CHEMICAL ENGINEERING TRANSACTION, Volume 10, 2006
Edited by Eliseo Ranzi

 ISBN88-901-915-7-0

The results of 7-year monitoring of the biogenic components of atmospheric aerosol in Southwestern Siberia

A.S. Safatov[1], I.S. Andreeva[1], G.A. Buryak[1], V.V. Marchenko[1], Yu.V. Marchenko[1], S.E. Olkin[1], I. K. Reznikova[1], V.E. Repin[1], A.N. Sergeev[1], V.V. Penenko[2], E.A. Tsvetova[2], M.Yu. Arshinov[3], B.D. Belan[3], M.V. Panchenko[3], G.N. Tolmachev[3], A.M. Baklanov[4], K.P. Koutsenogii[4], V.I. Makarov[4] and S.A. Popova[4]

[1]Federal State Research Institution «State Research Center of Virology and Biotechnology «Vector», 630559, Koltsovo, Novosibirsk region, Russia

[2]Institute of Computation Mathematics and Mathematical Geophysics, SB RAS, 6 Lavrentieva, 630090, Novosibirsk, Russia

[3]Institute of Atmospheric Optics, SB RAS, 1 Akademicheskii, 634055, Tomsk, Russia

[4]Institute of Chemical Kinetics and Combustion, SB RAS, 3 Institutskaya, 630090, Novosibirsk, Russia

The present work is devoted to summarizing the results of 7-year studies of the biogenic component in atmospheric aerosol of Southwestern Siberia. The most important parts of this component are total protein (as a marker of all substances of biological origin) and culturable microorganisms.

The carried out analysis of changes in the concentrations of total protein and culturable microorganisms allowed us to reveal an expressed seasonal dependence of these concentrations at all altitudes at which measurements were performed and the unique altitude profile of these concentrations. At the altitudes up to 7 km, atmospheric air samples contain a large variety of culturable microorganisms. Potential danger for man was evaluated for some of them. The portion of potentially pathogenic microorganisms can reach 88% for some microorganism groups.

The data presented in the work show that, besides different chemical pollutants, atmosphere always contains various biological pollutants. Some of them such as plant pollen cause allergic reactions. Besides, many types of culturable microorganisms present in atmosphere are potentially pathogenic for man.

1. Introduction

The knowledge of characteristics of the biogenic components of atmospheric aerosol is essential for solving the problems of ecology (the data on microbiological background of atmosphere of the region and its potential danger for the population), meteorology (the determination of possible microbiological markers proving the found long-term transport of air masses from different Earth regions to the Southwestern Siberian as well as the contribution of remote aerosol sources to the aerosol pollution of the region atmosphere) and applied microbiology (new microorganism strains and their variants with different phenotypes detection). Here the results of the studies of the atmospheric

bioaerosols in the South of Western Siberia are presented. We paid our main attention to two components of these bioaerosols: total protein and culturable microorganisms at the altitudes up to 7000 m (Andreeva, et al., 2002; Borodulin, et al., 2005).

2. Materials And Methods

Altitude sampling was performed 50 km south to Novosibirsk with "Optic-E" laboratory mounted on an Antonov-30 airplane during the last ten days of each month. The airplane was flying over the forested area at the altitudes of 7000, 5500, 4000, 3000, 2000, 1500, 1000 and 500 m in the daytime. Air samples were collected on AFA-HA type filters at a flow rate of approximately 250 L/min and impingers at a flow rate of 50 L/min. On-land samples were collected settlement Klyuchi (25 km to Novosibirsk) every 24 hours for a month during different seasons on the same filters and impingers at the same flowrates. Besides, 4 samplings were performed on a selected day in the middle of the month to determine the intraday variations of the measured values. Aerosol particles were collected on a 5-stage low-pressure impactor to analyze the distribution of the total protein within different size fractions of aerosol particles (Berner, et al., 1979).

Total protein content was analyzed in a laboratory according to the fluorescent method using a dye described in (You, et al., 1997); the method sensitivity was 0.1 μg/ml of the sample, and the error of the measured concentration value did not exceed 20%. To determine the total protein, the value attributed to polyaromatic compounds and determined with another method was subtracted from the measured values of the total fluorescence (The detection…, 1991).

For detection of culturable microorganisms the following agarized nutrient media in Petri dishes were used: LB (Miller, 1976) and impoverished LB (diluted 1:10 to prevent the growth of detected microorganisms and their hindering each other) – for detection of saprophyte bacteria; starch ammonium medium (Saggie, 1983) – for detection of Actinomycetes; soil agar, Sabouraud medium (Saggie, 1983) – for detection of lower fungi and yeast. The sowings were incubated in a thermostat at the temperature of 30^0C for 3-14 days. Morphological properties of the detected microorganisms were studied visually with light microscopy. For this, fixed Gram-stained cell preparations and live preparations of cell suspensions were made, and observed by the phase contrast method. Taxonomy of the detected microorganisms was determined to the Genus (Starr, et al., 1981; Methods…, 1982). Calculation of the number of culturable microorganisms in the samples was made according to standard methods (Ashmarin and Vorobyov, 1962), the number of microorganisms being averaged by 2-3 parallels scattered in 4-5 different culture media. The numbers and combinations of microorganisms varied in different samples. The group named "nonsporiferous bacteria" includes various microorganisms, which do not form endospores: gram-variable and gram-positive coccobacilli, various nonsporiferous rods having gram-negative staining such as pseudomonas, intestinal bacteria, etc. We also included bacteria whose cells had an irregular form such as *Mycobacteria* and *Nocardia* in this conventional group.

In some cases, for an indirect estimation of pathogenicity of the detected microorganism cultures, in *in vitro* experiments their plasmocoagulative activity was measured (Smirnov, et al., 1983). For this, dry citrate rabbit plasma was diluted 1:5 with sterile

physiological saline and poured out into tubes, 0.5 ml each. The one-day old culture was suspended in diluted blood plasma and incubated for 1, 2, 3 or 24 hours at 37^0C. Depletion of plasma in the mixture in the culture under study, irrespective of the plasma coagulation degree, was considered as positive reaction. As control, experiments with test strains of staphylococci having or not having positive reaction, were run simultaneously.

3. Results And Discussion

The data obtained allowed us to determine the altitude profile and seasonal variations of the concentrations of the total protein and culturable microorganisms at the altitudes up to 7 km. Normalized over each flight (to avoid seasonal changes) data on total protein and culturable microorganisms concentrations are presented at Fig. 1. The concentrations of the total protein and culturable microorganisms did not decrease at higher altitudes while the concentration of aerosol with the diameter exceeding 0.4 μm at the attitude of 7000 m lower than that in the on-ground layer by more than an order of magnitude (Panchenko and Pol'kin, 2001). Such profiles of admixture concentrations in the atmosphere can be formed due to very powerful remote sources, such as large plant massifs, reservoirs and soils. Aerosol particles from these sources, rising to a considerable altitude, are mixed and transported all over the northern hemisphere, creating the observed profiles as particles settle.

The behavior of altitude dependences of measured values gives a reason to build up the time course of change of the mean values for the concentration and numbers across the altitudes of 0.5 – 7 km, Fig. 2. As it follows from these graphs, there is a pronounced seasonal variability of the total protein concentration and the number of culturable microorganisms in the atmosphere.

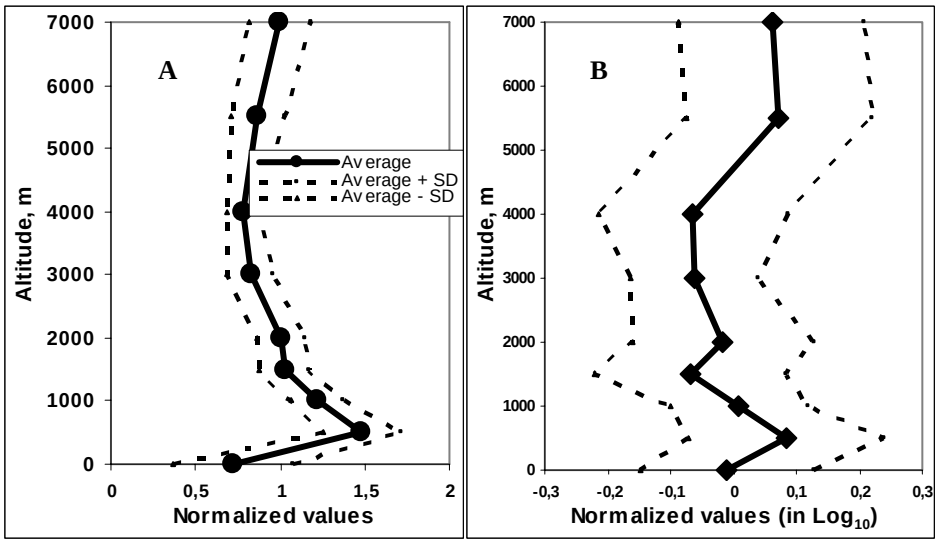

Fig. 1. Normalized altitude dependencies of total protein (A) and culturable microorganisms (B) concentration in atmosphere in the South of Western Siberia.

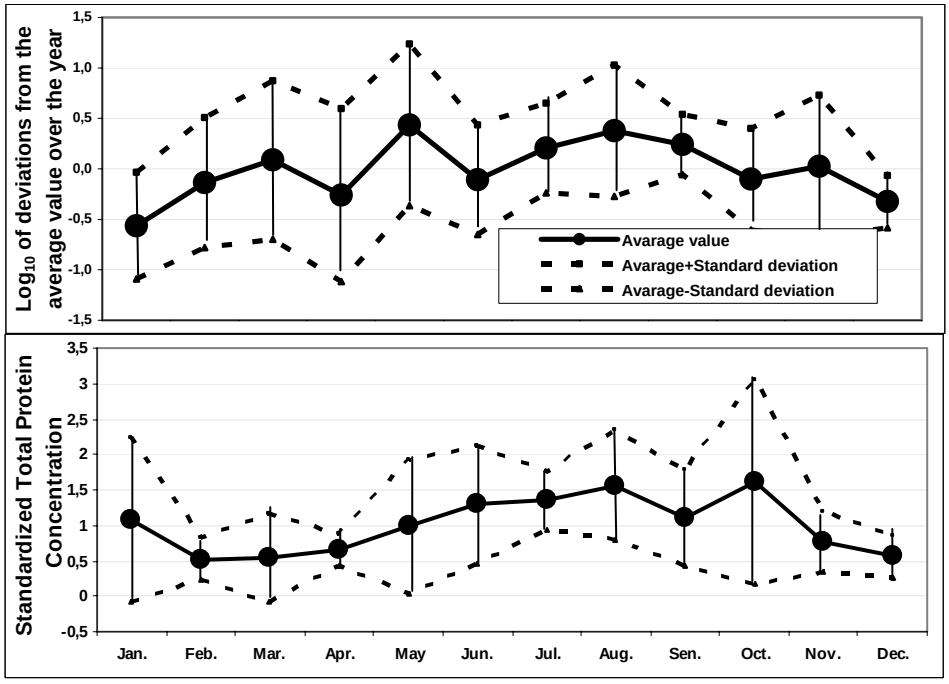

Fig. 2. Year changes of total protein and culturable microorganisms concentrations.

It was found out that the differences observed in the concentrations of the total protein and culturable microorganisms in samples of atmospheric air collected during a day of measurements are statistically invalid. In other words, these values on average do not change during the day. The carried out analysis did not reveal values on average any notable difference in the change of representation of culturable microorganisms in samples collected at different times of the day either.

The evaluation of the portion of the biogenic component in the whole atmospheric aerosol mass is a very important. Our measurements carried out in various seasons demonstrate that the portion of the total protein usually makes up 0.1 – 5% of the mass of all the atmospheric aerosols measured by the gravimetric method at respective time (Safatov, et al., 2003). At the same time, there are data where the mass portion of bioaerosols in the region of Lake Baikal makes up about 10 to 80% during the year (Mattias-Maser, et al., 2000). Moreover, according to (Artaxo et al., 1990) this portion of biogenic particles may be as large as 95%. It is natural that biogenic particles consist not only of protein; however, since protein is the foundation of any living matter, it usually makes a considerable part of the total mass of biological material. That is why the observed difference can hardly be accounted for by only this cause. Probably, those biogenic particles that have been studied in the above-mentioned works consisted not wholly of biological material (like, e.g., particles of plant pollen). Detailed information about the composition of bioaerosols could make it possible to establish more reliably its possible remote sources. The estimated total number of microorganisms in the observed concentrations of the total protein in the same aerosol taking into account that

the portion of culturable microorganisms in aerosol makes up 0.02 - 10.6% (Lighthart, 1997), should be considered quite representative.

Let us consider the distribution of the observed total protein in the atmosphere by the aerosol particles sizes that contain it. Preliminary experiments (Safatov et al., 2003) revealed that the greatest number of protein molecules is in the fraction of particles with aerodynamic diameters of 0.16 - 0.4 μm. But the portion of protein in the bulk particles is maximal for the fraction of 2.1 – 10 μm and makes up approximately 0.3 %. The presented results are preliminary as they are not quite statistically reliable. Nevertheless, they correlate well with the results presented in the work of (Tong and Lighthart, 2000), where it was shown that the fraction of the atmospheric aerosol with the diameter exceeding 2 μm is most enriched with microorgansisms (which always contain a considerable number of protein). Let us note that mechanical destruction of the dead biogenic material (remains of plant parts, animals and their individual cells) to very small pieces requires much energy. That is why it is natural that aerosol fraction with diameters of more than 0.1 μm is most enriched with the biogenic components.

Microorganisms of widespread genera such as *Micrococcus*, *Staphylococcus*, *Bacillus*, *Nocardia*, *Arthrobacter*, *Rhodococcus*, *actinomycetes*, *yeast* and mold *fungi* were detected in the samples. There are some strains of microorganisms found in aerosols which do not correspond to any of known taxons by their phenotypical characteristics also. Generally, variability of microorganism percentage are changing from month to month (as at on-land level, as at higher altitudes) and from one altitude to near by one. But we still have not enough data to construct reliable dependencies individual species concentration changes in time and at different altitudes.

The plasmacoagulation and hemolytic activities of some found microorganism cultures were determined for the direct evaluation of their potential pathogenicity in experiments *in vitro* according to method mentioned above. Among them, more than 40% of coccoid bacterial strains and 32% of actinomycetes showed the plasmacoagulation activity. 88% of the tested actinomycetes had the ability to hemolysis (16.5% - to strong hemolysis). This is indicative of high potential pathogenicity of microorganisms in the atmosphere and their possible effect on the morbidity of the region's population.

4. Conclusions

Let us consider one more interesting aspect indirectly proving the hypothesis of the dominating character of remote sources in the formation of the biogenic component of atmospheric aerosol in the South of Western Siberia. Fig. 1 shows that the concentrations of total protein measured on the earth are usually lower than those measured at the altitudes of 500 – 7000 m; and for culturable microorganisms these values are quite comparable. Really, such situation can be observed only in the case when the biogenic component "is falling from above" into the studied atmospheric layer, which, taking into account a considerable concentration of the biogenic component, could be caused by powerful remote sources such as vegetation, soil and water reservoirs of equatorial latitudes. The time needed for the air masses to transport aerosols from remote sources, the observed retardation in the peaks of the total protein and culturable microorganisms concentrations and seasonal activity of nature in the Northern Hemisphere becomes quite understandable.

Acknowledgement. The work was partially supported by ISTC grant #3275.

5. References

Andreeva, I.S., A.I. Borodulin, G.A. Buryak, V.A. Zhukov, S.V. Zykov, Yu.V. Marchenko, V.V. Marchenko, S.E. Ol'kin, V.A. Petrishenko, O.V. P'yankov, I.K. Reznikova, V.E. Repin, A.S. Safatov, A.N. Sergeev, V.F. Raputa, V.V. Penenko, E.A. Tsvetova, M.Yu. Arshinov, B.D. Belan, M.V. Panchenko, A.N. Ankilov, A.M. Baklanov, A.L. Vlasenko, K.P. Koutsenogii, V.I. Makarov and T.V. Churkina, 2002, Chem. Sustainable Develop. 10, 523.

Artaxo, P., W. Maenhaut, H. Storms and R. Van Grieken, 1990, J. Geophys. Res. 95, 16971.

Ashmarin, I.P. and A.A. Vorobyov, Eds., 1962, Statistical Methods in Microbiological Studies. Medgiz, Leningrad. (in Russian).

Berner, A., C.H. Lürzer, L. Pohl, O. Preining and P. Wagner, 1979, Sci. Total Environ. 13, 245.

Borodulin, A.I., A.S. Safatov, A.N. Shabanov, A.A. Yarygin, O.G. Khutorova, B.D. Belan, M.V. Panchenko, 2005, Aerosol Sci. 36, 785.

Lighthart, B., 1997, FEMS Microbiol. Ecology. 23, 263.

Mattias-Maser, S., V. Obolkin, T. Khodzer and R. Jaenicke, 2000, Atmos. Environ. 34, 3805.

Methods of experimental mycology. Reference book. Naukova dumka, Kiev, 1982.

Miller, G., 1976, Experiments in Molecular Genetics. Mir Publ., Moscow. (in Russian).

Panchenko, M.V. and V.V. Pol'kin, 2001, Atmos. Ocean. Opt. 14, 478.

Safatov, A.S., I.S. Andreeva, A.N. Ankilov, A.M. Baklanov, B.D. Belan, A.I. Borodulin, G.A. Buryak, N.A. Ivanova, K.P. Koutsenogii, V.I. Makarov, V.V. Marchenko, Yu.V. Marchenko, S.E. Ol'kin, M.V. Panchenko, V.A. Petrishenko, O.V. P'yankov, I.K. Reznikova and A.N. Sergeev, 2003, Atmos. Ocean. Opt. 16, 491.

Saggie, E., 1983, The Methods of Soil Microbiology. Kolos Publ., Moscow. (in Russian).

Smirnov, V.V., S.R. Reznik, I.B. Sorokulova, 1983, Methodical recommendations for isolation and identification of bacteria of Bacillus genus from human and animals organisms. Publishing House of the D.K. Zabolotny Institute of Microbiology and Virology AS USSR (in Russian).

Starr M.P., H. Stolp, H.G. Truper, A. Balows, H.G. Schlegel, Eds., 1981, The Prokaryotes. A Handbook on Habitats, Isolation, and Identification of Bacteria. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo. 2596 p.

The detection of polycyclic aromatic hydrocarbons (the method of high performance liquid chromatography). Guidance for atmospheric pollution control. RD 52.04.186-89, State Committee of USSR for Hydrometeorology, Ministry of Health of USSR, Moscow, 1991, 647 (in Russian).

Tong Y. and Lighthart B., 2000, Aerosol Sci. Technol. 32, 393.

You W.W., R.P. Haugland, D.K. Ryan, R.P. Haugland, 1997, Annal. Biochem. 244, 277.