockholm, Sweden; Mulhouse, France)	PGs exposed to polluted air
<i>Dactylis/Betula</i>		SEM/EDX
Observation of modification of the morphology of PGs		
Adhesion of particles to the surface of PGs and degradation of the exine structure		
Behrendt et al. (1992)	Industrial, roadside and park areas (location not mentioned)	Pollen collected from plants
<i>Dactylis glomerata</i> / <i>Corylus</i> L.		SEM
Observation of PM accumulated on the surface of PGs		
Cerceau-Larrival et al. (1991)	Roadside and industrial areas (Stockholm, Sweden)	Pollen exposed in Teflon bags to polluted air (outdoor) and to cigarette smoke (in laboratory)
<i>Dactylis glomerata</i> / <i>Betula verrucosa</i>		SEM/EDX
Observation of small particles on the surface of PGs and bigger particles between PGs		
Cerceau-Larrival et al. (1990)	Urban, roadside and industrial areas (Stockholm, Sweden)	PGs exposed in Teflon bags to polluted air
<i>Betula verrucosa</i> *		SEM/EDX

Table 1 continued

PGs exposed to heavy traffic are loaded with particles

Nilsson (1988)	Urban area and direct exposure to vehicle exhaust	Airborne sampling of PGs with a HVS
<i>Dactylis glomerata/</i>	(Stockholm, Sweden)	SEM/EDX
<i>Betula</i>		
<i>Fraxinus/Plantago/</i>		
<i>Galium</i>		

Adhered particles to the exine are rich in Al, Ca, Cl, Fe, K, Mg, P, Si and S

No heavy elements are detected

PGs exposed to vehicle exhaust are not polluted with Pb

Twenty-one articles report the presence of PM adhered to pollen surfaces based on a qualitative assessment only. These works are listed by reverse chronological order

Plant names marked with an asterisk (*) indicate which pollen species are shown in Fig. 2

PM particulate matter, PG pollen grain, EPMA electron probe microanalysis, SEM scanning electron microscopy, LM light microscopy, HVS high-volume sampler, HTS Hirst-type sampler, TEM transmission electron microscopy, EDAX energy-dispersive analysis of X-ray, RMS Raman microspectrometry, DEP diesel exhaust particle, RMS Raman microspectrometry

entomophilous pollen may promote PM adhesion (Dobson 1988; Pacini and Hesse 2005).

Pollen grains from polluted areas often have more particles on their surface than pollen grains from low-pollution areas. However, none of the studies on pollen harvested on plants reported statistical data on the relative proportion of polluted grains observed or the average number of particles adhered to pollen. Sedghy et al. (2016) provided an estimate of the fraction of *Platanus orientalis* pollen polluted by particles but with the total number of counted pollen not given and with very high uncertainties: 25–70% of pollen grains from polluted areas have particles on their surface compared to 0–9% of pollen grains from unpolluted areas. Unexpectedly, no soot particles were observed in this study, whereas sampling areas were chosen due to proximity to heavy traffic (Sedghy et al. 2016). Guedes et al. (2009) did find diesel exhaust particles on *Chenopodium alba* pollen collected in an urban area with heavy traffic.

There is a lack of studies reporting measurements of PM concentrations in close vicinity of the sites where pollen was harvested. Without the PM mass concentrations, no correlation could be made between the levels of particulate matter in the atmosphere, the meteorological conditions and the number of particles adhered to pollen grains. Moreover, individual particle chemical analysis by aerosol mass spectrometry or scanning electron microscopy coupled to energy-dispersive X-ray spectroscopy (SEM/EDX) may give valuable information about any preferential adhesion

on pollen depending on size or chemistry (Choël et al. 2007).

Anthers are known to provide protection from gaseous pollution to pollen; pollen exposed to air pollutants in vivo showed indeed better germination capability than pollen exposed in vitro (Wolters and Martens 1987). To what extent anthers offer protection to pollen from PM is not yet determined. Optical images of anthers of *Chenopodium album* collected in heavy traffic areas have been taken and no particle pollution could be detected in these observations (Amjad and Shafighi 2012).

1.2.2 Pollen collected and then exposed to air pollution

Pollen is collected from low-pollution areas and then exposed either in small bags or on adhesive tapes to polluted air in urban or industrial areas (Cerceanu-Larrival et al. 1990, 1991, 1996; Chehregani et al. 2004; Peltre 1998; Wang et al. 2012). Pollen exposed in polluted zones was systematically more polluted with PM than the one exposed in low-pollution zones. Polluted areas can be compared with the exact same batch of pollen, eliminating possible confusing parameters. One of the main advantages is the control of the pollen exposition time. Moreover, these experiments could provide samples of polluted pollen grains of several mg, or even grams, which could be very useful for further chemical analysis or for health-related studies. None of the studies provide

quantitative data neither on the specific pollution of pollen by PM, nor on the background pollution, nor about the PM concentration in the vicinity of the pollen.

1.2.3 Pollen sampled in the atmosphere

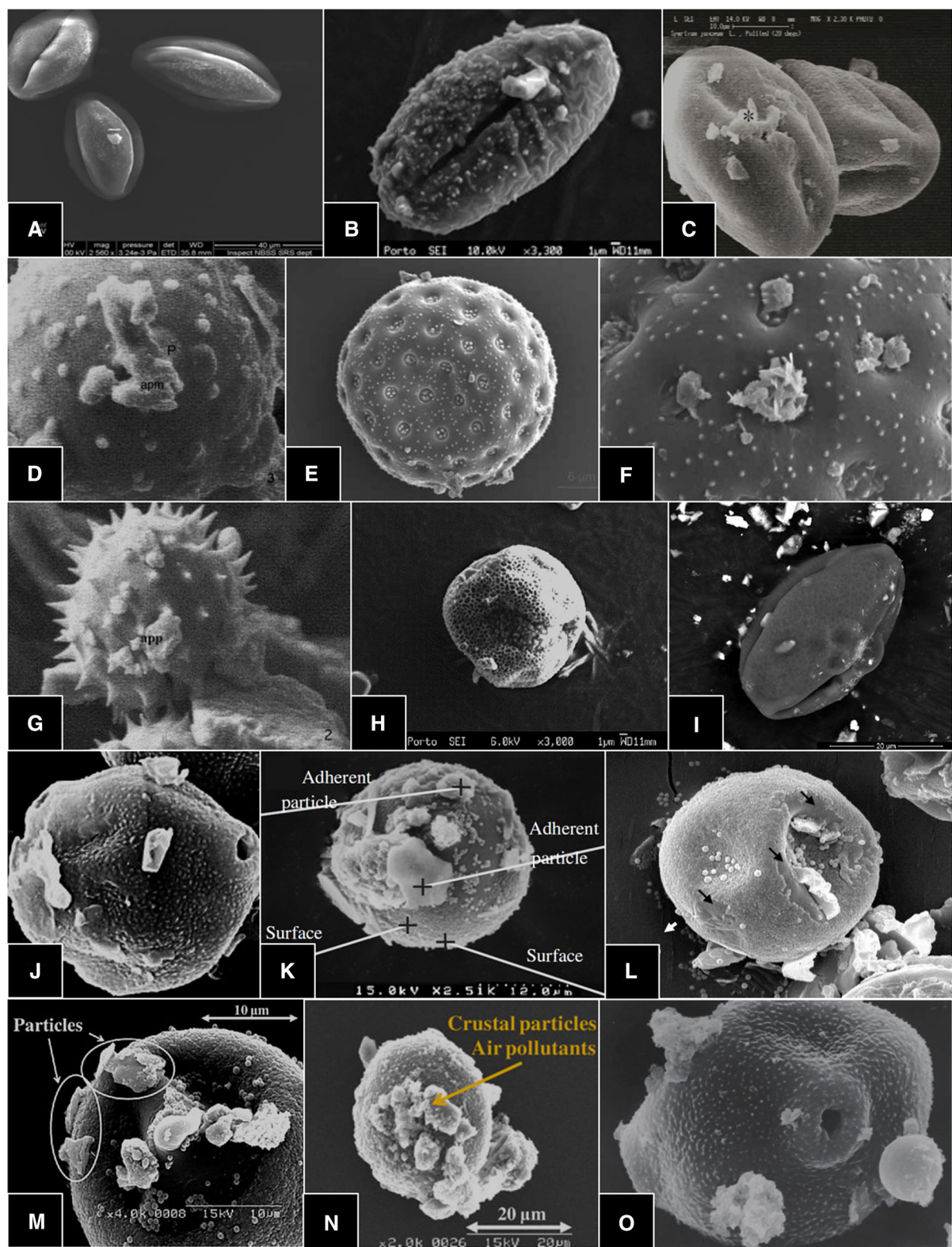
Direct sampling of airborne pollen provides information about the pollen that is actually inhaled. However, all particle adhesion processes depicted in Fig. 1 may add up, and it is impossible to infer the process of particle sticking. Pollen were collected on filters with suction pumps (Nilsson 1988), Andersen cascade impactors (Schäppi et al. 1996), Durham-type pollen collectors (Okuyama et al. 2007), high-volume

Fig. 2 SEM images of particles adhered onto the surface of pollen grains. A variety of PM-polluted pollen species are shown: (A) *Cassia siamiae* L. (Kalkar Surekha and Renu 2014), (B) *Acer* (Ribeiro et al. 2014), (C) *Spartium junceum* L. (Rezanejad 2007), (D) *Canna indica* (Majd et al. 2004), (E) and (F) *Chenopodium album* L. (Bianchimano et al. 2014), (G) *Zinnia elegans* (Chehregani et al. 2004), (H) *Platanus* spp. (Duque et al. 2012), (I) *Acer* spp. (Duque et al. 2013), (J) *Betula verrucosa* (Cerceanu-Larrival et al. 1990), (K) *Cryptomeria japonica* (Okuyama et al. 2007), (L) *Cupressus arizonica* (Shahali et al. 2009), (M) and (N) *Cryptomeria japonica* (Wang et al. 2012), (O) *Betula* (Behrendt et al. 1991) (Reproduced with permission)

samplers (Behrendt et al. 1991), and Hirst-type samplers (Duque et al. 2012, 2013; Ribeiro et al. 2014, 2015). Particles were found on pollen wall in all eight studies of airborne pollen sampled for various

Table 2 Summary of the literature on pollution of pollen by PM

Sedghy et al. (2016) 3 urban and 2 non-polluted areas (<i>Mashhad, Iran</i>)		Pollen collected from trees
<i>Platanus orientalis</i>		SEM
Particles of 150–550 nm adhered on the surface of PGs from polluted sites		
Aggregation of particles on the surface of PGs from urban area		
In polluted areas, 25–70% of PGs have attached particles, compared to 0–9% in unpolluted areas		
Statistical data, number of PGs and number of adhered particles were not provided		
Ribeiro et al. (2015)	Urban area	Airborne pollen collected with a HTS
<i>Platanus/Pinus/Acer</i> (<i>Porto, Portugal</i>)		SEM/EDX
Observation of 419 PGs (140 PGs of <i>Acer</i> , 123 of <i>Pinus</i> and 156 of <i>Platanus</i>); 7683 particles counted.		
Average number of particles on a PG: <i>Acer</i> 16 ± 12 , <i>Pinus</i> 39 ± 32 , <i>Platanus</i> 4 ± 4		
Size range of adhered particles: 0.1–25.8 μm		
Ultrafine particles ($< 0.1 \mu\text{m}$) were present but not counted		
Ribeiro et al. (2014)	Urban area (<i>Porto, Portugal</i>)	Control pollen collected from trees in public parks
<i>Acer</i> *		Airborne pollen collected with a HTS
		SEM/EPMA
144 PGs analyzed, 2666 particles characterized		
Counting of 0 to 47 particles adhered on the surface of PGs		
Behrendt et al. (1991)	Urban and industrial areas (<i>Duisburg and Düsseldorf, Germany</i>) and in vitro study	Airborne pollen collected with a HVS
<i>Betula</i> */ <i>Dactylis glomerata</i>		LM + TEM + SEM/EDX
Marked degree of agglomeration of particles on the surface of PGs in highly polluted area. Up to 75% of PGs heavily polluted by particles		
Particle agglomeration is less frequent and almost absent in pollen samples collected in less-polluted city. About 15% of PGs highly polluted		
Chemical composition of particles is involved in their sticking capability		
Four articles provide quantitative data about PM adhesion to pollen surfaces. These works are listed by reverse chronological order		
Plant names marked with an asterisk (*) indicate which pollen species are shown in Fig. 2		
PG pollen grain, EPMA electron probe microanalysis, SEM scanning electron microscopy, LM light microscopy, HVS high-volume sampler, TEM transmission electron microscopy		



species (incl. *Artemisia vulgaris*, *Urtica dioica*, *Phleum pratense*, *Betula*, *Fraxinus*, *Galium*, *Platanago*). Adhered particles were chemically characterized in several studies, mostly by SEM/EDX (Behrendt et al. 1991; Duque et al. 2013; Nilsson 1988; Okuyama et al. 2007; Ribeiro et al. 2014, 2015). Only Ribeiro et al. (2015) provided a statistical analysis of the number of particles adhered on pollen grains collected with a Hirst sampler: 419 pollen grains counted and 7683 adhered particles counted. The average number of particles per pollen grain was 16 ± 12 for *Acer*, 39 ± 32 for *Pinus* and 4 ± 4 for *Platanus* for a daily average PM₁₀ concentration during samplings of $86 \mu\text{g}\cdot\text{m}^{-3}$. The particles adhered to pollen grains were mostly below $5 \mu\text{m}$ (Ribeiro et al. 2014, 2015) (Table 2).

2 Discussion about sampling artifacts

Tables 1 and 2 list 25 papers reporting SEM observations of particles adhered to pollen for various plant species, using different sampling strategies and at various locations around the world. The surface coverage of pollen by adhered particles was found important in two studies (Behrendt et al. 1991; Ribeiro et al. 2015). Only four studies give quantitative data about PM adhesion on pollen (Table 2) and the other studies do not provide any statistical counting of pollen or adhered particles (Table 1). Furthermore, some sampling artifacts could introduce a mis-estimation of PM adhesion on pollen.

Regarding the approach where pollen is directly sampled on plants, particles adhered to anther walls may contaminate pollen grains during harvesting and sieving of the pollen. Microscopic imaging of anthers to check the presence of particles on anther walls before pollen extraction by sieving may circumvent this artifact.

In a similar way, when pollen is exposed on adhesive tape the average number of particles per pollen grain unit surface should be compared with the average number of particles on the surface of adhesive around pollen grains. Without this comparison of surface density of particles ($\text{particles}\cdot\text{cm}^{-2}$), conclusions on the affinity of particles to pollen surface are impossible to draw. Moreover, use of adhesive tape could be problematic due to electrostatic charges that

may affect pollen–particle interactions (Kweon et al. 2015; Vaknin et al. 2000).

Hirst-type samplers (HTS) were designed to efficiently collect spores or pollen grains for subsequent counting and identification, independently of PM analysis (Hirst 1952). After collection inside an HTS, the pollen is not protected from impaction of smaller particles on its surface. The air velocity at the inlet is $6 \text{ m}\cdot\text{s}^{-1}$ for HTS [rectangular orifice of $2 \times 14 \text{ mm}$, flow of $10 \text{ L}\cdot\text{min}^{-1}$ (Hirst 1952)] and $3.3 \text{ m}\cdot\text{s}^{-1}$ at the inlet for a typical PM₁₀ cascade impactor (Dekati, circular orifice of 8 mm , flow of $10 \text{ L}\cdot\text{min}^{-1}$). With such an inlet velocity for HTS, the collected particles are finer than a PM₁₀ cascade impactor. The cut size of HTS, i.e., the aerodynamic diameter of particles collected with 50% efficiency, is reported as $3.7 \mu\text{m}$ (Khatab and Levetin 2008). Pollen trapped in the HTS are therefore “bombarded” by these particles and the distinction between particles adhered to pollen before or during sampling is not possible. In the SEM image shown in Duque et al. (2013) (Fig. 3), a high number of small particles were clearly visible on the collection tape around pollen grains. These particles could statistically land on the tape or on the pollen causing a sampling artifact. The comparison of the surface density of particles between the adhesive sampling tape and the pollen grains were not provided in this study with quantitative data about PM adhesion on pollen. As a result, the number of adhered particles on pollen grains is likely to be

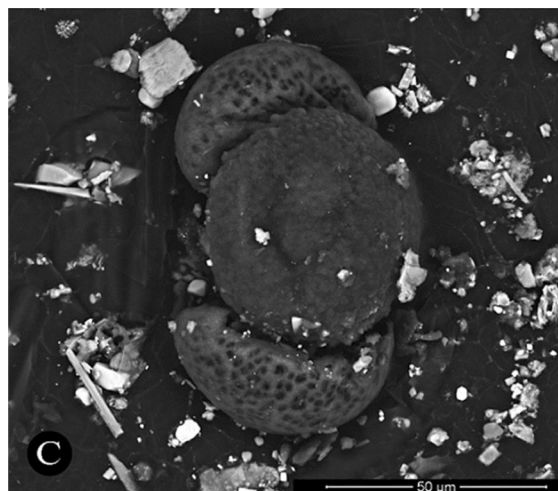


Fig. 3 *Pinus* pollen sampled with a Hirst-type sampler. Smaller particles are clearly trapped on the collection surface around the pollen grain (Duque et al. 2013) (Reproduced with permission)

overestimated. Conventional HTS should not be used when pollen sampling is conducted with the aim of characterizing particles adhered onto pollen surface. Instead, cascade impactors with cutoff diameters higher or equal to 10 μm are efficient in preventing the contamination of pollen by particles during sampling.

Another source of overestimation of PM on the surface of pollen is the presence of nanometer-sized ultrafine particles (0.2 μm) observed on pollen from several species (Ruggiero and Bedini 2018). Those particles were not orbicules, and the pollen grains were collected from an Herbarium and were not subjected a priori to PM exposure. Those particles of unknown origins could be easily mistaken with PM adhesion on the pollen wall. In addition, pollen damages were reported even until rupture and release of sub-pollen particles (Azzazy 2016; Cerceau-Larival et al. 1991; Majd et al. 2004; Peltre 1998; Rezanejad et al. 2003; Shahali et al. 2009). Clear differentiation between PM and sub-pollen particles should be guaranteed in the case of damaged pollen grains to accurately assess PM pollution of pollen.

3 Conclusion and suggestions of guidelines for research

Pollen-PM interactions have received attention since the 1990s. Adhesion of particles on pollen grains has been repeatedly reported in the literature since then. However, these pollen-particle atmospheric interactions are complex to study (Behrendt et al. 1991). Pollution of pollen grains by particles was documented for plant exposure and atmospheric coagulation of particles onto pollen grains. To the best of our knowledge, deposition of pollen grains on leaves and ground and subsequent resuspension of polluted pollen grains by wind have not been studied yet although such mechanisms provide a direct contact between pollen and dust.

Moreover, the extent of pollution of pollen grains by PM is indeed largely unknown. A very limited number of studies report pollen grains heavily polluted by PM but we suspect that sampling artifacts may hinder the proper quantitative assessment of such PM pollution on pollen grains. Although PM was shown to increase airway inflammation and favors Th2 response (Sénéchal et al. 2015), whether they synergize with

pollen to exacerbate allergic sensitization and symptoms requires more studies to unravel and quantify their specific interactions and help to understand the links between allergy/asthma and the atmospheric environment. Analysis of the adjuvant activities to the allergenic reaction of adhered PM triggered by pollen grains requires a reliable quantitative assessment of pollen-particle complexes as well as a report of the chemical composition of adhered particles.

We give here several suggestions for future research in the field of quantitative assessment of adhered particles to pollen grains.

1. Possible artifacts during sampling and handling of anthers must be avoided (for example by collecting just before dehiscence and washing the anthers). A microscopic observation of anther walls is required to ensure that no contamination of pollen grains by PM adhered on the surface of anthers could occur during pollen extraction by sieving. For suction samplings of airborne pollen grains, collection surfaces should have a minimum contamination by PM, *i.e.*, the surface density of PM adhered on pollen grains should be at least an order of magnitude greater than the surface density of PM on the collection surface. Samplers used to characterize particles adhered on pollen grains must have a cutoff diameter higher or equal to 10 μm to prevent contamination of pollen grains by PM during sampling.
2. Comparison of pollen from airborne sampling and pollen harvested in plants should be avoided. Firstly, the size of the pollen sample collected by airborne sampling is different from that obtained by plant harvesting. In the case of airborne sampling, the number of pollen grains is at most several hundreds. In the case of plant harvesting, the sample mass can reach milligrams or even grams of pollen (millions or billions of grains). This raises questions about the representativeness of samples: are they homogeneous? If individual analysis of hundreds of grains is feasible, it cannot be carried out on millions of grains. Secondly, control pollen grains (*i.e.*, non-polluted) have to be collected in the same conditions as polluted pollen grains. Sampling artifacts could then be comparable. Thus, pollen collected from plants should be compared among plants growing in different locations and under different environmental

conditions. Accordingly, airborne pollen sampling should be conducted in areas with different levels of air pollution. As well, the contribution of pollen from long-range transport should be controlled since long-range transported pollen is expected to be more polluted with PM due to a higher residence time in the atmosphere. Sampling outside local pollination period could give information on PM pollution for pollen from long-range transport and resuspension. Side measurements should include ambient PM mass concentrations, size distributions, and microscopic analysis of particles including SEM/EDX.

3. A better knowledge of pollen deposition and resuspension is needed for a better knowledge of the total residence time of pollen in the atmosphere. Moreover, resuspension data are also needed to improve modeling of pollen atmospheric concentrations (Sofiev et al. 2013). The airborne residence time of pollen is in the order of hours but some pollen may suffer several cycles of deposition/resuspension. Such pollen could accumulate PM and other pollutants leading to “super-polluted” pollen like the ones observed in literature with very high surface coverage by PM (Behrendt et al. 1991; Ribeiro et al. 2015; Sedghy et al. 2016).
4. The health effects of such pollen-particle complexes need more attention regarding symptoms and allergic sensitization. In health-related studies, surface density of particles on pollen grains and chemistry of adhered particles are crucial parameters.
5. The use of individual pollen samplers could give a more accurate depiction of the degree of pollution of pollen that is actually inhaled. Stationary pollen sampling is generally performed on the roof of buildings. Sampling within human reach should give a more realistic picture of pollen exposure (Peel et al. 2013).

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