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## Impact of Environmental Stressors on Birch Pollen Allergenicity and Fertility in Northern France

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### Aim of the study:

Although its main purpose is to ensure sexual reproduction, birch pollen grain is also responsible for allergic rhinitis and asthma, also known as pollinosis. In Northern Europe, *Betula pendula* is the birch species with the most allergenic pollen (P. Beck et al. 2016; G. D'Amato et al. 2007; Emberlin et al. 2002). Alteration of pollen fertility, changes of protein and lipid content, modifications of the chemical composition of the surface of birch pollen grains (I. Beck et al. 2013; Bychkova and Khlebova 2019; Cerceau-Larival et al. 1990; Franze et al. 2005; Ramírez-Aliaga et al. 2022) : numerous studies highlight the interactions between atmospheric pollutants and pollen grains (Sénéchal et al. 2015). In addition, many studies have highlighted the exacerbation of pollen allergies symptoms by atmospheric pollution (Bowler and Crapo 2002; Burte et al. 2020; Gennaro D'Amato et al. 2016; Lubitz et al. 2010). Birch trees are widespread in Northern France, a densely populated, highly urbanised and industrialised region. For these reasons, the goal of my thesis is to assess the impact of specific environmental stressors in Northern France on the pollen grains allergenicity and fertility. To do so, we aim to characterize the pollution at the vicinity of the trees and to know the state of the pollen from the trees. The followed study shows preliminary results and the establishment of a germination protocol.

### Material and method:

The first step was to identify contrasting sampling sites with issues specific to the region. Once the trees were selected, the soil was sampled at the foot of the tree and pollen was collected from the catkins. To characterise the environment at the vicinity of the trees, the heavy metal content in soil was quantified using Inductively Coupled Plasma Mass Spectrometry. To determine the state of the pollen, we measured its allergenicity pollination by quantifying the allergen bet v 1 in each sample. Also, the pollen fertility can be assessed by counting the germination rate of the pollen. As pollen grains need rehydration and nutrients, germination protocol has been set up to obtain the best results of germination rate so that all factors are controlled to compare pollen fertility of each tree.

### Results:

A total of 97.28 g of pollen from 12 birch trees was harvested at 6 sites different sites in Northern France. Samples were taken at 2 urban sites (localised in a metropolitan area), 2 industrial sites (a brownfield and an active metallurgical industrial site) and 2 rural sites (one near a slag heap and one in countryside).

As the pollen samples had been stored at -80°C, they had to be thawed before being rehydrated. The results show that the germination rate is the highest when thermal shocks are avoided, i.e. a gradual return to room temperature is recommended. Moreover, pollen germination kinetics experiments have also shown that the rate of germinated pollen stagnates after 4 hours. After this period, the pollen tubes continue to elongate and tangle, making image analysis unworkable.

The average Bet v1 concentration in the pollen samples are 91 091ng/10mg of pollen from rural sites, 66 942ng/10mg of pollen from urban sites and 77 811ng/10mg of pollen from industrial sites.

Means concentrations of heavy metals in soil show a very high levels of Cu, Zn, Pb, Cd, Se and As in industrial sites : Cu : 299 mg.kg<sup>-1</sup>, Zn : 8754 mg.kg<sup>-1</sup>, Pb : 859 mg.kg<sup>-1</sup>, Cd : 42 mg.kg<sup>-1</sup>, Se : 5.6 mg.kg<sup>-1</sup>, As : 141 mg.kg<sup>-1</sup>; followed by rural sites : Cu : 65 mg.kg<sup>-1</sup>, Zn : 235 mg.kg<sup>-1</sup>, Pb : 105 mg.kg<sup>-1</sup>, Cd : 0.46 mg.kg<sup>-1</sup>, Se : 1.3 mg.kg<sup>-1</sup>, As : 24 mg.kg<sup>-1</sup>; and then urban sites Cu : 33 mg.kg<sup>-1</sup>, Zn : 145 mg.kg<sup>-1</sup>, Pb : 101 mg.kg<sup>-1</sup>, Cd : 0,9 mg.kg<sup>-1</sup>, Se : 0.4 mg.kg<sup>-1</sup>, As : 11 mg.kg<sup>-1</sup>.

### Conclusion:

In conclusion, preliminary results show that despite the high concentrations of heavy metals in the industrial soils, allergenicity of pollen from tree growing on this such sites doesn't show higher concentration of the

allergen Bet v1. One of the reasons for these results may be the robustness of birch, which means it can grow in metal-rich soils without being impacted. Another next analysis will be to quantify heavy metal content in pollen samples to know if heavy metal in soil could influence heavy metal concentration in pollen grain in catkins. However, IgE reactivity of birch pollen-sensitized patients will be measured to assess allergenic risk for each pollen sample. The germination tests now allow us to assess the ability of pollen to germinate under optimum conditions and to observe differences in germination rate between the sampling sites.

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