**Building an automatic Pollen Monitoring Network (ePIN): Selection of optimal sites by clustering pollen stations**

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**Abstract**

**Introduction:** Pollen monitoring provides a tool for allergy diagnosis and prevention. A disadvantage of current manual pollen monitoring networks is the workload required for obtaining information on time. Knowledge on the optimal distribution of pollen traps for adequately representing an area is lacking.

**Objective:** To determine the optimal number and location of monitoring stations within an automatic online pollen monitoring network (ePIN).

**Method:** We established and run a dense pilot pollen monitoring network of 27 manual Hirst-type pollen traps across Bavaria, Germany, during 2015. Based on its data, Ward clustering analysis was then performed to select the optimal locations for the sites in the final pollen monitoring network ePIN.

**Results:** Bavaria can be clustered into three large pollen regions with eight zones. Within each zone, pollen diversity and abundance among different locations do not vary significantly. Based on the determined pollen zones, we opted to place one automatic monitoring station per zone. The described work led to the installation of eight automatic pollen monitors across Bavaria, serving 13 million inhabitants.

**Conclusions:** Here we have standardized the method for establishing an aerobiological network. The method defines homogenous aeropalynologically locations, which reduces redundancy within the network and subsequent costs. Thus, allergic citizens can therefore be informed in a timely and effective way even in larger geographical areas.

**Keywords:** Aerobiology; air quality; automatic pollen monitoring; BAA500; Bavaria; biomonitoring network; clustering; pollen

**Introduction**

Pollen is part of the biological exposome carrying allergens, fungi and bacteria able to activate the human immune system ([1-3](#_ENREF_1)). There are a number of networks that routinely monitor airborne pollen worldwide. These were built for a range of purposes ([4](#_ENREF_4)), such as examining gene flow ([5](#_ENREF_5)), allergy prevention ([6-10](#_ENREF_6)), crop forecasting, pest control, and impacts of land use changes ([11-15](#_ENREF_11)), climate change impacts ([16-21](#_ENREF_16)) and monitoring biodiversity ([22-25](#_ENREF_22)).

The simplest way to sample airborne pollen and fungal spores is to collect the particles deposited or impacted on certain surfaces ([26](#_ENREF_26)). First generation traps (1G) do not provide volumetric information ([27-29](#_ENREF_27)), although some have been termed semi-volumetric because they were calibrated with help of an anemometer ([30](#_ENREF_30)). On the other hand, second generation samplers (2G) use a range of different sampling principles ([31](#_ENREF_31)) and provide volumetric data that is comparable. The system designed by Hirst more than 65 years ago is based on the impaction principle ([32](#_ENREF_32)), and is the standard in pollen and fungal spore monitoring networks in countries such as Germany ([33](#_ENREF_33)). The Hirst-type trap has several advantages, e.g. it delivers pollen concentration data at a temporal resolution of up to 2 h, it provides volumetric information (i.e. pollen/m3) and it has a long autonomy of up to 7 days.

There are currently more than 600 Hirst-type traps actively running worldwide, mostly in Europe (<https://www.zaum-online.de/pollen-map.html>); ([34](#_ENREF_34)). A standardized sampling system and working methodology is essential for a network, and there are numerous publications on the standardization of this monitoring method ([33](#_ENREF_33), [35-44](#_ENREF_35)). The “Minimum requirements for aerobiology” by Galán et al. ([45](#_ENREF_45)), supported by the European Aerobiology Society, is recognized as an international standard for pollen monitoring using the Hirst-type trap. Other devices currently used in active networks include the Cour method ([46](#_ENREF_46)), Rotorod samplers ([47](#_ENREF_47)) and Durham traps ([48](#_ENREF_48)).

The main disadvantage of all manual methods is that data production is highly time consuming and thus it is not feasible to provide timely information with respect to the current, real-life situation. A time lag of several days between actual pollen flight and reported pollen flight is common. A solution could be automation, but the complexity of pollen identification has made this impossible until now. New technologies allow for fully automatic-online pollen monitoring with third generation automatic traps (3G). The main advantages of these traps are that they can provide almost real-time information and the data are free of random errors produced by human interferences. Such 3G automatic systems are starting to be used for routine pollen monitoring, e.g. the KH-3000 in Japan ([49](#_ENREF_49)), the Plair PA-300/Rapid E in Switzerland ([50](#_ENREF_50)), Pollen Sense in the USA (<http://pollensense.com/>), the BAA500 in Germany ([51](#_ENREF_51)) or Wibs-4 in the Republic of Ireland ([52](#_ENREF_52)).

The main reason for building the Bavarian pollen monitoring network ePIN was to reduce delays in the dissemination of pollen information and to inform allergic citizens, physicians, and health organizations operationally, in a timely manner. Another aim of the ePIN network was to serve as extensive sensor of environmental changes. To do so, we had to increase the representativeness of pollen monitoring across Bavaria. The whole federal state has actually a population of 13 million inhabitants, but airborne pollen has routinely been monitored at only three locations. A handicap of classical pollen monitoring networks is that they are often built on the basis of stations set up by individuals, but this does not guarantee an optimal choice of positions for pollen monitoring stations. There is a general lack of studies on the optimization of monitoring locations, and so the decision to install a station has usually been based on personal preferences often in large urban areas.

The aim of this study was to standardize the procedure of setting up an operational automatic pollen monitoring network. To do so, we answered a series of standard questions for any network: What is the aim of the network? How many traps are needed and where should they be installed? Which are the optimal locations for a trap? The mains outcome of this study was to decide on an effective method for selecting the optimal pollen monitoring locations within an extensive area, such as Bavaria, comprising more than 70.000 km2.

**Material and Methods**

**Study area**

Bavaria with a surface of 70.553 km2 is the largest federal state in Germany and is located in the south of the country (Figure S1). Distance between locations in Bavaria can be more than 400 km. The environmental zones are quite heterogeneous, containing a wide range of climates from the cold Bavarian Alps in the South (Zugspitze with an annual mean temperature of -4.3°C and mean precipitation of 2071 mm) to the warmer-dryer Franconian wine area in the Nord-West (Würzburg with an annual mean temperature of 9.6°C and mean precipitation of 601 mm). The annual mean temperature at Munich is 8.7°C with a mean annual precipitation of 834 mm and the annual mean temperature at Nuremberg is 9.3°C and mean precipitation is 637 mm (all climate data from the German Meteorological Service for the reference period 1981-2010). These different conditions render representative pollen monitoring across the state rather complex.

**>> Figure S1**

**Steps of building the network**

1. **Selection of pollen monitoring methods**

We reviewed the advantages and disadvantages of the current options for pollen monitoring. For the final (permanent) network ePIN, an automatic pollen monitoring system was selected, whereas for the pilot (test) network we chose the Hirst-type monitoring method ([32](#_ENREF_32)).

1. **Review on the optimal site conditions for installing a pollen monitoring device**

In 2014 we performed an international survey among the administrators of pollen monitoring networks about the main factors affecting the decision of trap location at the local (site) scale and reviewed the already published information.

1. **Selection of the optimal pollen monitoring network design (number and location of devices)**

To select the optimal number and location of monitoring stations across Bavaria, we first built a dense network of 27 Hirst traps throughout the country and, based on the results obtained, we then reduced the number of locations with mathematical methods until a representative optimum was reached.

***a) Selection of monitoring locations in the pilot network***

The pre-selection of 27 pollen monitoring locations was performed in order to increase the regional representativeness of the pollen sampling. It was based on a semi-quantitative method aimed at building a redundant network covering the majority of the Bavarian territory, population and pollen sources. For the pre-selection, the following factors were considered: demography, availability of historical time series, climate, land use types, topography and proximity to local pollen sources. The pre-selection was done trying to satisfy most of the specific features that the network should have:

1. Must conserve historical time series, so historical locations were included.
2. Must be informative for the bulk of the population, so the most populated areas were closely monitored. Bavaria is unevenly populated, with several major urban agglomerations (Figure S2a). The 25 km radius buffer around all the pre-selected stations covered 94% of the Bavarian population.
3. Must be capable of detecting pollen episodes early, so source areas and borders of the country were preferred (Figure S2b). The places near main pollen sources are optimal locations to perform a better forecasting of airborne particles. Broad-leaved forests, which incorporate spring, early-flowering allergenic trees, like alder and birch, are present in patches, spread over Bavaria and near its borders. This suggests the possibility of setting stations closer to the borders of Bavaria to earlier catch the moments of the forests starting flowering out of the boundaries. Grasses are common in Bavaria; their pollen is known to be less efficiently transported than those from anemophilous trees.
4. Must provide data for model-based forecasting, for data assimilation in near-real-time and for the model evaluation.
5. Must cover the major biogeographic environments existing in Bavaria. Temperature is important from for season start and duration: plants the warmer parts tend to flower earlier. In this sense, the warmest regions are north-west and south-east. The colder and wetter areas are the alpine mountains in the south and the area of the Bavarian Forest in the east (Figure S2c, S2d).

**>> Figure S2.**

The pre-selection of the locations for new stations was carried out for zones with a 25 km radius. The selection of the specific location was then done by screening for optimal monitoring conditions inside each pre-selected zone. To ensure a proper coverage of all pollen sources in Bavaria, an analysis with the System for Integrated modeLling of Atmospheric coMposition (SILAM, <http://silam.fmi.fi>) was conducted, ensuring a large coverage of Bavarian sources ([53](#_ENREF_53), [54](#_ENREF_54)). The footprint covered in 2015 is shown in Figure 1.

A footprint is the area comprising the sources that affected the monitored parameter during a specific observation period (in the current case, pollen data of a specific day). Such a footprint delineates the area where the sources would contribute to the monitor readings, if emitting pollen during the corresponding time. Areas outside the footprint do not affect the pollen monitor. This “negative” part of the footprint message makes it a handy tool for delineating the network weaknesses: if regions are not covered by the footprints during the flowering period, the network will not record emission that might happen there. Footprints have a direct relation to the fraction of air reaching the monitor. Formally, the convolution of emission E, and the footprint “intensity” *φ*\* over space and time is equal to the mean concentration *C* at the specific place during the specific period (monitor location and time of activity) (function 1.



(1)

Thus, a footprint delineates the area where the sources would contribute to the monitor readings if emitting pollen during the corresponding time and shows which sources can affect the monitor during the specific observation period. The sum of the footprints of all daily measurements by the 27 ePIN stations in 2015 is shown in figure 1. Areas outside the footprints do not affect the monitors. Therefore, this “negative” part of the footprint message makes it a handy tool for delineating the network weaknesses: if some region is not covered by the footprints during the flowering, the network will not record emission that might happen there. The footprints were calculated over three days backwards from the observation day, i.e. covered the areas from where the emitted pollen needed up to three days to reach the monitor.

**>> Figure 1.**

The exact location to set up a pollen trap was selected by a site visit of potential places within the 25km-radius zones. Criteria for a site were reviewed in step 2 and are listed in Table 2. This resulted in 27 locations described in Table 1.

**>> Table 1.**

***b) Establishment and quality control in the dense pilot pollen monitoring network***

A dense network of 27 Hirst pollen traps was set up across Bavaria at the selected locations (Table 1) during the winter of 2014-2015. The network then was operated from 15 March until 15 September 2015. All traps were located following the optimal monitoring conditions (see Table 2; e.g. all stations were built homogeneously at 12 m a.g.l. (±3 m), all traps were located at 1.5 m above roof level by a standard tower (Figure S3a) and at least 2 m from the building edge). Flow rates of the pollen traps were calibrated using the same flowmeter thereby reducing intra-rotameter variability ([41](#_ENREF_41)), Drums and microscope slides were processed centrally by a single laboratory (figure S3b) under homogeneous conditions. The drums were sent bi-weekly to the 27 monitoring stations. Slides were processed using the standard operating procedure described by Galán et al. ([55](#_ENREF_55)).

Each pollen slide corresponded to one independent day (Figure S3c). All slides had a blue line, marking midday (12:00). Four blue dots were set 1mm apart from each other at the center of the slide with a standard own-made tool to guide the analyst for the starting point of each horizontal line. Pollen microscopic identification and counting were conducted in four continuous horizontal sweeps along the whole slide under 400x magnification, each sweep starting from each one of the marked points.

Sub-sampling of the slide is essential for reducing workload. The area of the slide sub-sampled during the analysis was at least 7% of the slide, following the recommendations of VDI norm ([33](#_ENREF_33)). The use of a standard 12.5 mm net micrometer reduced the area of the slide examined to 9% when examining 4 transects (or 7% when using a 10mm net micrometer) (figure S2d). A standard correction factor was used to reduce error. Pollen counts (raw data) were entered into a specially designed computer program to reduce typing errors (figure S2f). Data were exported from this program and stored in an online SQL ZAUM database.

In total, 13 pollen types were analyzed: *Alnus*, *Ambrosia*, *Artemisia*, *Betula*, *Carpinus*, *Fraxinus, Picea*, *Pinus, Plantago*, Poaceae*, Populus*,Cupressaceae*,* and *Urtica.* Pollen were reported as 12-hour concentrations for each station during the study period. Pollen not falling within the 13 specified pollen types were reported as “unspecified” pollen grains.

**>> Figure S3.**

An external Quality Control program of the analysts was performed with a novel method, to be published in detail by Smith et al. ([43](#_ENREF_43)).

**4. Establishment of the automatic final pollen monitoring network**

***a) Selection of the definitive number of traps by clustering analysis***

We clustered the information obtained from all pollen traps in order to determine areas with a similar distribution in pollen loads. Due to the complexity of data (daily pollen monitoring of 27 stations and 13 pollen types), we applied a multivariate statistical method able to consider all the variables at the same time.

**First**, we preselected the part of the database to be included in the analysis. We selected the most abundant pollen types for the clustering analysis (>1000 pollen grains/season on average): *Betula*, Cupressaceae, *Fraxinus*, *Pinus*, *Picea*, Poaceae and Urticaceae. Those are the pollen types with the largest spread of annual pollen values, whereas less abundant pollen types do not show big differences among the locations and so it makes little sense to cluster pollen zones based on them (Figure 2). From the 27 monitoring stations, DEERLA was excluded from the analysis due to technical malfunctions. Cupressaceae pollen was excluded from the analysis due to the incomplete monitoring of the whole season. *Pinus* was excluded from the clustering analysis to avoid overrepresentation of Pinaceae family for designing the network, *Picea* was included. The pollen season for each pollen taxon was defined for the whole Bavaria as following, excluding the long tales before and after the season with zero values: *Betula* (from 1/4 to 14/5); *Fraxinus* (from 25/3 to 2/5); *Picea* (from 22/4 to 26/5); Poaceae (from 8/5 to 6/8); *Urtica* (from 1/6 to 8/9).

**>> Figure 2.**

**Second**, we calculated all Pearson correlations in daily pollen concentrations between pairs of stations (26 stations). We applied this correlation analysis for each selected pollen type (5 pollen types in total: *Betula*, *Fraxinus*, *Picea*, Poaceae and Urticaceae). For each pollen taxon, we only included in the analysis the stations with >80% of the data during the season. When a correlation is too low, as could happen by chance, all correlations <0.5 are equalized to 0 in the correlation matrixes for the clustering analysis.

**Third**, we applied a clustering analysis to the correlations’ coefficients (Hierarchical clustering by Ward method) ([56](#_ENREF_56), [57](#_ENREF_57)). In the analysis, 26 cases were included to be conglomerated (each monitoring station). Each variable was defined as the correlation coefficient between one station (for each pollen type) and each one of the 26 stations (for the same pollen type). Twenty-six cases were included in the analysis (one per station). A correlation coefficient is not a metric of distance *per se*, but the combination of them allows us to calculate Euclidean distances. Furthermore, a visualization of the five closest Euclidean distances for each element is shown by a network plot.

***b) Determination of the final monitoring locations***

Within each cluster calculated in step 4a, one station was then selected. We selected the most relevant station of each cluster using the following selection criteria:

1. The station with the highest **population** was selected (If two or more stations differ by **<0.5% population**, they are all selected at this stage).

2. In the case of a draw (similar population), the station **closest to the border of Bavaria** was selected.

3. Two selected stations cannot be located closer than **70 km apart** (ensuring a proper coverage of the whole surface). If two stations are closer than 70km, then the most populated location is selected in one sub-cluster and the next by population is selected in the other cluster.

4. Four stations using the Hirst method were kept as a parallel **manual network** to maintain a historical time series (DEOBER, DEMUST, DEBAMB and DEUFS) and were not selected for the automatic network.

An automatic network was then built in Bavaria based on these calculations.

**Results and Discussion**

The main goals of a pollen monitoring network are: 1. to provide near real-time health information to pollen allergic individuals and health care practitioners. 2. To serve as a valuable source of biological data for (but not limited to) long-term biodiversity and climate change studies. Hence, the network must allow for, if possible, the continuation of historical time series and monitor the whole spectrum of airborne pollen types, not only the allergenic ones.

A permanent network of Hirst-type traps may provide pollen information with a minimum lag time of 1 to 2 days, involving a colossal human effort and huge costs (arising mostly from the need of experienced personnel). We therefore focused on an alternative, automated, detection system able to provide near-real-time information. There is a well-established monitoring network of 120 traps based on an automatic-online monitoring system in Japan, the KH-3000 ([49](#_ENREF_49)), but this automatic system until now has been unable to provide accurate information on the complete range of pollen diversity. Other systems look promising for the development of a complete and reliable automatic recognition system of pollen: e.g. Pollen Sense in the USA (<http://pollensense.com/>), BAA500 in Germany ([51](#_ENREF_51)) or Plair PA-300 Rapid E ([50](#_ENREF_50)) and Swisens ([www.swisens.ch](http://www.swisens.ch)) in Switzerland. Of these, to date, only two pollen monitoring systems are currently probed as functional for automatic pollen monitoring: BAA-500 ([51](#_ENREF_51)) and Plair PA-300 ([50](#_ENREF_50)).

The BAA500 is the preferred monitoring system for the ePIN network because it has specific features that make it a good candidate for the transition from manual to automatic monitoring. The BAA500 uses image recognition emulating the process of a human using a microscope, and can provide 2-hour pollen concentrations online and the averaged identification error rate is below 10% ([51](#_ENREF_51)).

**Optimal pollen monitoring conditions**

The results of the survey among European experts about optimal conditions for pollen monitoring are shown in Table 2. Most of these criteria are extracted from the pre-established knowledge about pollen monitoring ([31](#_ENREF_31), [45](#_ENREF_45), [55](#_ENREF_55), [58](#_ENREF_58)).

**>> Table 2.**

Some criteria, such as the optimal height of the trap location, have not been defined quantitatively because the vertical distribution of airborne pollen has not been exhaustively studied, as reviewed by Damialis et al. ([59](#_ENREF_59)). In this sense, they should not be termed as optimal but more motivated by the necessity of defining comparable standard conditions and homogeneous for the whole network. The reason of locating traps at roof level is to have a more uniform information over a greater area in comparison to measurements on the ground with an extreme influence of plants nearby.

**ePIN network**

At a regional scale, the pollen stations had to be placed in areas of interest to the general population, thus demography was a main criterion. Other factors that strongly affect air quality such as topography ([60](#_ENREF_60)), land use ([61](#_ENREF_61)) or weather ([62](#_ENREF_62), [63](#_ENREF_63)), which modify the interpretation of pollen monitoring, were also considered.

The pilot pollen monitoring network based on these criteria is shown in Figure 3. Based on these criteria, the steps followed to perform the selection were:

* Existence of historical time series was considered for monitoring climate change impacts. **9 Stations** with historical time series were selected at first: DEZUSM, DEMUST, DEBAMB, DEERLA, DEBAYR, DEDONA, DEMUNC, DEGAIS and DEBERC.
* Existence of other independent stations running during 2015 in Bavaria were also considered as part of the pilot network. **5 Stations were added:** DEBIED, DEAUGS, DEPFRO, DEGARM and DEUFS.
* The network must be capable of detecting pollen episodes early, so source areas got particular attention. The places closer to the main pollen sources are optimal locations to perform a better forecasting of airborne particles. **4 Stations were added:** DEALTO (Close to the Austrian sources), DEVIEC (Bavarian Forest), DEMARK (North-West Franconian forest) and DEOETT (Schwaben-South Franconia forest).
* The network must be informative for the bulk of the population, so the most populated areas were closely monitored when necessary. **4 Stations were added:** DEFEUC (Close to Nürnberg), DEKITZ (Close to Würzburg), DEKOES (Close to Ingolstadt) and DEMIND (populated area in Schwaben).
* Must cover the major biogeographic environments existing in Bavaria. Most of the environments are already covered. **5 Stations were added:** DELAND (middle-stream Danube area), DETROS (South East Bavaria), DEPASS (downstream Danube area), DEWEID and DEHOF (North-East Bavaria).

Out of those stations, five where independently managed: DEAUGS (UNIKA-T, first data: 1999), DEBIED (private-ZAUM, first data: 2003), DEOBER (private-PID, first data: 1982), DEGARM (TUM, first data: 2008) and DEUFS (TUM, first data: 2008). Two stations were managed by PID with long time series: DEMUST (first data: 1990) and DEMUNC (first data: 1987). The other 20 stations were managed at the central lab of ZAUM. From these 20 stations, 7 correspond to historical PID locations with long time series but previously discontinued: DEBAMB (first data: 1989), DEBAYR (first data: 1989), DEERLA (first data: 1987), DEDONA (first data: 1989), DEZUSM (first data: 1987), DEGAIS (first data: 1992) and DEBERC (first data: 1987). Thirteen Stations corresponding to new locations commenced in 2015: DEHOF, DEMARK, DEKITZ, DEWEID, DEFEUC, DEOETT, DEVIEC, DEKOES, DELANDS, DEPASS, DEALTO, DETROS and DEMIND.

**>> Figure 3.**

**Clustering Bavarian pollen zones and selected locations**

The result of the analysis is summarized as a dendrogram (Fig. 4a), starting with 26 elements and ending with one (senseless) big cluster. We had to determine the number of clusters that represented similarities between locations and clustering distance. Elbow plot suggest the existence of 4 main clusters. After 4 clusters, the variance explained by additional clusters is smaller (Figure S4).

**>> Figure S4.**

Figure 4a shows four clearly defined clusters: A Central Cluster (C1) with 12 stations distributed in the center of Bavaria; A Cold Cluster (C2) with 6 stations distributed in the colder areas (Alps and Bavarian Forest); An Outlier Cluster (C3) with 3 stations without apparent connection; A Franconian Cluster (C4) with 5 stations distributed in the North. The abovementioned names of each cluster are provisional and serve only for providing a better understanding of the grouping.

**>>Figure 4.**

There were not always well-defined boundaries between clusters and, so, some regions could be considered as transitional or subclusters (8) inside the bigger four clusters. For instance, in the cluster C4 (Franconian cluster), DEHOF and DEBAYR are in a small sub-group (East-Franconia) because of bioclimatical similarities (lower temperatures, see Figure S3c) with respect to West-Franconia. In the same way, DEBIED and DEMUNC are the nearest stations in the first cluster C1 (Central cluster) and indeed both traps are located within the same city (Munich). DEALTO, DEPASS and DETROS showed similar data, the three locations are quite close to the southeast of Bavaria. DEAUGS and DEMIND showed similar data too, both locations are close in southwest Bavaria. DEGARM and DEUFS constitute also a small subcluster inside the C2 (Cold cluster), both locations being only 10 km apart in the Alps region, however altitudinally differing by 1900 m. Three stations, DEMUST, DEZUSM and DELANDS (Cluster C3) were considered as outliers (i.e. the cluster was formed of stations located in distant and different bioclimatic areas).

Figure 4b shows a visualization of the Euclidean distances between locations (based on all the pairs of correlations), for a better understanding of sub-clusters and transitional areas. For visualization, we represented only the edges with the five closed elements. Figure 4b is a way of visualizing a multidimensional space into two dimensions by rescaling the weight of the edges, so the result does not necessarily match with the dendrogram produced by Hierarchical clustering. This visualization allows us to understand that inside C4, the stations of East-Franconia (DEHOF and DEBAYR, colder area) are closer to the Cold cluster (C2) and the stations of the warmer area are closer to the Central cluster (C1). Inside the cluster C2, DEVIEC is closer to the central cluster, indeed this station is at the border of C1 surrounded by DEWEID, DEDONA and DEPASS-DEALTO-DETROS. As can be observed, DEUFS is the station farthest away from the rest of the network inside C2, as this station is located under extreme conditions at the top of the Alps at 2656 m a.s.l., being the highest pollen monitoring station in the world (<http://www.schneefernerhaus.de/startseite.html>). The three stations included into the Outlier cluster (C3) appear isolated also in Figure 4b. DEWEID appears connected with the colder stations of C4 and the 5 stations of C2, and indeed this station is also located in the Bavarian Forest, under transitional conditions between C1 and the stations DEHOF-DEBAYR (C4) and DEVIEC (C2). The hierarchical clustering put this station in a subcluster together with DEFEUC (both are the northern stations of C1).

For each of the 8 subclusters we selected only one station for automation in the permanent network. To select the most relevant station inside each sub-cluster we followed a series of selection criteria. Table 3 shows the final selection.

**>> Table 3**

The station DEOBER would have been selected for the permanent network by population, however this station has one of the longest time series in Bavaria ([64](#_ENREF_64)), and so, the station was already been selected for a parallel permanent manual network. To avoid double sampling at the same location and to save resources, we defined the fourth criteria, the station was changed for the next suitable station inside the alpine sub-cluster according to the selection criteria (DEGARM). This station has a special touristic-economic interest in a changing environment ([65](#_ENREF_65)). Figure 5 shows the final selected network with 8 automatic locations.

**>> Figure 5.**

During the last 60 years of pollen monitoring, the standardization of methods was an important issue and all the efforts ended into a high degree of comparability between pollen data across the globe ([45](#_ENREF_45)). At the same time, there was no evolution in the sampling technology, predominantly using the same pollen sampler (i.e. the Hirst-type trap) with the same features as the original design ([41](#_ENREF_41)). The building of a network based on an automatic system is an alternative and promising option. First, it provides information about airborne pollen in almost real time, eliminating the workload and the delay of the information, which are the main disadvantages of classical pollen analysis for health purposes. It also eliminates human variability and personal bias during routine monitoring, increasing the comparability of the data. Different 3G automatic systems will probably coexist during the following decades without any becoming dominant. Pollen experts will be essential to calibrate, supervise and support machines to be adapted to changing environments. In our vision, classical pollen monitoring will not disappear, but will be performed more selectively by pollen experts and only for specific scientific purposes. Although the automatic data flow is already creating new problems, like the necessity of filtering the disseminated information ([66](#_ENREF_66)), the advantages provided by the automatic monitoring was always an ambition of aerobiologists, now becoming possible.

We established a method to determine how many traps are needed to represent the pollen flight in a certain area. Our method minimizes effort and operational costs whilst providing a representative picture of the pollen flight within an area. Of course, we would be able to improve monitoring of pollen flight if we had unlimited budgets, but by automating the selection of monitoring sites we were able to obtain more rapid data delivery and provide better service to allergic individuals.

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**Table 1**. Selected 27 locations in the ePIN pilot network run in 2015 with Hirst-type traps. Population coverage (%) within 30 km distance from each station is indicated

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Code** | **Location** | **Latitude**  **(°N)** | **Longitude**  **(°E)** | **Elevation**  **(m a.s.l.)** | **Population**  **coverage (%)** |
| DEALTO | Altötting | 48.23 | 12.68 | 398 | 2.5 |
| DEAUGS | Augsburg | 48.33 | 10.90 | 497 | 6.4 |
| DEBAMB | Bamberg | 49.90 | 10.89 | 238 | 3.6 |
| DEBAYR | Bayreuth | 49.94 | 11.53 | 419 | 2.8 |
| DEBERC | Berchtesgaden | 47.64 | 13.01 | 573 | 0.8 |
| DEBIED | Munich | 48.16 | 11.59 | 510 | 15.2 |
| DEDONA | Donaustauf | 49.04 | 12.21 | 425 | 3.4 |
| DEERLA | Erlangen | 49.60 | 11.01 | 284 | 9.7 |
| DEFEUC | Feucht (Nuremberg) | 49.38 | 11.20 | 365 | 8.9 |
| DEGAIS | Gaissach | 47.75 | 11.58 | 717 | 2.7 |
| DEGARM | Garmisch-Partenkirchen | 47.49 | 11.10 | 821 | 1.2 |
| DEHOF | Hof | 50.32 | 11.90 | 531 | 1.9 |
| DEKITZ | Kitzingen | 49.74 | 10.14 | 246 | 3.6 |
| DEKOES | Kösching | 48.82 | 11.51 | 391 | 3.2 |
| DELANDS | Landshut | 48.54 | 12.14 | 397 | 3.0 |
| DEMARK | Marktheidenfeld | 49.85 | 09.63 | 216 | 3.4 |
| DEMIND | Mindelheim | 48.04 | 10.50 | 610 | 3.3 |
| DEMUNC | Munich | 48.13 | 11.56 | 538 | 15.1 |
| DEMUST | Münnerstadt | 50.25 | 10.18 | 347 | 2.6 |
| DEOBER | Oberjoch | 47.52 | 10.40 | 870 | 1.7 |
| DEOETT | Oettingen | 48.96 | 10.60 | 431 | 1.8 |
| DEPASS | Passau | 48.56 | 13.44 | 318 | 2.1 |
| DETROS | Trostberg | 48.03 | 12.56 | 483 | 2.7 |
| DEUFS | Umwelt Forschungsstation Schneefernerhaus (UFS) | 47.42 | 10.99 | 2650 | 0.8 |
| DEVIEC | Viechtach | 49.08 | 12.87 | 459 | 2.0 |
| DEWEID | Weiden | 49.68 | 12.17 | 403 | 2.0 |
| DEZUSM | Zusmarshausen | 48.40 | 10.61 | 483 | 5.7 |

**Table 2.** Optimal conditions for pollen monitoring

|  |  |  |
| --- | --- | --- |
| **OPTIMAL CONDITIONS FOR POLLEN MONITORING** | | |
| **Logistic** | **Trap location** | **Emission sources** |
| Safety at the location | Flat and horizontal surface | Absence of overrepresentation of some species in surrounding 500 m area |
| Easy access | Higher than surrounding roofs and other wind walls | Absence of anemophilous sources in surrounding: no uncut grass areas within 50 m and no birch/olive trees within 100 m |
| Access to electric network | Not at ground level. Between 9 m and 15 m from the ground (on a roof or an elevation tower). This criterion aims to build the whole Bavarian network under homogeneous conditions, but it is not supposed to be a worldwide standard. | Absence of proximity to non-biological and biological particle sources of high emission (e.g. waste disposal plant) |
| Access to internet (For 3G systems) | Not placed at the edge of a building (> than 2 meters) to avoid turbulent flow | Absence of proximity to wind distortion sources (e.g. solar panels, refrigeration systems…) |
| Temporal sustainability | Elevated more than 150 cm from the roof or elevation surface | Consider whether land use change will have an effect on pollen concentrations in future |

**Table 3**. Subclusters and selection criteria (indicated in material and methods, section 4.b.). Within each subcluster (zone), the station with the highest population was selected. In the case of draw, the selection criteria were applied for tiebreaking and selecting the most representative station within the subcluster.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ward Sub-Cluster** | **Code** | **% of covered population** | **Selected** | **Criteria** |
| 1.1 | DEAUGS | 6.4 | No | Standard Criteria - <70km apart from the closest station (DEMUNC) |
| **1.1** | **DEMIND** | **3.3** | **Yes** | **Standard Criteria** |
| 1.2 | DETROS | 2.7 | No | Population draw |
| **1.2** | **DEALTO** | **2.5** | **Yes** | **Population draw, Closer to the border** |
| 1.2 | DEPASS | 2.1 | No | Standard Criteria |
| 1.3 | DEBIED | 15.2 | No | Population draw |
| **1.3** | **DEMUNC** | **15.1** | **Yes** | **Population draw, Closer to the border** |
| 1.3 | DEDONA | 3.4 | No | Standard Criteria |
| 1.3 | DEKOES | 3.2 | No | Standard Criteria |
| 1.3 | DEOETT | 1.8 | No | Standard Criteria |
| **1.4** | **DEFEUC** | **8.9** | **Yes** | **Standard Criteria** |
| 1.4 | DEWEID | 2 | No | Standard Criteria |
| **2.1** | **DEVIEC** | **2** | **Yes** | **Standard Criteria** |
| 2.1 | DEBERC | 0.8 | No | Standard Criteria |
| 2.2 | DEGAIS | 2.7 | No | Standard Criteria - <70km apart from the closest station (DEMUNC) |
| 2.2 | DEOBER | 1.7 | No | DEOBER was already selected for manual monitoring in a parallel manual network |
| **2.2** | **DEGARM** | **1.2** | **Yes** | **Standard Criteria** |
| 2.2 | DEUFS | 0.8 | No | Standard Criteria |
| 3 | DEZUSM | 5.7 | No | Outlier |
| 3 | DELAND | 3 | No | Outlier |
| 3 | DEMUST | 2.6 | No | Outlier |
| 4.1 | DEBAMB | 3.6 | No | Population draw |
| 4.1 | DEKITZ | 3.6 | No | Population draw |
| **4.1** | **DEMARK** | **3.4** | **Yes** | **Population draw, Closer to the border** |
| 4.2 | DEBAYR | 2.8 | No | Standard Criteria - <70km apart from the closest station (DEFEUC) |
| **4.2** | **DEHOF** | **1.9** | **Yes** | **Standard Criteria** |
| --- | DEERLA | 9.7 | No | Standard Criteria |

**Figure 1.** Footprint of System for Integrated modeLling of Atmospheric coMposition (SILAM, <http://silam.fmi.fi>) for the Birch pollen season of 2015 for the 27 ePIN locations.



**Figure 2.** Boxplot showing the Seasonal Pollen Integral of the considered 13 pollen types at the 26 ePIN locations (all excluding DEERLA).



**Figure 3**. ePIN pilot pollen network set up during 2015 across Bavaria and composed of Hirst-type pollen traps at 27 locations.



**Figure 4. a)** Dendrogram result of hierarchical clustering by Ward method of Euclidean distances. **b)** Network plot representing the position of the 26 considered ePIN stations (nodes) at a bi-dimensional space based on the 5 shorter Euclidean distances of each station (edges).



**Figure 5.** Final selected ePIN network with 8 locations to perform permanent automatic pollen monitoring in Bavaria.



**Figure S1.** Topographical map of (a) Germany with its federal states and (b) Bavaria with its seven administrative districts (CGIAR SRTM).



**Figure S2. a)** Demographic conditions over Bavaria (CIESIN, 2010). **b)** Forest distribution over Bavaria (CORINE Land Cover, 2006). **c)** Average annual temperature (ºC) (WorldClim, 1960-1990). **c)** Annual precipitation (mm) (WorldClim, 1960-1990).



**Figure S3.** Equipment used during the ePIN pilot network. **a)** 1.5 m standard own-made tower for supporting Hirst pollen trap. **b)** Central pollen lab at ZAUM (Munich), capable to manage 25 pollen stations / week. **c)** Draft of pollen slide, each slide corresponds to one independent day. **d)** 12.5 mm net micrometer used for reducing the area of the slide examined to 9% when analysing 4 transects, a net micrometer of 10 mm was also allowed following the national standards (4 lines = 7% sub-sampling) **e)** Examples of own developed equipment: flowmeter without air resistance, ruler with support for cutting samples, petrolatum coating machine with a rotating motor, guide for drawing standard limits into the slides. **f)** Computer program to use the keyboard of as a pollen counter. Screenshot of the online SQL database.



**Figure S4.** Elbow plot suggesting the appropriate number of clusters in the dataset.

