Estimate of the biological component of the atmospheric aerosol in Southwestern Siberia

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Among aerosol forms of pollution, biogenic aerosols and the biological components of aerosol particles occupy a special place. Unfortunately, in Siberia, with the exception of pollen aerosols, the various components of biogenic aerosols have not been systematically investigated. The present paper reports preliminary estimates of the biological component of atmospheric aerosols in Southwestern Siberia: bacteria, other micro-organisms, and also protein pollutants.

Introduction

It has been reliably established that atmospheric aerosols, by redistributing radiative fluxes and being nucleation centers, have a significant effect on the climate.[1] Through the intermediary of climate and directly, aerosols also have a significant effect on human health.[2,3] Obviously, in this regard, the task of monitoring atmospheric aerosols and identifying their sources as well as trends in their spreading and transformation in the atmosphere is extremely important. Intense efforts toward the fulfillment of such tasks are underway both on a regional scale, as the Fifth Session of the Working Group on Siberian Aerosols testifies, and on a global scale, as indicated, for example, by the global atmospheric observations carried out under the auspices of the World Meteorological Organization.

A quite unexpected result of earlier studies was the fact that the biological component of an atmospheric aerosol can make up as much as 95% of the total number of particles with diameter greater than 2 μm (Refs. 4 and 5). However, the biological component of atmospheric aerosols has, until recently, from our viewpoint, received insufficient attention. Indeed, despite the fact that aerobiological studies of the atmosphere were being undertaken already in the last century (see, for example, Ref. 6, cited in Ref. 7), they have still not been done in a systematic way. An exception is long-time observations of bacterial flora in Sweden[7] and Canada,[8] of saprophytic fungi in Finland,[9] and of dusty flora in Siberia.[10] However, these studies were dedicated to only one kingdom of organisms and did not touch on the others. At the same time it is known, for example, that the resistance of humans to infectious diseases is a function not only of "internal" factors, such as immunity, but also "external" physical and chemical factors acting on the human organism or on the micro-organism in question, and also the combined action of these factors and such micro-organisms on man.[11–13] Consequently, the most informative form of monitoring, from the point of view of estimating the effect on man, would be simultaneous monitoring of all biological components of an atmospheric aerosol in combination with measurement of the physical-chemical characteristics of the aerodispersed system (including the most "significant" gaseous impurities) and meteorological parameters governing the transport of aerosols in the atmosphere.

It should be noted that the problem of transport of an atmospheric aerosol is important not only on the local scale (on the order of tens of kilometers), but also on an interregional scale (on the order of thousands of kilometers).[1] Mathematical modeling of processes of aerosol transport allowing for its transformation in the atmosphere enables one to reliably determine its possible sources, including remote sources. Here it should be mentioned that in the literature one finds descriptions of cases of transport over great distances not only of pollen and macromolecular compounds, but also of living micro-organisms. Thus, Ref. 14 describes drift of the virus causing foot-and-mouth disease during an epidemic of this disease in La Manche, Ref. 15 describes the transport of soil bacteria from the region of the " lack Sea into Sweden, and Ref. 16 discusses the hypothesis of transcontinental transport of the flu-virus aerosol in connection with epidemics. Data on local spreading in the atmosphere of aerosols containing micro-organisms are much more numerous, see, for example, Refs. 9, 17–19.

On the basis of the above-said it is clear that to obtain reliable information about the direct action of the biological component of atmospheric aerosols on the health of a local population, it is necessary to track simultaneously all components of this component and at the same time determine the physical-chemical

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characteristics of the aerosol and a number of meteorological parameters.

The present paper contains a discussion of the first results of studies, which we have initiated within the scope of the indicated problem.

Materials and methods

The physical characteristics of an aerosol system and the concentration of some of its gaseous components were measured with the help of a mobile laboratory outfitted at the Institute of Chemical Kinetics and Combustion. The laboratory was set up in a ZIL-131 heavy-duty van. The laboratory included the following apparatus (a significant part of which was developed at the Institute of Chemical Kinetics and Combustion[20–23]):

1. Aerosol diffusion spectrometer, which measured particles with diameters in the range from 3 to 200 nm at concentrations of 10^2 to 10^6 particles/cm^3.

2. A photoelectric aerosol analyzer. The range of measurement of the particle diameters extended from 0.3 to 10 μm.

3. Fluorescent sulfur-dioxide analyzer with a measurement range of 1–750 ppb.

4. A CLD 700 AL chemiluminescence nitrogen-oxide analyzer with a measurement range of 0.001 to 100 ppm.

5. A thermal-denuder system for determining the concentration of sulfuric acid and sulfates in the atmosphere. The sensitivity of the instrument for a sampling time of 1 hour was around 0.02 μg/m^3.

6. A thermal-denuder system for determining the concentration of nitric acid and nitrates in the atmosphere. The sensitivity of the instrument for a sampling time of 0.5 hour was around 0.1 μg/m^3.

7. An LGMI chemiluminescence analyzer with a measurement range of 1 to 100 ppb.

8. Nephelometers of the type FAN and ECN Model 1550".

9. An instrumentation complex for measuring the most important meteorological parameters: wind direction and velocity, temperature, humidity, and air pressure.

All measuring devices and sampling units were linked together in a computer-controlled measuring system. Measurement, sampling, data processing, and storage of information on the physical characteristics of the aerosol and the concentrations of the tracked gaseous impurities were all completely automated. The periodicity of data acquisition in this case depends on the purpose and conditions of the experiment. The possibility exits of continuous observations extending over many days.

The biological component of atmospheric aerosols was sampled using three systems located at a height of 1 m above the underlying surface. The first of these consisted of a fibrous filter of the type AFA-KhA, the flow rate through which was 50 l/min. It is intended for analysis in the presence of specific and unspecific proteins or other biological macromolecules using the techniques described below. The second system was a one-step impinger (MTs-0 microcyclone dust separator) analogous to those described in Ref. 24. The flow-rate through it was also 50 l/min. Fifty ml of a physiological solution ensuring viability of bacteria and fungi in the absence of multiplication up to startup of sample analysis. The third system was the same kind of microcyclone as in the second system, but with the following additives in the physiological solution: antibiotics (penicillin in the amount of 100 mg/ml and streptomycin in the amount 100 units/ml to suppress growth of bacteria and fungi), 2% KRS serum (for improved preservation of the sampled virus particles) and an antifoaming agent. Sampling took place over one hour. After that, the microcyclones were cooled down to +4°C and stored at this temperature until the beginning of sample analysis.

The biological and protein components of the sample were analyzed in the laboratory of the Scientific-Research Institute of Aerobiology of the State Scientific Center for Virology and "iotechnology "Vector." No special analysis for pollen was performed within the scope of the given study.

The protein content of the samples was determined as follows. The aerosol filter was placed in a sample bottle and wetted with 3 ml of a solution containing 3.2 g of boric acid and 1.6 g of potassium hydroxide in 500 ml of distilled water. Protein desorption took place during the course of a day with constant mixing on an automated shaker at room temperature. The protein content in the obtained liquid was determined by the "radford method.[25] To 1 ml of desorbing solution with protein, 4 ml of reagent solution was added, containing 100 mg of brilliant blue G-250 kumass dye, 50 ml of a reference standard, and 100 ml of 85% phosphoric acid added to 1 liter of distilled water. The reaction mixture was stored for 1 hour and then photometrized at the wavelength 595 nm on a Cary 219 Varian spectrophotometer. As the standard for quantitative determination of protein, we used bovine serum albumen (protein standard solution with a concentration of 1 mg/ml in 0.15 M sodium chloride, Sigma Chemical Company).

Analysis of living micro-organisms. The species and concentrations of the viable bacteria in the samples were determined by successive decanting of tenfold diluted samples onto agar culture media with subsequent Gram staining and examination of the micro-organisms in the nascent colonies under a microscope.[26]

Titration of enteroviruses, which we had hoped to discover in the samples, was performed by the method of platelet formation in HeLa cells under an agar coating after successive tenfold dilutions of the virus-containing suspensions.[27]

Identification of the fungi detected in some of the samples, and searches for other micro-organisms (yeast, algae, etc.) were not carried out within the framework of the given study.

Results and discussion

Preliminary results of our study of the biological components of atmospheric aerosol obtained in 1998 are summarized in Tables 1 and 2. Table 1 displays results of studies carried out in June–July of that year in an area located 5 km north-northwest of Chik village in the Novosibirsk region. The closest possible local source of bio-aerosols was the settling tank of the Chik poultry farm (and it was specifically for this reason that we hoped to detect enteroviruses), located about 1 km from the working area.

Table 2 presents results of studies carried out in September–October in an area near the Institute of Chemical Kinetics and Combustion in Novosibirsk Academic Township. No possible local sources of virus bio-aerosols were identified in the vicinity of this area; therefore, we did not sample here to detect viruses in the aerosols. In addition, two other differences should be noted. First, the variation in the protein concentration of the air in this area was markedly lower than in the first area: on average it was 0.39±0.21 μg/m^3. "y the way, if we ignore the one outlier value of the protein concentration (sample 03.07 at 23:24) then the variation of the protein concentration was of the same order of magnitude. This significant increase in the protein concentration was probably due to some intense source located downwind. At the same time, for similar wind directions and speeds a greater air protein concentration was not recorded. This data point was probably just a random excursion. Second, because of a lapse of the program we lost some meteorological data, including measurements of the coarsely dispersed fraction of atmospheric aerosol. Therefore, Table 2 contains less information than Table 1.

Let us dwell now in more detail on analysis of the microbiological components of atmospheric aerosol. Out of 30 samples taken in the presence of bacteria, in 26 of those samples bacteria were detected. In one case, growth of bacteria was suppressed by fungi. In three cases, the amount of bacteria in the samples did not exceed the sensitivity threshold of the technique.

Gram-positive bacteria were most frequently encountered in the investigated samples: various cocci (cocci, diplococci, streptococci, and in one case staphylococci) and bacilloform bacteria. Gram-negative bacteria were detected only in two samples, and in one of these no other micro-organisms were detected.

Table 1. Results of measurements made in the region of the Chik settlement.

Data	Sampling startup time	Mean wind speed, m/s	Mean temperature, °C	Protein concentration, μg/m^3	Form of micro-organisms in order of frequency of occurrence	Concentration of particles with diameter greater than 0.5 μm, particles/cm^3	Concentration of particles with diameter greater than 2 μm, particles/cm^3	Ozone concentration, ppb	SO_2 concentration, ppb	Nephelometer reading, arb. units[a]
06.30	10:10	–	–	0.69	cocci, diplococci, (–)bacilli [b]	– [c]	9 [d]	–	38.7	–
06.30	15:24	–	–	0.48	cocci, diplococci	12.7	–	–	–	–
06.30	18:24	0.0	20.8	0.53	–	39.8	–	9.56	15.3	0.3
06.30	21:24	0.0	18.5	0.52	cocci, (+)bacilli	137.8	–	5.93	–	0.4
06.30	22:54	0.0	17.7	0.30	cocci, diplococci, (+)bacilli	71.4	22	5.40	5.9	0.4
07.01	01:54	0.0	16.8	0.46	cocci, diplococci, (+)bacilli	27.3	–	5.81	3.0	0.4
07.03	17:24	–	–	0.38	–	42.0	–	–	4.0	–
07.03	18:24	–	–	0.30	cocci, diplococci, streptococci	40.7	–	–	3.9	–
07.03	23:24	2.7	16.5	3.19	–	23.3	–	9.27	3.2	0.5
07.04	00:54	4.1	16.0	0.45	–	10.7	–	9.98	2.2	0.4
07.04	06:54	3.7	15.7	<0.17	–	9.1	–	8.58	2.5	0.4
07.04	07:54	5.8	15.4	0.62	–	9.2	–	9.50	3.0	0.4
07.05	16:24	2.2	25.3	0.27	–	8.0	22	10.52	4.6	0.3
07.05	17:10	2.5	25.3	0.19	–	–	–	10.81	3.6	0.3
07.08	15:24	2.0	27.0	0.53	cocci, (+)bacilli	13.2	36	17.98	3.9	0.3
07.08	16:24	2.5	26.6	0.38	–	9.7	–	19.99	–	0.3
07.08	17:24	–	–	0.36	cocci, (+)bacilli	25.2	–	18.96	–	–
07.09	06:24	–	–	0.30	cocci, diplococci	22.2	–	10.50	–	–
07.09	07:24	–	–	<0.17	–	30.9	–	10.42	–	–
07.09	08:10	–	–	<0.17	–	–	–	10.47	–	–
07.09	09:10	–	–	<0.17	cocci, diplococci	–	–	11.45	–	–

Remarks: (a) arbitrary units corresponding to visibility range, expressed in 10^{-1} km^{-1}; (b) for bacilli (+) and (–) are for gram-positive and gram-negative bacteria, respectively; (c) dash indicates quantity not determined; (d) the data in this column were kindly provided by A.L. Vlasenko.

Table 2. Results of measurements made in the region of the Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences.

Data	Sampling startup time	Form of micro-organisms in order of frequency of occurrence	Mean temperature, °C	Ozone concentration, ppb	Nephelometer readings, arb. units
09.21	11:15	cocci, diplococci, (+)bacilli	12.1	–	0.3
09.21	12:15	fungi	12.6	2.9	0.4
09.21	13:15	streptococci, cocci, diplococci	13.3	7.1	0.3
09.21	14:15	streptococci, cocci, diplococci	13.7	9.3	0.3
09.24	11:40	not found	11.1	2.3	0.6
09.24	12:55	not found	11.5	8.8	0.3
09.24	13:55	(+) large bacilli	11.8	16.8	0.6
09.24	14:55	not found	11.9	19.7	0.5
09.28	10:25	staphylococci, cocci	4.9	13.6	0.3
09.28	12:40	cocci, (+)bacilli	5.5	12.5	0.3
09.28	13:40	(+)bacilli, diplococci	5.6	11.8	0.3
09.28	14:45	(–)bacilli	5.6	11.2	0.2
09.30	10:30	streptococci, cocci	5.9	24.0	0.3
09.30	13:00	cocci, diplococci	6.8	25.6	0.5
10.01	10:45	cocci, diplococci	6.1	12.9	0.3
10.01	14:00	(+)bacilli	6.2	16.3	0.3
10.02	09:30	cocci, (+)bacilli	–	–	–
10.02	14:20	cocci, diplococci, streptococci	–	–	–
10.05	10:40	streptococci, cocci, diplococci	5.8	12.4	0.4
10.05	14:25	cocci, diplococci, (+)bacilli	6.3	13.2	0.3

Note. Designations are the same as in Table 1.

Note that a detailed identification of the species of bacteria and their forms was not carried out. However, microscopic studies allow us to conclude that the gram-positive bacilloform bacteria belong most likely to the species "acillus, and the gram-negative, to the species Pseudomonas.

Our results are in good agreement with the microfloral composition of the atmosphere, both its upper and lower layers, where gram-positive bacteria are frequently found and gram-negative bacteria, fungi, and yeasts are found significantly more rarely.[17,18,28] At the same time, it is our understanding that not all viable bacteria were revealed by the implemented techniques: according to the data in the literature it is necessary to use a much greater number of different kinds of culture media, on which various species of bacteria grow.[7,29]

Our analysis of the enterovirus content of the samples did not reveal their presence in quantities exceeding the sensitivity of the titration technique.[27] Probably, there were not strong enough sources of viral aerosols near the sampling locations. At the same time, since the test systems for virus titration were quite specific,[27] no other viruses were detected in the investigated system.

Future studies of the viral component of the biological component of atmospheric aerosols probably need to be more closely referenced to predetermined sources of such aerosols. Only after selection of suitable high-sensitivity test systems for identifying the virus from a given source and the development of a technique for its quantitative determination, will it be possible to carry out extended monitoring of the atmosphere, which in combination with predictions of the spreading of aerosols from various sources will make it possible to track the concentration of a viral aerosol in a given region.

In conclusion it is necessary to dwell on one more problem connected with monitoring of the microbiological component of atmospheric aerosol. This is, first of all, the need to develop new, highly efficient sampling devices, stable to changes in the conditions of sampling (wind direction and velocity). Second, this also includes the development of real-time methods for identifying micro-organisms in the collected samples. These techniques should be, on the one hand, very non-specific in order to identify the presence in the sample of unknown micro-organisms, considering that according to the data summarized in Ref. 30, at present only around 4% of all viruses, 12% of all bacteria, and 5% of all fungi in existence on the Earth are known. On the other hand, the techniques should be highly specific in order to allow a detailed identification of the various micro-organisms detected (including their species and strain). Without subsequent development of such techniques, further progress in aerobiology will hardly be possible.

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