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Quantification of DNA in PM_{10} fraction of aerosols from rural ambient air

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ABSTRACT Although existing guidelines and standards for measurement of biotic components in Germany are dominated by cultivation dependent-analyses more and more genome-based methods are used to address the limitations of cultivation-dependent investigations and to gain deeper insights into the composition of bioaerosol particles. However, the amount of DNA in the outdoor air can be a limitation for genome-based analysis. So, in this study it has been tested whether after collecting the PM_{10} fraction DNA is present on filters in sufficient amounts to follow the course of the DNA concentration for one year in rural ambient air. In the PM_{10} fraction of the investigated air annual median DNA concentrations ranged from 49 to 60 pg/m³. The highest median DNA concentration was measured in summer and DNA concentration was positively correlated with temperature, ozone level, radiation as well as negatively with relative humidity. These observations might be a result of increased natural emission as well as intensified anthropogenic activities in the agricultural sector. Overall, the applied methods for determination of DNA concentration proved to be appropriate to detect general exposures to bioaerosol particles.

Quantitative Erfassung der Gesamt-DNA in der PM_{10}-Fraktion von Aerosolen des ländlichen Raums

ZUSAMMENFASSUNG Die in Deutschland bestehenden Richtlinien und Standards zur Erfassung biotischer Aerosolkomponenten sind dominiert von kultivierungsabhängigen Analysemethoden. Jedoch werden vermehrt genom-basierte Methoden eingesetzt, um Limitierungen von kultivierungsabhängigen Analysen zu vermeiden und einen tieferen Einblick in die Zusammensetzung der Bioaerosole zu erhalten. Allerdings kann der DNA-Gehalt in der Außenluft eine Limitierung für genom-basierte Untersuchungen sein. Daher wurde in dieser Studie untersucht, ob nach Sammlung der PM_{10}-Fraktion genug DNA auf den Filtern vorhanden ist, um den Verlauf des DNA-Gehalts in der Außenluft über ein Jahr zu verfolgen. In der PM_{10}-Fraktion der untersuchten Luft wurden jährliche mediane DNA-Konzentrationen von 49 bis 60 pg/m³ gemessen, die höchste im Sommer. Die DNA-Konzentration korreliert positiv mit Temperatur, Ozonlevel und Strahlung sowie negativ mit Luftfeuchtigkeit. Diese Beobachtungen sind womöglich das Ergebnis von verstärkter natürlicher Emission und erhöhter anthropogener Aktivität im Landwirtschaftssektor im Sommer. Insgesamt haben sich die angewandten Methoden zur Bestimmung der DNA-Konzentration als geeignet erwiesen, um die generelle Exposition gegenüber Bioaerosolpartikeln zu detektieren.

1 Background

The investigation of abiotic components of airborne solid or liquid aerosol particles has a long tradition in assessment of risks at workplace or environmental settings. The existing measurement strategies are therefore laid down in corresponding standards or guidelines and limit values are established for several parameters. Although, a differentiation between workplace and environment exist, and different particular matter fractions have to be considered due to the specification of different guidelines [1] the subsequent analytical procedures seems to be quite comparable.

Investigation of the biotic components of bioaerosol particles also takes place in quite different scientific disciplines of aerosol research worldwide. Outdoor air analyses are performed e.g. in connection with meteorology, atmospheric chemistry, allergology, human, animal or plant pathology but also in the field of biogeography and biodiversity research [2 to 6].

The investigation of biotic components of aerosol particles (bioaerosol particles or synonym „bioaerosols“[1]) in comparison to abiotic components is far less established in the field of regulation at workplace or environmental settings e.g. in Germany. Actually, there are no measurement obligations, nor have limit values been derived for components of bioaerosol particles. Not only between environment and workplace different particular matter (PM) fractions have to be investigated (see above). Different PM fractions also have to be considered according to the specification of different guidelines for environmental emission and immission analyses (compare VDI 4257 Part 2 and 4252 Part 2 and 3) as well as between fungi or bacteria for immission analyses (compare sampling in VDI 4252 Part 2 and Part 3) [7 to 9]. The PM fraction for investigation of bacteria at workplaces is actually rather unidentified whereas for moulds and en-

[1] Bioaerosols are defined in the VDI standards as „... all airborne accumulations of particles to which fungi (spores, conidia, hyphal fragments), bacteria, viruses and/or pollen as well as their cell wall components and metabolic products (e.g. endotoxins, mycotoxins) adhere or contain or form“ (e.g. VDI 4253 Part 2), are understood as „airborne particles of biological origin“ according to DIN EN 13098, and specified in occupational health and safety with reference to their effect as „airborne particles and droplets of biological origin which can influence human health by infectious, allergic or toxic mechanisms of action“ (e.g. the TRBA 500).

dotoxin analyses the inhalable aerosol particle fraction has to be considered [10 to 12].

In general, the defined PM fractions for bioaerosol particle analyses differ mostly from those actually used in abiotic component analyses. This phenomenon seems to be a result of the subsequent cultivation based analytical approach, because the sampling methods are often adopted to the cultivation-based analyses and consider the sampling tolerance of bacteria or moulds. However, the less strictly defined sampling strategy for biotic particles seems over-all less surprising, since it has not yet been possible to derive dose-response relationships with sufficient accuracy from the existing measurement parameters [13].

The few guidelines and standards existing in Germany, are dominated by cultivation-dependent analyses for the detection of sum parameters as mentioned above [14; 15]. The only cultivation-independent investigations are the methods for total cell count and endotoxin analyses [12; 16; 17]. Both methods have the advantage that they are applicable independent of the used sampling method provided that the particles can be detached from the collection medium.

In the field of scientific investigations, cultivation-based methods as well as endotoxin and cell count analyses are used. However, the trend is increasingly moving towards genome-based analyses to address the limitations of cultivation-dependent investigations and, most importantly, to gain deeper insights into the composition of bioaerosol particles. Those approaches in future may allow a more common and better comparable sampling strategy because the sampling stress can be neglected.

The basis of the genome-based analysis methods is the genetic material present in aerosol samples, i.e. the DNA. For the analysis of bioaerosol particle exposures at highly polluted workplaces, DNA is usually present in efficient amounts in the bioaerosol particles of a low aerosol volume, so that downstream analysis for quantification by established methods are possible [18 to 23]. In the context of possible genome-based analyses in the future, in this study, it should be recorded whether DNA is present on filters in sufficient amounts to follow the course of the DNA concentration in a year in the rural ambient air after collecting the PM_{10} fraction according to well established regulation VDI 2463 Part 11: 1996–10 [24]. VDI 2463 Part 11: 1996-10 has been withdrawn recently. However, the sampling method is now described in VDI 2463 Part 2: 2021–05 [25].

2 Material and methods

2.1 Sampling

Three different sampling spots in Saxony, Germany, were chosen: Radebeul (51°7‘10“N, 13°40‘30“E, 246 m AMSL), Brockau (50°36‘29“N, 12°12‘40“E, 430 m AMSL) and Niesky (51°17‘7“N, 14°44‘59“E, 148 m AMSL). These sampling spots belong to the air monitoring network of the Saxon State Office for Environment, Agriculture and Geology [26]. Brockau and Niesky are defined as rural, Radebeul as rural and peri urban. In a 500 m radius the land-use around the sampling spots is mainly dominated by agriculture, residential buildings and recreational areas. The volume of traffic in a 100 m radius is < 2,000 vehicles/day, < 100 vehicles/day and no traffic for Brockau, Niesky and Radebeul, respectively. According Zensus 2011 Atlas the population density in a 500 m radius around the sampling spots for Brockau is < 250 residents, in Niesky < 150 residents and in Radebeul < 600 residents [27]. In regard to routine air monitoring these sampling spots are regarded as background monitoring spots. In the course of one year (between September 2018 and September 2019) approximately every two weeks PM_{10} samples were taken with a high volume sampler (HVS, DIGITEL DHA-80, Fa. Riemer Messtechnik, Hausen/Röhn) at a flow rate of 500 l/min for 24 h according DIN EN 12341 and VDI 2463 Part 11 [24]. Glass fibre filters (MN85/90 BF, Fa. MACHEREY-NAGEL, Düren) used for aerosol particle sampling were heated beforehand at 180 °C for 3 h and transported sterile to the sampler. After sampling the filters remained in the sampler for 2 – 9 days before being transported to the laboratory where they were frozen at –20 °C until DNA extraction.

2.2 DNA extraction

Filters were cut in app. 3 x 3 mm pieces and split up in twelve tubes. In each tube 900 µl Proteinase K solution (0.5 mg/ml, ≥ 30 mAnson U/mg, Fa. Carl Roth, Karlsruhe) were added. After an incubation for 30 min at 1,000 rpm and 50 °C 0.5 ml Zirconia/Glass-Beads (0.1 mm, Fa. Carl Roth, Karlsruhe) were added. Then the GeneElute Plant Genomic DNA Miniprep Kit (Fa. Merck KGaA, Darmstadt) was used according to the manufacturer’s protocol with the following adaptions. After addition of the lysis buffers samples were vortexed for 2 min at max speed (Vortexer-Genie 2T, Scientific Industries, Inc., New York, USA). After precipitation of debris the supernatants from twelve tubes were merged on nine filtration tubes. When loading lysate onto the binding columns the content of three filtration tubes was used for one collection tube leading to three collection tubes per filter. Finally, the same 50 µl elution solution was used for elution of DNA on all three collection tubes. DNA was stored at -80 °C until further analyses.

2.3 DNA quantification

DNA was quantified fluorometrically (Quantus Fluorometer, Fa. Promega, Walldorf). For concentrations below 0.3 ng/µl DeNovix Ultra High Sensitivity Assay (DeNovix Inc., Wilmington, USA) was used and for higher concentrations the QuantiFluor ONE dsDNA System (Fa. Promega, Walldorf) was used according to the manufacturer’s instructions.

LOQ (limit of quantitation) and LOD (limit of detection) were determined as follows based on 15 independent measurements:

LOD or LOQ = F * SD / m

where

F: factor of 3.3 and 10 for LOD and LOQ, respectively

SD: standard deviation of the blank

m: slope regression line at low concentrations

2.4 Other measurement parameters

Meteorological parameters (temperature, humidity, radiation, air pressure, wind speed), ozone and other air quality parameters (PM_{10}, NO_x, NO, NO_2) were monitored simultaneously [28 to 36]. Daily data for rain fall and snow height were obtained from the German National Meteorological Service from monitoring sites < 9 km from the bioaerosol monitoring sites (Station ID: 7329, 1693, 7423) [37; 38].

2.5 Statistics

All statistical analyses were conducted in R version 4.0.3 [39]. For plotting group comparisons ggpubr and ggforce were used.

Kruskal-Wallis test was used for multiple group comparisons and Wilcox test with Benjamin & Hochberg adjustment for posthoc pairwise comparisons [40; 41]. Correlation analysis was carried out with Hmisc and corrplot [42; 43].

3 Results

For determination of LOQ and LOD a regression line was created. The standard deviation of n=6 measurements per concentration level was ≤ 10 % in the concentration range of 9 and 1,700 pg/µl of sample extract. Considering the fluorescence-based DNA quantification method of DNA in aqueous extract and an aerosol sample volume of 720 m³, there was thus an LOD and LOQ of 0.3 pg/m³ and 0.9 pg/m³ of air, respectively. Consideration of all achieved test results of 399 samples collected between September 2018 and September 2019 at the sites Brockau, Niesky and Radebeul in the federal state of Saxony showed that the DNA concentrations in all of the samples were above LOQ and LOD.

Median annual DNA concentrations between 49 and 60 pg/m³ were observed in the PM_{10} fraction of the investigated rural air. Comparing the results between the three sites, no significant difference can be found (**Figure 1**).

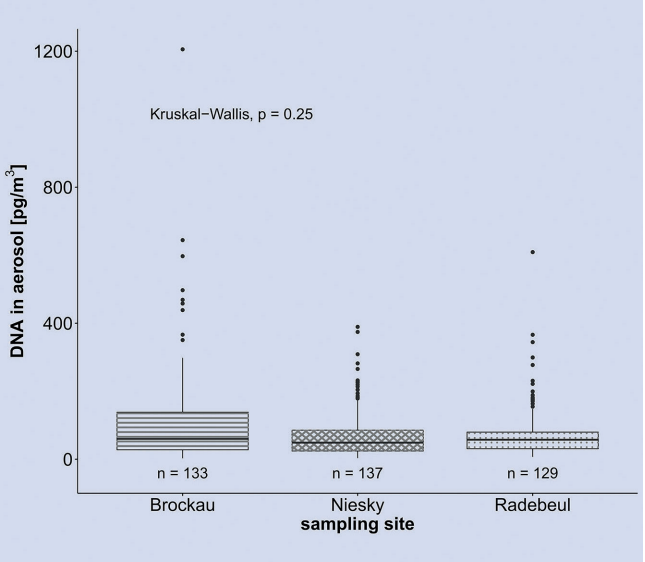

Figure 1 DNA concentration in rural outdoor air at different sampling spots. *Graphic: authors*

The obtained DNA levels were grouped by meteorological seasons (spring: March to May; summer: June to August; autumn: September to November; winter: December to February). Comparison of these groups showed significant seasonal differences, which were also independent of the measurement site (**Figure 2**). Seasonal grouping across all sites revealed that the median concentrations were lowest in winter with 18.3 pg/m³, and highest in summer with 151 pg/m³. In contrast, median concentrations of 45.7 and 66.1 pg/m³ were determined in spring and fall, respectively.

DNA concentration in the rural outdoor air is also moderately correlated with temperature (r_s = 0.68; p ≤ 0.0001), ozone level (r_s = 0.48; p ≤ 0.0001), radiation (r_s = 0.64; p ≤ 0.0001) and relative humidity (r_s = −0.61; p ≤ 0.0001) (**Figure 3**).

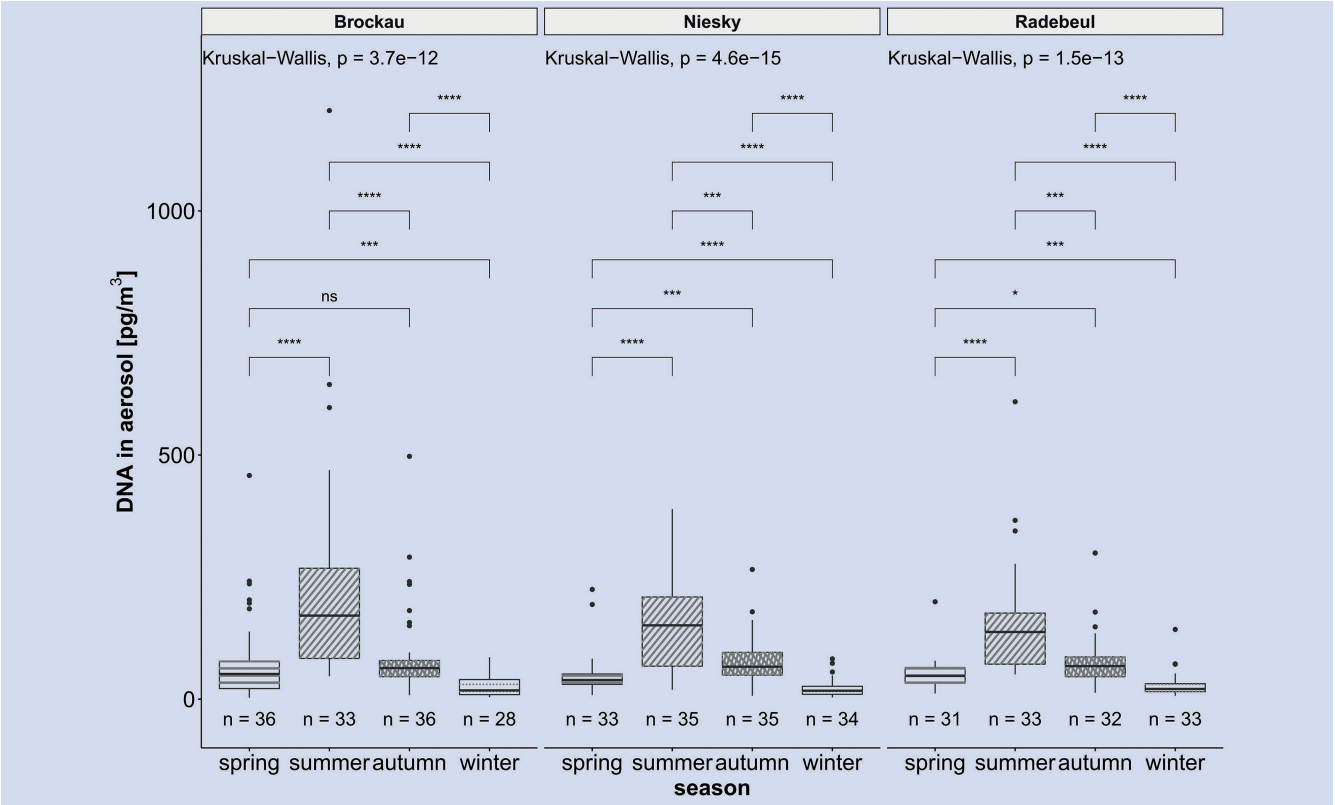

Figure 2 DNA concentrations in rural outdoor air at different seasons. Mean comparisons showed significant differences between DNA concentrations in different seasons (* p <= 0.05; *** p <= 0.001; **** p <= 0.0001). *Graphic: Authors*

4 Discussion

Investigation of the PM_{10} fraction of rural air revealed comparable DNA levels at three sampling spots in Saxony. So, median annual background levels between 49 and 60 pg/m³ air can be assumed for rural air in Saxony. For an estimation of the number of microbial cells present in the air, the exact distribution of the species present in the sample as well as their corresponding genome sizes would have to be known. This is not possible based on the current range of methods. The average genome size of bacteria and fungi are approx. 3.65 Mb and 42.30 Mb, respectively [44; 45]. This corresponds to a genome weight of approx. 4 – 46 fg. Assuming all sampled DNA would be either of bacterial or fungal origin, the median detected DNA amounts per m3 in summer correspond to the amount of DNA that would be present in approx. 3.3 x 10^3 – 3.8 x 10^4 cells. Compared to previously by total cell counting investigated working environments with up to 10^9 microbial cells/m³ [46; 47] the background numbers are relatively low. Similarly, in a first attempt to investigate the amount of DNA in the PM_{10} fraction from the exhaust air of a pig house values up to 10^4 times higher than in the background air could have been determined (data not shown).

The DNA content within the PM_{10} fraction of the investigated rural air followed a certain seasonal pattern. The median DNA concentration was over two times higher in spring, three times higher in fall and seven times higher in summer than in winter. Likewise in a previous study in West Siberia the extracted DNA yield from air sampled with a SASS 3100 was on average 39-fold higher in summer than in winter [48]. Moreover, seasonal variations in the air have already been observed by measuring other parameters such as culturable moulds, bacteria, endotoxins and bacterial activity [49 to 58]. Furthermore, the higher concentration of pollen in the air in spring-autumn may cause an increase of the DNA concentration. Especially given the fact that pollen have a significantly larger genome compared to bacteria and fungi (bacteria 3.65 Mb, fungi 42.30 Mb and pollen 6540 Mb) [44; 45; 59]. Although pollen are predominantly assumed to have aerodynamic diameters above the collection system's cut-off, it cannot be excluded that a certain amount of pollen and parts of pollen are still sampled. However, the individual influence of moulds, pollen or bacteria to the DNA content of the investigated air and the observed seasonal variations cannot be deduced with certainty without further detailed analysis of the DNA.

Moreover, from spring to autumn activities in the agricultural sector like ploughing or mowing may lead to an increase in bioaerosol release. Yet, at this point no clear answer can be given whether the seasonality is driven by a change in agricultural activities and other anthropogenic sources or whether it is a result of a natural source changing during the year.

In this measuring campaign DNA concentration was correlated positively with temperature, ozone level and radiation as well as negatively with relative humidity. These findings coincide with results by Tong and Lighthart, who investigated total, culturable and particulate-associated bacteria in rural air. Moreover, in previous studies decreased relative humidity and wind speed as well as increased temperatures were associated with higher endotoxin levels in the air [49; 52; 56; 60]. In another study by Zhong and colleagues enzyme activity of microorganisms measured via fluorescein diacetate hydrolysis method was positively correlated with temperature and negatively with windspeed [58].

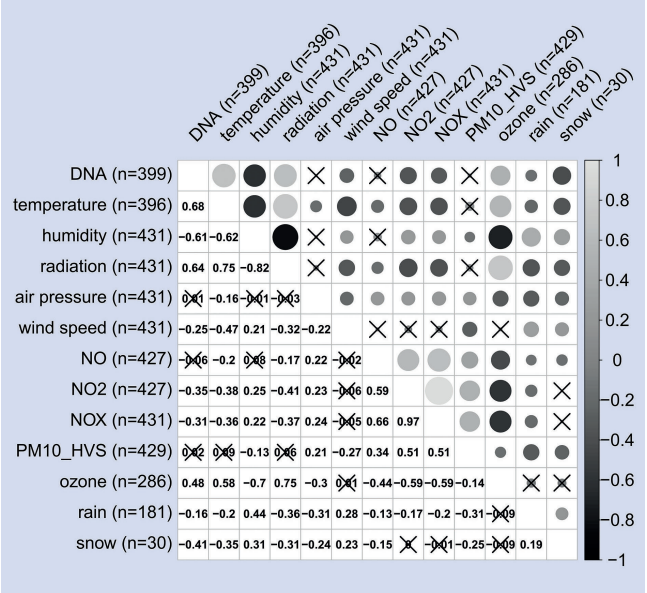

Figure 3 Spearman correlation between DNA concentration in rural outdoor air and different meteorological and air quality parameters. Insignificant correlations ($p > 0.05$) are crossed out. *Graphic: Authors*

From all of these results it can be assumed that the higher concentrations of bioaerosol particles in warm and dry seasons might be a result of increased natural emission (e.g. from dry soil by wind erosion) as well as intensified anthropogenic processing of natural microbial habitats like soils and plants.

Overall, the determination of DNA concentration seems to be a very robust method to detect general exposures to bioaerosol particles. The transfer of the method to filters exposed to other particle fractions is probably feasible. The main limitation of the method so far has been the DNA extraction efficiency since a fixed method cannot extract DNA from all organisms in the same way and completely. Automation of DNA extraction from large filters would be desirable, as manual crushing of filters prior to extraction is very time consuming. In this study DNA was quantified via fluorometric measurements. Compared to other commonly used DNA quantification methods, like measurement of UV absorbance at 260 nm, fluorometric DNA quantification is less influenced by contaminations (e.g. proteins and phenols) and more sensitive [61; 62]. Furthermore, fluorometric measurements are able to discriminate between RNA and DNA as well as double-stranded and single-stranded oligonucleotides [61; 62]. However, fluorometric DNA quantification requires creation of a standard curve. Often lambda DNA is used for this purpose, but currently real DNA reference material is lacking. This can lead to difficulties in comparing results from different laboratories. Especially, because not only the choice of standard DNA but also the level of fragmentation might influence fluorometric DNA quantification [63]. Nevertheless, the greatest advantages in using DNA concentration as a marker for bioaerosol load are certainly the relatively simple measurement principle and the possibility to freeze the DNA extracts over a long period of time to obtain reserve samples. Furthermore, and as mentioned above the DNA extract represents the basis for a more detailed bioaerosol analyses. Still, whether the method is robust enough to be used as a standard method in the field of guidelines must be tested in the future in interlaboratory tests. ■

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