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1 **ATMOSPHERIC PARTICULATE MATTER DEPOSITION ON BIRCH CATKINS**  
2 **AND POLLEN GRAINS BEFORE POLLINATION**

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9 Keywords: Pollen allergy, *Betula*, industrial air pollution, Catkins

10  
11 **Abstract**

12 Birch is the most allergenic tree species in northern and central Europe. Airborne  
13 birch pollen concentrations are observed to be on the rise for several decades. Health  
14 hazard due to birch pollen grains (BPGs) can worsen due to particulate air pollution.  
15 The prevalence of the intimate mixture of BPGs with atmospheric particulate matter  
16 (APM) at the single pollen grain level is still unraveled. In this study, APM transfer  
17 mechanisms to birch catkins and BPGs prior to pollination were investigated at an  
18 urban-industrial and a peri-urban site in northern France. The surface of catkins was  
19 heavily polluted with micrometer-sized particles. Conversely, BPGs were relatively  
20 unpolluted with on average 0.1 particle.BPG<sup>-1</sup>. Differences in the chemical  
21 composition of adhered particles were observed as a function of the sampling sites.  
22 In contrast, no significant difference was found in terms of surface concentrations of  
23 APM adhered to BPGs and catkins between the two sites. Comparison of the number  
24 of particles deposited per pollen grain according to whether they were harvested  
25 from catkins or collected while airborne suggests that particulate pollution of pollen  
26 occurs preferentially after pollen shedding, either by impaction or coagulation  
27 mechanisms with other suspended APM or by pollen settling on dust-contaminated  
28 surfaces followed by resuspension.

## 30 1. Introduction

31 Birch is the tree from the Betulaceae family that is the main source of allergenic tree  
32 pollen in northern and central Europe (Biedermann et al. 2019). Birch pollen is  
33 produced in the flowers grouped in male catkins; each catkin can produce 7 to 37 mg  
34 of pollen (Erdtman 1943; S. C. Jochner et al. 2011; Thomas 2000). Considering that  
35 an adult tree has at least several hundred catkins, considerable quantities up to a few  
36 tens of grams of pollen are emitted by each birch tree in a short period of time (a few  
37 days) during pollination (Erdtman 1943; Proctor et al. 1996). Birch pollen in the  
38 atmosphere very frequently reach concentrations in the order of 1,000 to 10,000  
39 BPGs per cubic meter of air (Ranta et al. 2008). Allergy to birch pollen is widespread  
40 with more than 100 million people worldwide sensitive to this pollen (Lavaud et al.  
41 2013). Symptoms can start in the pollen season with an ambient pollen concentration  
42 as low as 30 BPG.m<sup>-3</sup> (Caillaud et al. 2014), and between 0.1 to 0.5 mg BPGs can be  
43 inhaled per individual per week during pollination (Lavaud et al. 2013).

44 Air pollution can affect different aspects of birch metabolism, including  
45 reproduction; the effects of ozone on birch have been most extensively studied  
46 (Darbah et al. 2008; Fry and Black 1973; Günthardt-Goerg et al. 1993; Karlsson et al.  
47 2003; Matyssek et al. 1991, 1997; Mortensen and Skre 1990; Oksanen et al. 2005).  
48 For example, ozone was correlated with delayed birch reproductive phenology and  
49 growth (S. Jochner et al. 2015; Karlsson et al. 2003). Severe industrial pollution (SO<sub>2</sub>  
50 and heavy metals) did not have significant observable effects on the reproduction of  
51 *Betula pubescens* in terms of number of reproductive trees, reproductive effort and  
52 catkin weight (Kozlov and Zvereva 2004). A similar conclusion was reached on the  
53 effects of industrial pollution on the amount of major allergens in *Betula* pollen  
54 (Helander et al. 1997). An increase in carbon dioxide concentration had a largely  
55 positive effect on the reproduction and regeneration of *Betula papyrifera*, whereas  
56 high ozone concentrations had an inverse effect on this species (Darbah et al. 2008).  
57 Increased concentrations of Bet v 1, the major birch pollen allergen, were observed in  
58 *Betula* pollen on trees located in urbanized areas compared to trees located at the  
59 urban fringe (Žiarovská et al. 2013). This increased load of allergens is to be placed in  
60 a context of increasing exposure to BPGs due to pollen concentrations observed to be  
61 on the rise for several decades in different places (Besancenot et al. 2019; Frei 1998;  
62 Frei and Gassner 2008; Hoebeke et al. 2017).

63 To our knowledge, the deposition rate of atmospheric particulate matter (APM) on  
64 birch has not been studied, whereas the deposition of APM has been the subject of a  
65 large number of studies on many other trees (Litschke and Kuttler 2008). Plant  
66 exposure to APM can result in the following direct damage: plant growth reduction,  
67 altered leaf surface structure, abrasion, radiative heating, reduction of  
68 photosynthesis (Grantz et al. 2003; Rai et al. 2010). Particles can also settle on pollen  
69 in the inflorescences before or during pollination (Visez et al. 2020). Particles  
70 deposited on PGs may affect their germination capacity and allergy risk (Visez et al.  
71 2020).

72 Under field conditions, no relationship could be established between APM  
73 concentrations and the germination of PGs from *Cichorium intybus* (Jaconis et al.  
74 2017). Pollen viability in *Pinus nigra* was negatively correlated with SO<sub>2</sub>, PM<sub>10</sub> and  
75 NO<sub>x</sub> concentrations (Gottardini et al. 2004). However, laboratory exposures have  
76 identified certain types of particles with effects on the reproduction capacity of PGs:  
77 fullerene nanoparticles totally inhibit PG germination in *Camellia japonica* (Aoyagi  
78 and Ugwu 2011), silver nanoparticles decrease viability in *Actinidia deliciosa*  
79 (Speranza et al. 2013), graphene oxides nanoparticles affect germination in  
80 *Nicotiane tabacum* (Carniel et al. 2018), silver nanoparticles significantly reduce PG  
81 germination in *Peltophrum ptecocarpem* (Dutta Gupta et al. 2019), palladium  
82 nanoparticles strongly inhibit germination in *Actinidia deliciosa* (Speranza et al.  
83 2010). These laboratory experiments also raise the crucial question of the dose of  
84 particles to be applied to pollen to represent a realistic *in vitro* environmental  
85 exposure. However, quantitative studies on the deposition of APM on PGs, which  
86 would make it possible to determine these doses, are particularly lacking or present  
87 positive artefacts related to the sampling method of PGs (Choël et al. 2020; Visez et  
88 al. 2020). A link between atmospheric concentrations of APM and plant  
89 reproduction has not yet been formally demonstrated, although the deleterious effect  
90 of gas pollution on pollen performance is widely accepted (Jaconis et al. 2017;  
91 Sénéchal et al. 2015; Wolters and Martens 1987).

92 The lack of quantitative data on APM polluted PGs does not allow to understand the  
93 role of such surface pollution by particles in allergy and asthma (Visez et al. 2020).  
94 However, co-exposure to APM and bioaerosols is associated with increased  
95 inflammation, lung disease and exacerbation of symptoms (Mack et al. 2019).  
96 Laboratory exposure of birch-allergic basophils to organic extracts from urban

97 aerosols suggests a role of particle-borne organic compounds in the aggravation of  
98 IgE-mediated allergic diseases (Schober et al. 2006). There is no doubt that APM  
99 worsens asthma, although the underlying mechanisms are not fully elucidated  
100 (Polosa 2001).

101 Recently, sampling of BPGs in low-polluted urban air (average  $PM_{10} = 26.3 \mu\text{g}\cdot\text{m}^{-3}$ )  
102 has enabled to estimate the average pollution of BPGs at 0.86 particles deposited per  
103 PG (Choël et al. 2022). Airborne sampling of BPGs does not allow to elucidate timing  
104 of pollution of BPGs surface by APM. This work contributes to a better  
105 understanding of APM transfer mechanisms to PGs. Male catkins were collected just  
106 before pollination on two birch trees, one located in a city with a strong industrial  
107 influence and the other one located in a peri-urban area. Catkins and PGs extracted  
108 from inflorescences were observed by scanning electron microscopy (SEM) for  
109 examination of particles adhering to their surfaces. Elemental mappings of  
110 particulate pollutants on catkins and BPGs were performed by X-ray detection  
111 (EDX). This study aims at exploring possible correlations between the particulate  
112 contamination of catkins and the pollution degree of PGs from which they were  
113 extracted from, depending on the environment in which the parent trees grow.

## 114 **2. Materials and methods**

### 115 **Sampling locations**



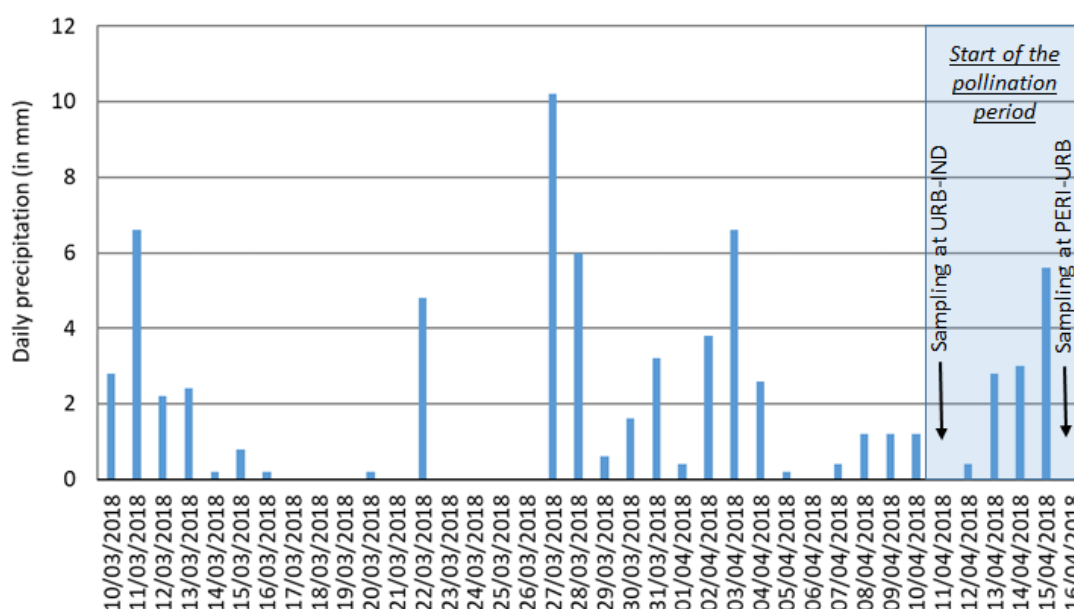
116

117 Figure 1: The two sampling locations situated 4.71 km apart from each other in  
118 Dunkirk conurbation and local wind rose from July 2017 to April 2018. The urban-  
119 industrial (URB-IND) and peri-urban (PERI-URB) areas are delimited in blue and  
120 red, respectively.

121 The air quality in the port of Dunkirk is influenced by both industrial (metallurgical  
122 plants, oil refineries, chemical factories) and urban (>200,000 inhabitants) activities  
123 close to two busy motorways (Figure 1). However, the average concentrations of  
124 regulated pollutants (Table 1) remain below the WHO guidelines owing to a good  
125 dispersion of pollutants by prevailing southwestern winds (Figure 1). Catkins were  
126 collected at two separate sites shown on the map (Figure 1), one located in the city  
127 with a strong industrial influence (URB-IND site: 51°01'36.1"N 2°20'03.0"E), the air  
128 quality monitoring station being located 580 m north of this site, and the other in the  
129 peri-urban area (PERI-URB site: 51°00'27.1"N 2°23'39.1"E). Catkins were collected  
130 during the pollination period, just before dehiscence, on the 11<sup>th</sup> and 16<sup>th</sup> April 2018  
131 for the urban-industrial and peri-urban sites, respectively. Rainfall intensity prior  
132 and during this period was relatively low (Figure 2).

133 Table 1: Mean daily values of PM<sub>10</sub>, NO<sub>2</sub>, SO<sub>2</sub> and O<sub>3</sub> from 1<sup>st</sup> January to 16<sup>th</sup> April  
134 2018 measured at the air quality monitoring station located in Dunkirk (data from  
135 Atmo Hauts-de-France).

Pollutant	Mean daily value ( $\pm 1$ s.d. in $\mu\text{g}\cdot\text{m}^{-3}$ )	WHO air quality guidelines (in $\mu\text{g}\cdot\text{m}^{-3}$ )
PM <sub>10</sub>	16.5 ( $\pm 12.2$ )	45 (24-hour mean)
NO <sub>2</sub>	20.1 ( $\pm 9.5$ )	25 (24-hour mean)
SO <sub>2</sub>	1.0 ( $\pm 1.4$ )	40 (24-hour mean)
O <sub>3</sub>	50.4 ( $\pm 17.8$ )	100 (8-hour mean)



136

137 Figure 2: Daily precipitation just before the pollination period marked by a blue area.

138 **Analysis of atmospheric particles adhered on catkins and pollen grains**

139 After sampling, catkins were allowed to dry in laboratory room temperature. Pollen  
 140 grains were extracted from catkins by tapping catkins above a glass Petri dish. Before  
 141 analysis, samples were stored at  $-80^{\circ}\text{C}$  in a sealed glass Petri dish to avoid  
 142 contamination by atmospheric particles. Pollen grains were sprinkled over carbon  
 143 adhesive tape and catkin bracts were gently spread on carbon adhesive tape. A 10-nm  
 144 layer of chromium was sputtered on samples (Q150T ES sample preparation system,  
 145 Quorum Technologies).

146 Individual analyses of particles adhered on pollen and bract surfaces were performed  
 147 by Scanning Electron Microscopy with Energy-Dispersive X-ray spectrometry  
 148 acquisition (SEM-EDX), using a JEOL 7100F FEG-SEM equipped with three  
 149 ultrathin-window ( $30\text{ mm}^2$ ) energy-dispersive X-ray detectors (Bruker XFlash 6/30),  
 150 enabling the analysis of elements with atomic number higher than boron ( $Z \geq 5$ ).  
 151 Spectral acquisitions were performed at 15 kV, 300 pA for 20 s. The particle  
 152 elemental composition is calculated by normalization of elemental mass percent  
 153 (PhiRhoZ quantification) without carbon and oxygen considered as major elements  
 154 in pollen and bracts. The total number of X-ray counts associated with the detected  
 155 elements (without C and O) gives their ratio compared to carbon and oxygen in the  
 156 analyzed particles. For the same particle size, a very low total number of counts  
 157 indicate a high contribution of carbon and oxygen.

158 The particle equivalent diameters were deduced from their projected area  
159 considering particles as spherical. Considering the low particle density on the surface  
160 of PGs, manual analysis of particles larger than 60 nm was performed on several  
161 thousands of individual PGs. For catkin inspection, particles larger than 500 nm  
162 were automatically detected and analyzed on the bracts. The resulting databases  
163 (particle size, morphology and elemental composition) were then statistically  
164 analyzed by Hierarchical Clustering Analysis (HCA) with the FactoMineR software,  
165 gathering in clusters particles with similar elemental compositions. Chemical  
166 elements with an average mass contribution lower than 5% in all clusters are not  
167 presented in this study.

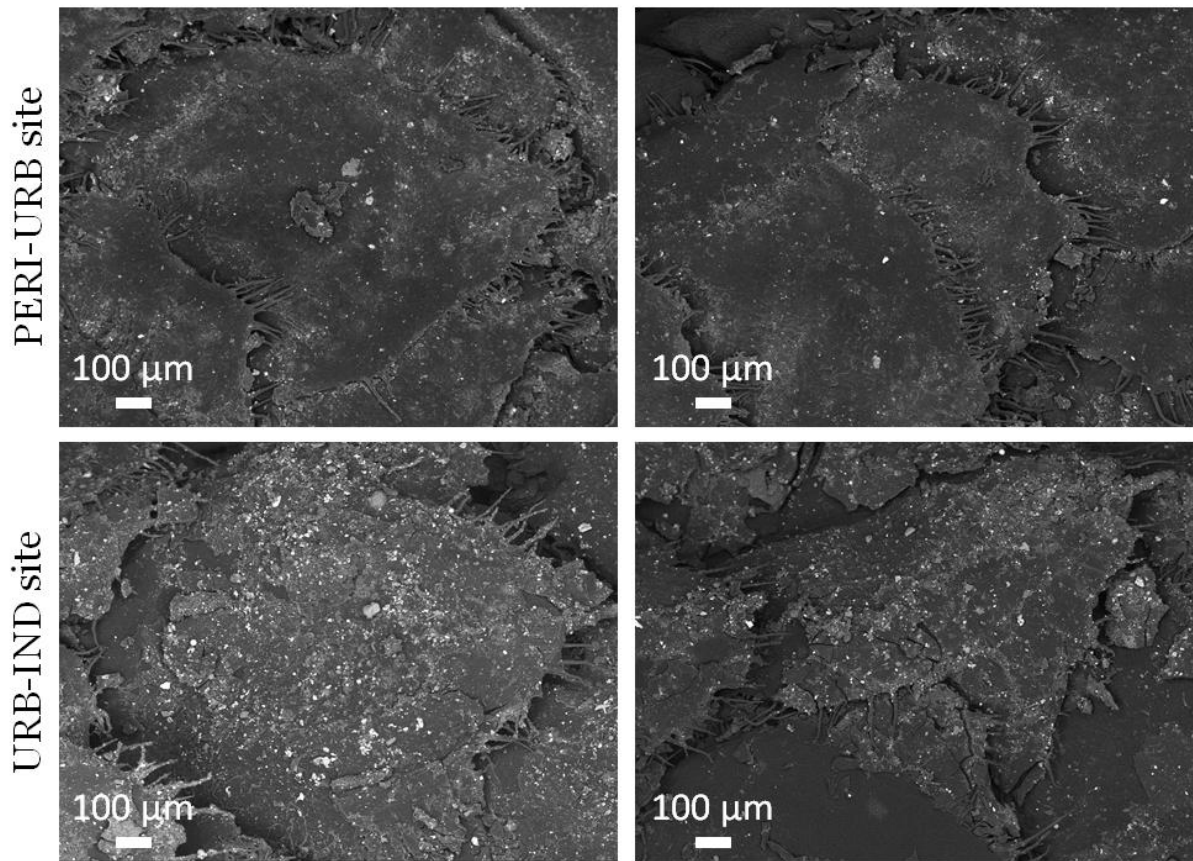
### 168 **3. Results**

#### 169 **Bract surface analysis**

170 Catkins were collected just before dehiscence and noticeably no BPG was observed  
171 on the surface of the bracts (Figure 3). APM deposited on the bract surfaces have  
172 average particle diameters consistent with an atmospheric origin:  $1.56 \pm 0.96 \mu\text{m}$  and  
173  $1.60 \pm 1.18 \mu\text{m}$  for the urban-industrial (URB-IND) and peri-urban (PERI-URB) sites,  
174 respectively. Particle spatial distribution on the surface of catkins is very  
175 heterogeneous within the same catkin (Figure 3). Thus, the particle surface density  
176 was not calculated on bracts but for PGs only. The particles observed on the surface  
177 of bracts were grouped into 9 clusters using Hierarchical Clustering Analysis  
178 (clusters C1 to C9 in Figure 4). Clusters C1 to C8 correspond to groupings of particles  
179 of similar compositions, while cluster C9 corresponds to a mixture of various  
180 particles not attributable to any of the other clusters. Clusters C1 to C3 are common  
181 to the particles deposited on catkins from the two sites and correspond to iron  
182 oxides, more or less enriched in calcium for C1 and C3, and iron-enriched  
183 aluminosilicates in the case of cluster C2. These particles represent respectively 60%  
184 and 33% of the particles observed on the surface of bracts of catkins from the URB-  
185 IND and PERI-URB sites. While clusters C4 and C5 made up of calcium-rich  
186 particles are characteristic of the URB-IND site, particles from clusters C6 to C8 are  
187 specific to the PERI-URB site and predominate at this site representing 64% of the  
188 particles analyzed. The low number of X-ray counts associated with these particles  
189 from clusters C6 to C8 (Figure 4) and their low chemical contrast in SEM images

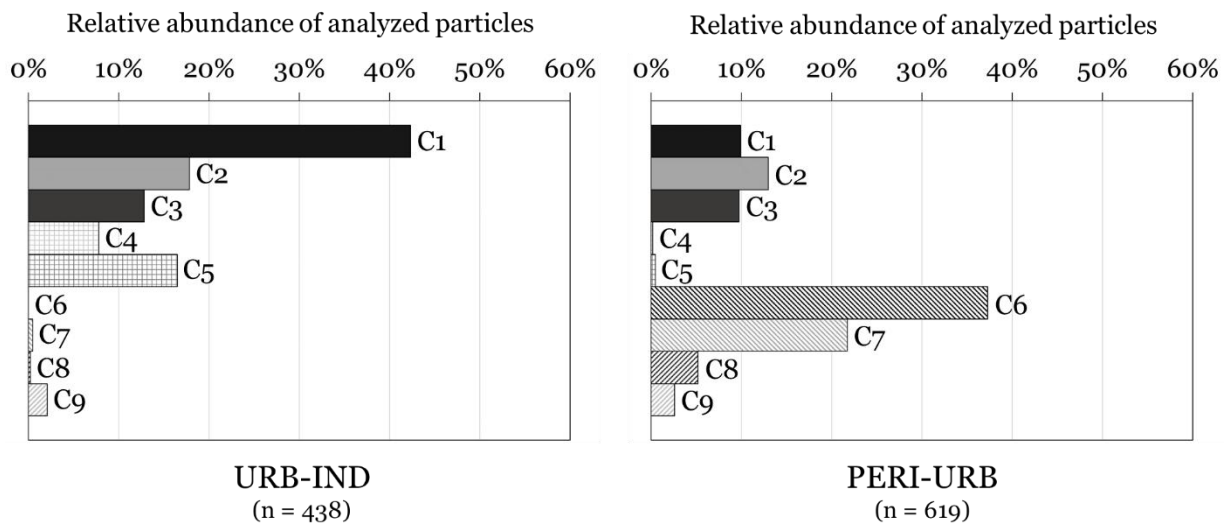


190 from the backscattered electrons indicate that they are carbonaceous particles  
 191 containing calcium, chlorine and potassium as minor elements.



192

193 Figure 3. Typical SEM images (chemical contrast mode) of catkin bracts sampled at  
 194 URB-IND and PERI-URB sites.



195

Clusters	Total counts	Elements (relative mass %)										Mean equivalent diameter (μm)
		Al	Ba	Ca	Cl	Fe	K	Na	P	S	Si	
C1												
C2												
C3												
C4												
C5												
C6												
C7												
C8												
C9												

C1	11302	0.02	0.00	0.04	0.01	0.86	0.01	0.00	0.00	0.01	0.04	1.7
C2	15013	0.15	0.00	0.09	0.04	0.13	0.08	0.01	0.01	0.01	0.45	1.7
C3	8426	0.04	0.00	0.19	0.03	0.53	0.04	0.01	0.01	0.02	0.10	1.5
C4	16909	0.10	0.00	0.32	0.02	0.14	0.03	0.01	0.01	0.03	0.23	1.4
C5	10431	0.03	0.00	0.72	0.03	0.06	0.02	0.02	0.00	0.02	0.08	1.4
C6	2141	0.03	0.00	0.31	0.26	0.05	0.18	0.04	0.04	0.02	0.05	1.6
C7	2587	0.00	0.00	0.21	0.44	0.00	0.22	0.07	0.00	0.00	0.00	1.2
C8	2415	0.01	0.00	0.27	0.08	0.02	0.16	0.03	0.24	0.13	0.04	2.4
C9	6851	0.03	0.00	0.04	0.03	0.28	0.03	0.01	0.02	0.01	0.05	1.8

196

197 Figure 4: Relative contribution and elemental composition (total counts and relative  
198 mass % without C, N and O) of particles larger than 500 nm observed on catkin  
199 surfaces at URB-IND site (619 analyzed particles) and PERI-URB site (438 analyzed  
200 particles).

### 201 Surface analysis of pollen grains

202 The level of particle deposition on PGs is identical between the two sampling sites  
203 (Table 2). Between 5 and 7% of the PGs were observed with at least one particle on  
204 their surface. The average number of particles per pollen grain is 0.1 particle.PG<sup>-1</sup>.  
205 However, this is a minimum value because only the visible surface of PGs was  
206 observed; a reasonable approximation would be to double this value considering  
207 firstly that the analysis is carried out on half of the surface and secondly that the  
208 particulate pollution is homogeneous between the visible surface and the non-visible  
209 surface.

210 Particles deposited on PGs range in size from a few tens of nanometers to several  
211 micrometers. About 25% of the observed particles are smaller than 500 nm. As an  
212 example, Figure 6 shows particles observed on the surface of PGs with their  
213 corresponding EDX spectra. Orbicules, which are rather rare on *Betula* pollen, were  
214 not observed on the surface of the analyzed PGs (Ruggiero and Bedini 2018).

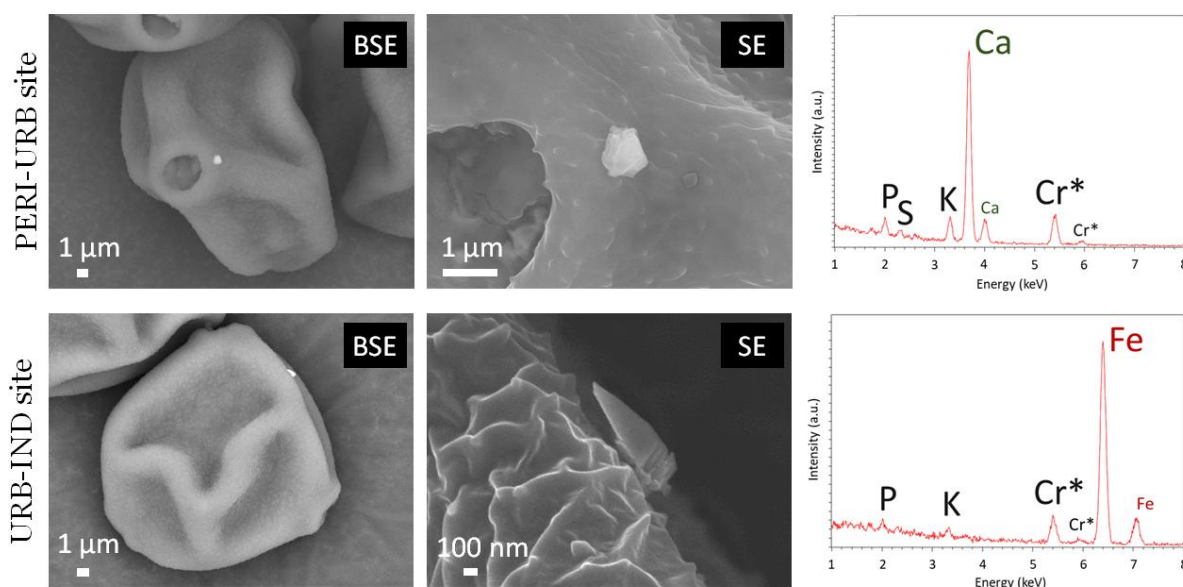
215 Table 2. Counts of particles deposited on pollen grains (PGs) extracted from catkins  
216 harvested in a peri-urban (PERI-URB) and an urban-industrial (URB-IND) site.

	PERI-URB	URB-IND
PGs analyzed	573	533
PGs with particles	30	38
PGs with particles (%)	5.2%	7.1%
Size range of particles (nm)	108 - 5275	62 - 7513
Particles larger than 500 nm	42	40
Particles smaller than 500 nm	16	14
Number of particles / PG with particles	1.9	1.4
Number of particles / PGs analyzed	0.10	0.10

217

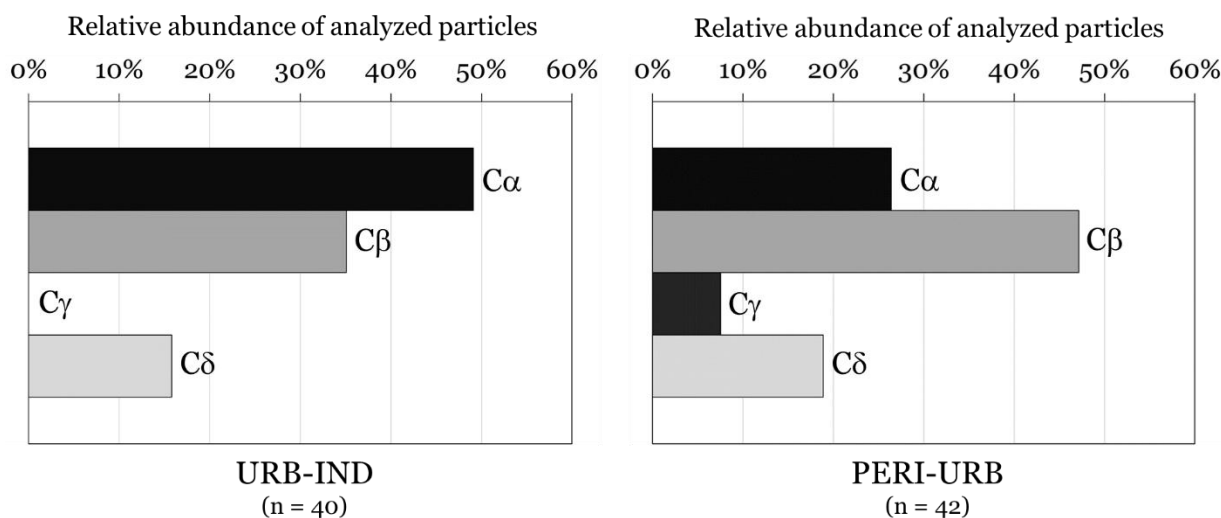
218 The particles deposited on the surface of BPGs were grouped into 4 clusters using  
 219 Hierarchical Clustering Analysis (clusters C $\alpha$  to C $\delta$  in Figure 7). Clusters C $\alpha$ , C $\beta$  and  
 220 C $\delta$  are common to samples from both PERI-URB and URB-IND sites. Cluster C $\gamma$  is  
 221 characteristic of the URB-IND site. Particles making up cluster C $\gamma$  were not detected  
 222 on the bracts but it should be noted that they correspond only to a very small number  
 223 of particles present on the surface of the BPGs (3 particles observed out of 42). Also,  
 224 considering the total number of particles observed on PGs, there is no statistically  
 225 significant difference in the relative contribution of clusters between PERI-URB and  
 226 URB-IND sites.

227 The main elements detectable on the surface of BPGs without particulate pollution  
 228 (i.e., P, S, K, Na and Ca) are consistent with analyses reported in the literature for  
 229 BPGs (Cerceanu-Larrival et al. 1991; Nilsson and Berggren 1991).



230

231 Figure 6. SEM images of a pollen grain from PERI-URB and URB-IND sites with a  
 232 Ca- and an Fe-containing particle attached, respectively (BSE: chemical contrast  
 233 mode, SE: topographic contrast mode), and EDX spectra of these particles (Cr\* is  
 234 from the sample metal coating).



235

Clusters	Total counts	Elements (relative mass %)										Mean equivalent diameter (μm)
		Al	Ba	Ca	Cl	Fe	K	Na	P	S	Si	
Cα	16691	0.00	0.00	<b>0.82</b>	0.01	0.00	0.05	0.02	0.04	0.03	0.02	1.6
Cβ	20052	0.06	0.00	0.09	0.02	<b>0.35</b>	<b>0.14</b>	0.02	0.06	0.04	<b>0.17</b>	2.1
Cγ	8277	0.01	<b>0.40</b>	0.07	0.03	0.01	<b>0.17</b>	0.01	<b>0.12</b>	<b>0.16</b>	0.02	2.5
Cδ	12599	0.00	<b>0.10</b>	<b>0.10</b>	0.03	0.00	0.30	0.15	<b>0.14</b>	<b>0.11</b>	0.03	2.0
Clean BPGs	-	0.00	0.00	0.09	0.00	0.00	<b>0.17</b>	0.14	<b>0.37</b>	<b>0.20</b>	0.00	-

236

237 Figure 7. Relative contribution and elemental composition (relative mass % without  
 238 C, N and O) of particles larger than 500 nm observed on birch pollen grain (BPG)  
 239 surface at PERI-URB site (42 analyzed particles) and URB-IND site (40 analyzed  
 240 particles). The average elemental composition (n=12) of clean BPGs (relative mass %  
 241 without C, N and O) is also presented.

## 242 4. Discussion

243 Atmospheric particles are known to settle on plants with an average deposition  
 244 velocity of 1 cm.s<sup>-1</sup> (Litschke and Kuttler, 2008). Surface analyses of catkins show  
 245 that male inflorescences of birch are actually contaminated with atmospheric  
 246 particles. The sampling at the urban-industrial site (URB-IND) is characterized by a  
 247 significant fraction of particles from cluster C1 with iron as dominant element; these

248 Fe-bearing particles most probably come from steelwork emissions from large  
249 metallurgical plants located in Dunkirk (Alleman et al. 2010; Cazier et al. 2011). The  
250 sampling at the URB-IND site is also marked by calcium-rich particles (cluster C5)  
251 which are emitted by cement factories and steelworks which use  $\text{CaCO}_3$  in  
252 desulphurization processes (Cazier et al. 2011). The clusters characteristic of  
253 carbonaceous particles observed in the peri-urban sample (clusters C6 to C8) have in  
254 common predominant calcium, potassium and chlorine. It is not possible to clearly  
255 determine the sources of these particles from these elements alone. At most, we can  
256 suggest a combined influence of industrial and urban activities. The low presence of  
257 marine aerosols (typically sodium-rich particles) may be due to their washout by  
258 rainfall during the few days prior to collection (Figure 2), as these particles are highly  
259 water-soluble.

260 BPGs collected from catkins harvested just before dehiscence show low particulate  
261 pollution in the order of 0.1 particle per PG (Table 2). The pollution level of PGs did  
262 not differ significantly according to the location of the tree (i.e. in urban-industrial or  
263 peri-urban areas). Moreover, the particles analyzed on the surface of PGs have  
264 relatively similar EDX spectra for the two sampling areas. Only a specific pollution by  
265 particles containing barium was observed in the samples from the URB-IND  
266 industrial site (cluster C, Figure 7), although the relative contribution of these  
267 particles is not significantly different between the two sites studied. Barium is a  
268 characteristic element of automobile transport, but barite ( $\text{BaSO}_4$ ) is also used in  
269 some industrial processes (Monaci and Bargagli 1997).

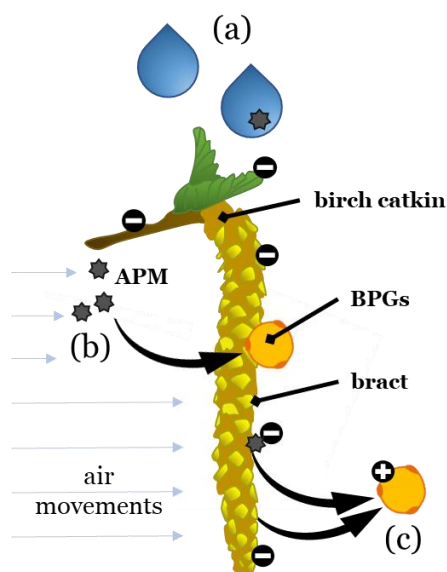
270 Adhesion of particles on PGs can take place under different circumstances: when  
271 pollen is still on the parent plant, during pollen atmospheric transport or during  
272 pollen settling and resuspension cycles (Visez et al. 2020). The relative contribution  
273 of these three mechanisms on the deposition of APM on PGs was previously  
274 completely unknown (Visez et al. 2020). By comparing the surface concentration of  
275 APM on BPG from this work and the one from the work of Choël et al. (2022), the  
276 deposition of APM on BPGs appears to take place mainly during the windborne  
277 transport of PGs. More precisely, a number of particles more than 8 times higher was  
278 observed on BPGs collected directly from the atmosphere (BPGs carried an average  
279 of 0.86 particles) compared to BPGs collected from catkins (0.1 particle per PG)  
280 (Choël et al. 2022). The study by Choël et al (2022) was carried out under conditions  
281 of similar air particulate load:  $26.3 \mu\text{g.m}^{-3}$  for  $\text{PM}_{10}$ . The relative significance of

282 contamination by coagulation processes between windborne PGs and APM by  
283 deposition-resuspension still remains unknown.

284 To our knowledge, no other quantitative study has investigated PM pollution of PGs  
285 directly in inflorescences. Several reports of particulate pollution of the surface of  
286 PGs have been made for several plant species and under different contamination  
287 conditions but without specifying the number of particles per PG (Visez et al. 2020).  
288 In this work, PGs were extracted from catkins following a protocol used in previous  
289 qualitative studies on the pollution of PGs in inflorescences (Azzazy 2016; Guedes et  
290 al. 2009; Namjoyan et al. 2020; Sedghy et al. 2016). This protocol may induce a  
291 positive artifact when handling catkins in the laboratory. Catkins are indeed covered  
292 in particles and shaken (in some earlier works catkins were sometimes crushed) in  
293 order to extract PGs. Some particles may detach from the surface of catkins during  
294 shaking and be redeposited on simultaneously released PGs. Moreover, as is  
295 common in this type of work, catkins were collected before dehiscence so as to collect  
296 a maximum of PGs. However, this practice could minimize particulate pollution of  
297 PGs which are in the case of birch still enclosed in the bracts at this stage of  
298 maturation. In real conditions, exposure of PGs to particulate air pollution occurs  
299 during dehiscence when bracts split open exposing enclosed PGs to air pollutants  
300 hence offering less protection to PGs. We suggest a future study involving pollen  
301 sampling with a PM<sub>10</sub> impactor located in the immediate vicinity of the parent tree  
302 and under prevailing winds. The presence of PGs transported from other more  
303 distant sources cannot be prevented using such sampling scheme, but most of them  
304 would come from the nearest source where concentrations much higher than  
305 ambient concentrations are expected.

306 We propose three mechanisms by which atmospheric particles can be deposited on  
307 BPG prior to atmospheric transport (Figure 8): (a) wet deposition, (b) dry  
308 deposition, and (c) electrostatic interaction during pollen blow-off. The surface of  
309 catkins was coated with particles with the impossibility of distinguishing between  
310 wet and dry deposition. At most, it could be hypothesized that PGs were probably  
311 protected from rain to prevent abortive germination within the catkin (Grote et al.  
312 2003). The influence of the electrostatic environment of a PG and its electrical  
313 charge on its transport and interactions with other atmospheric particles is far from  
314 being understood and relatively little studied (Harrison and Carslaw 2003). The  
315 deposition mechanism by electrostatic adhesion during dehiscence (Figure 8c) is

316 speculative, but is based on observed interactions in the atmosphere between  
317 positively charged combustion particles and globally negatively charged bioaerosols  
318 (Roos and Dutertre-Laduree 1990). Plants are indeed on overall negatively charged  
319 which could enhance the capture of some positively charged APM (Bowker and  
320 Crenshaw 2007a, 2007b; Niklas 1985; Vaknin et al. 2000). On the other hand, the  
321 electric charge of PGs just after shedding is slightly positively shifted for some plant  
322 species (Bowker and Crenshaw 2007a). Pollen sampling close to plants using an  
323 impactor, as previously suggested, could make it possible to provide insight into the  
324 effects of electrostatic interactions. Furthermore, it cannot be ruled out that the  
325 sampling method used in the laboratory could affect electrostatic interactions that  
326 could facilitate adhesion of metallic particles such as clusters C $\beta$  or C $\gamma$  containing  
327 iron or barium respectively (Figure 7).



328  
329 Figure 8. Schematic representation of the mechanisms of transfer of atmospheric  
330 particles to BPGs in catkins or during pollen blow-off: (a) wet deposition (b) dry  
331 transfer by impaction or sedimentation (c) electrostatic interactions during PG  
332 release from catkin.

333 The effects of particulate pollution of PGs on asthma and allergy are still unknown.  
334 Although the deposition rate observed is low (0.1 particle per PG) the chemical  
335 nature of particles could play a greater role than the total mass of deposited  
336 particulate matter. The correlation between pollen concentrations and daily clinic  
337 visits for pollinosis is even more significant with higher concentrations of some  
338 chemical constituents of PM<sub>2.5</sub> such as non-sea salts Ca<sup>2+</sup> or SO<sub>4</sub><sup>2-</sup> (Phosri et al.

339 2017). Several studies showed an enhancing or aggravating effect of particulate air  
340 pollution on allergy and asthma. For example, epithelial cells exposed to lead-  
341 contaminated BPGs showed an increase in IL-5 gene expression (Bellanger et al.  
342 2012). Furthermore, particles on the surface of PGs with particularly fine sizes (60  
343 nm minimum) were observed here for the first time. Health effects of nanoparticles  
344 in combination to allergens remain largely unknown.

345 As regards potential effects on germination, several examples of negative effects of  
346 particles containing various metals on the germination or viability of pollen of  
347 different species are reported in laboratory studies (Dutta Gupta et al. 2019;  
348 Speranza et al. 2007, 2010, 2013; Wang et al. 2014). More nuanced conclusions on  
349 the effect of industrial pollution on the germination capacity of PGs have been drawn  
350 from field works (Helander et al. 1997; Kozlov and Zvereva 2004). Laboratory  
351 studies seeking to evaluate the effects of APM on in vitro germination should use  
352 realistic amounts of particles deposited on pollen as close as possible to those  
353 observed in the atmosphere (0.86 particles per PG) (Choël et al. 2022). Finally, to  
354 understand the overall effects of APM on birch reproduction, the deposition of  
355 particles on female catkins should be further investigated.

## 356 **5. Conclusion**

357 PGs collected directly from catkins on trees growing in an urban-industrial or peri-  
358 urban environment are very little polluted (0.1 particle per PG) despite a significant  
359 contamination of the surface of catkins by particles, some of which are of industrial  
360 origin. In comparison, the particulate pollution of airborne BPGs suggests that they  
361 are polluted mostly during transport (0.86 particles per PG). The extent of  
362 particulate contamination might be correlated with the residence time of pollen in  
363 the atmosphere. The significance of particulate pollution of the pollen surface on  
364 allergic sensitization or on exacerbation of symptoms is not unraveled simply  
365 because individual exposure to polluted pollen is not known. At a time when  
366 automatic real-time counting and identification of pollen in the atmosphere is now  
367 operational, a next step in these instrumental developments would be to assess in  
368 real-time the pollution degree of single PGs.



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