Estimation of the content of biogenic components in fresh snow

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The previous study of snow cover, taken all the way down nearby some sources of anthropogenic aerosols at the end of winter, was carried out for to study of atmospheric aerosol biogenic component. To detect the pollutions from these sources, fresh snow can serve as the background pollution. The data obtained from the analysis of samples collected during 4 snowfall episodes in Vector settlement near Novosibirsk are presented. The samples were analyzed for the presence of viable microorganisms, total protein, ionic and the elemental composition of the melted snow. Besides, plasmacoagulation, fibrinolytic, hemolytic, and gelatinolytic activities, as well as some biochemical and physiological characteristics of the microorganisms were estimated to gain information on potential pathogenicity of the latter. The importance of such studies for understanding the processes of global atmospheric transport of bioaerosols and detecting possible sources and discharges of these aerosols have been demonstrated.

Introduction

Data on biogenic pollution of snow cover nearby anthropogenic sources of different nature give useful information about total long-term discharge and atmospheric transfer of pollutants. The snow cover can be polluted by different ways: first, through dry deposition of atmospheric aerosols, including deposition of pollutants from a considered source; second, by washing out of pollutants from the atmosphere. In this case, two variants are possible: capture of pollutants by snowflakes from the atmosphere nearby a considered source and formation of polluted snowflakes in the atmosphere. Data on biogenic pollution of discrete samples of fresh snow far from the sources under study allow accurate accounting for background concentrations of different pollutants in snow cover nearby anthropogenic sources of different nature.

Data on concentrations of total protein and viable microorganisms measured in atmospheric aerosol at different heights, as well as in samples of snow cover, taken at the end of winter, are given in Refs. 1–4 along with data on elemental composition of snow cover pollutants. In this work, the contents of these elements in fresh snow are estimated. To obtain more complete characteristic of fresh snow composition, the ionic composition and its micro- and macro-elemental content was determined.

Materials and methods

Analyzed samples were taken during four snowfall episodes at one site in Novosibirsk environment: 1) February 18, 2005, between 10:30 and 14:30 of the local time; 2) January 18, 2006, between 10:00 and 14:00; 3) February 27, 2006, between 10:30 and 14:30; 4) March 16, 2006, between 10:00 and 14:00. Every sample was taken from an area of 1 m^2. Totally, twenty three samples were taken; three groups of six of them were analyzed for the presence of viable microorganisms, total protein concentration, and ionic composition; and five of them – for the elemental composition.

To detect *viable microorganisms*, the samples were thawed in aseptic conditions and sown on agar nutrient media, i.e. LB[5] – to detect saprophytic bacteria; starch-and-ammonia medium SAM[6] – actinomycetes; soil agar and depauperated LB (dilution 1:10) – organics-excess inhibited microflora; and Sabouraud's medium[6] – to extract lower fungi and yeast. The samples were sequentially diluted when needed. The sowings were incubated in a thermostat at a temperature of 30°C during 3–14 days. Individual colonies, appearing on the agar surfaces and differing in morphological characters, were transported on fresh nutrient media and incubated in similar conditions.

The number of viable microorganisms in samples was calculated by standard techniques[7] and averaged with accounting for the number of microorganisms revealed in 2–3 parallel sowings in 4–5 different media.

Phenotypic characters of the extracted microorganisms (cells and colonies morphology, mobility, sporogenesis, biochemical characters, Gram's stain, and fermentation activity) were studied with standard techniques.[8] In addition, for some microbe isolates their capability to grow at increased temperatures (42 and 55°C) and NaCl concentration up to 10% was studied.

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Screening of strains for lipolytic activity was carried out at 23°C on L-agar, containing 1% of Tween-20 or Tween-40 with 0.01% of $CaCl_2$ (Ref. 8).

To detect *alkaline phosphatase*, 0.3 ml of cell thick suspension in (0.85% NaCl) was added to 0.3 ml solution of substrate, containing 0.04 M glytin buffer pH 10.5 and 0.01 M disodium – *n*-nitrophenyl phosphate ("Sigma"). The mixture was incubated during 3 hours at 37°C. Test samples free of bacteria were also under control. Appearance of yellow coloration of the reaction mixture confirms the phosphatase activity of the strain.[8] The level of enzyme activity was determined with an Uniplan device (Russia) using a 450-nm wavelength color filter.

Exonuclease activity was determined on a dense medium with thymus DNA and toluidine blue. The reaction was estimated from the appearance of a brilliant rose zone around a bacteria colony.[8]

Screening of strains for endonuclease restriction was carried out according to Ref. 9; as substrates for hydrolysis, the DNA of λcI857 and T7 phages were used. Postrestriction DNA electrophoresis was carried out in 1-% agarose ("Sigma").[10] The presence of restriction endonuclease in microorganism strains was judged from appearance of discrete substrate DNA fragments in the electrophoregram UV pattern.

The content of plasmid DNA in strains was determined by standard screening method.[8] To do this, cells with a dense nutrient medium were loop-suspended in 100 μl of buffer liquid with saccharose (50 mM tris pH 8.0; 50 mM Na_2-EDTA, 15-% saccharose), added by 200 μl of alkaline solution (0.2N NaOH, 1-% SDS) and then 150 μl of 3M sodium acetate pH 5.0. Then this was centrifuged at a table centrifuge during 5 min. The residue was added with 1 ml of 96-% ethanol. The obtained DNA was analyzed in 0.8-% agarose and tris-borate buffer pH 8.0.[8]

Pathogenicity of isolated strains was determined using tests for plasmacoagulation, fibrinolytic, hemolytic, and gelatinolytic activities.[8]

The total protein content was determined from fluorescence analysis with the use of the reagent, described in Ref. 11; the sensitivity of the method was 0.01 μg/ml and the error was less than 20%. For analyzed earlier samples taken in the end of winter nearby the TPP, being the source of polyaromatic compounds, the magnitude referred to polyaromatic compounds and determined independently,[12] was subtracted from the total fluorescence value.

The *ionic composition* was determined by the following procedure: the analyzed snow sample was thawed, filtered, and then divided into two parts. One part served for determination of concentrations of NH_4^+, Na^+, K^+, F^- + $HCOO^-$, Cl^-, NO_3^-, and SO_4^{2-} ions by the HPLC method, while the second part – for measuring pH and specific conductivity. Then the concentrations of Ca^{2+} and Mg^{2+} ion sum and HCO_3^- ions were determined by the method of conductometric titration.

For *elemental analysis*, snow sample was quickly thawed and then concentrated on a graphite collector. In this work, a uniform analysis technique for graphite microelement concentrate, developed at the Analytical Laboratory of the Institute of Inorganic Chemistry SB RAS, was applied.[13] To take into account the matrix effect due to a complex composition of the analyzed sample, the method of suspension varying (0.2, 1.0, and 5.0-ml aliquots) was used. Elemental analysis of fresh snow samples was carried out by the method of atomic emission spectroscopy with arc spectrum excitation at a diffraction spectrometer PGS-2 (Carl Zeiss Jena, Germany).[13] Emission spectra were recorded using a linear photodiode array (SPA "Optoelectronica"), spectral data were computer-processed with the "ATOM" program (developed by SPA "Optoelectronica" in collaboration with IIC SB RAS).

Results and discussion

Data on weather conditions during the sampling and some parameters of snow samples under study are given in Table 1. The obtained results show a noticeably lower concentration of microorganisms in fresh snow as compared to snow samples taken in the end of winter: 0.9–1.8 Log_{10} in 1 g of fresh snow and 2.1–4.8 Log_{10} in 1 g of "old" snow in different years and different source regions.[1–3] Total protein content in 1 g of fresh snow is between 0.7 and 3.4 μg, which agrees with the previous results,[1–3] i.e. 0.4–5.25 μg/g. Study of the morphology of cells and microorganism colonies in fresh snow samples of episode 1 revealed the prevalence of nonspore-forming bacteria (70% of all the microorganisms in samples), usually colored with various tints of yellow, orange, and rose. Among them are *Pseudomonas, Flavobacterium, Arthrobacter, Nocardia*, and other microorganisms. The Coccus (21%), a small number of spore-forming bacteria *Bacillus* (1.5%), actinomycetes (1.5%), and mold fungi (6%) were also revealed in the samples.

Nonspore-forming bacteria (64%) prevail in snow samples of episode 4 as well; in this case the prevalence of pigmented bacteria was not so pronounced as in episode 1, the content of Coccus was 5%, 7% of bacillus, and 3% of mold fungi; yeast were also revealed in the samples. Coccus prevail in snow samples in episodes 2 and 3 (43 and 71%, respectively), while the content of other microorganisms are: 28 and 7% for nonspore-forming bacteria, 28 and 14% for bacillus, 2 and 5% for actinomycetes, 0 and 3% for mold fungi, respectively.

Note, that, according to data obtained in previous years, nonspore-forming bacteria also prevail in snow cover,[1–3] while atmospheric aerosol contains a large amount of coccus and bacillus. The content of nonspore-forming bacteria in snow cover probably increases due to both their income into fresh snow, and dry deposition of atmospheric aerosol containing microorganisms.

Table 1. Parameters of snow samples

Parameter	Sampling time	Episode 1	Episode 2	Episode 3	Episode 4
Temperature, °C	Beginning	−18.5	−19.5	−11.5	−11.0
	Middle	−16.8	−17.7	−10.6	−8.6
	End	−15.0	−15.6	−9.5	−7.0
Relative humidity, %	Beginning	78	78	73	89
	Middle	78	72	72	89
	End	78	73	71	85
Mean wind speed, m/s	Beginning	6	2	4	6
	Middle	6	2	4	6
	End	5	2	4	6
Wind direction	Beginning	SE	NE	S	S
	Middle	SE	NE	S	S
	End	S	NE	S	S
Pressure, hPa	Beginning	775	753	760	754
	Middle	774	753	761	754
	End	773	753	762	753
Low boundary of cloudiness, m	Beginning	450	500	800	500
	Middle	3000	800	800	800
	End	450	800	800	800
Sample mass, g/m^2		790±320	470±30	280±40	950±30
Total protein concentration, μg/g		1.32±0.07	1.29	0.71	1.68
Viable microorganisms concentration, Log(#)/ml		1.2±0.6	1.7	0.8	1.3

N o t e . Weather data were taken from the site http://meteo.infospace.ru/

In addition, for episodes 1 and 4, microorganisms in fresh snow were tested for plasmacoagulation, fibrinolytic, hemolytic, and gelatinolytic activities, which describe their potential pathogenicity, because of their reproducibility in blood and other human tissues. It was revealed for episode 1, that 62% of studied microorganisms exhibit at least one of the above four activities (38% of them exhibits two or more activities). Most these microorganisms relates to nonspore-forming bacteria and bacillus; coccus, revealed in fresh snow samples, seldom exhibit such activities. In episode 4, only 13% of the detected microorganisms exhibit more than one of the above activities, while about 50% exhibit none of them.

Capability of microorganisms to grow at different temperatures and in media with increased NaCl concentrations reflect their survival capabilities in atmosphere during long time. It has been shown, that 96% of microorganisms, revealed in episode 1, can grow at 28°C, 78% — at 37°C, 52% — at 42°C, and 26% — at 55°C. Besides, 87% of microorganisms can grow in a medium with 1-% concentration of NaCl, 91% — in a medium with 2-% NaCl, 65% — with 5-% NaCl, 57% —with 7-% NaCl, and 13% can grow in a medium with 10-% NaCl concentration. Only about 10% of microorganisms, revealed in episode 4, turned out to be incapable to grow in a medium with 10-% NaCl. All these microorganisms (100%) can grow at 30°C, 74% — at 37°C, 61% — at 42°C, and 9% — at 55°C. The obtained results point to a large body of microorganisms in fresh snow samples, which are resistant to different environmental conditions. Note, that not only *Bacillus* (being endospore-forming) are among them, but also some strains of coccus, nonspore-forming bacteria, etc.

The difference between parameters of ionic composition in each sample is evident from Table 2. They also differ from the parameters, measured at another observation point.[14] The distinctions for each observation point can be referred to variations of weather conditions, while the distinctions for parameters, determined for different observation points, — to their local environment and closeness to Novosibirsk. In the samples under study, the total content of hydrosoluble salts, estimated by electroconductivity values, was higher for episodes 3 and 4, probably, due to more effective ion washing out with snowflakes. Low concentrations of ammonium and high concentrations of calcium and magnesium cations in all samples are noticeable. Sources of the former in snow precipitations can be fine aerosol particles and, to a less degree, gaseous ammonia; while probable sources of the latter, having no volatile precursors, are coarse aerosol particles, including biogenic compounds and microorganisms.

Concentration of a number of elements, revealed in fresh snow (Table 3), as expected, turned out to be significantly lower than the values, obtained for snow cover samples within the area of anthropogenic source action (TPP-4, Novosibirsk) (Ref. 4, Table 4).

High concentrations of aluminium, silicon, calcium, and magnesium in fresh snow in episode 1 are noticeable. Combination of these elements is typical for aluminum silicates, hence, their presence in snow precipitations can be referred to action of erosive sources. Anomalies of element composition were not revealed for episodes 2—4. High aluminium concentration in fresh snow in episode 1 could be caused by the fact, that initial air masses were within a zone of action of some industrial enterprise or a gas flare, located near the air mass trajectories.

Table 2. Parameters of the ionic composition of fresh snow, %-equiv.

Episode	Conductivity, mS/m	pH	NH_4^+	$\sum Ca^{2+} + Mg^{2+}$	Na^+	K^+	H^+	HCO_3^-	F^-	Cl^-	NO_3^-	SO_4^{2-}
1	1.39	6.19	6.4±0.1*	79.2±1.5	12.6±2.0	3.3±0.4	0.51±0.04	21.6±1.3	5.5±0.9	13.5±0.8	31.3±2.6	28.1±2.1
2	0.75	5.66	н/о	77.1	14.7	5.0	3.1	25.1	10.6	14.0	39.0	11.2
3	1.72	5.92	12.2	68.0	14.4	4.6	0.8	15.2	11.8	16.4	32.5	24.2
4	1.83	5.72	13.3	61.2	19.1	5.1	1.2	11.6	6.3	33.5	17.1	31.6

* By two measurements; other parameters for episode 1 are determined by three measurements; one measurement was carried out for each of episodes 2–4.

Table 3. Concentrations of some elements in fresh snow, μg per 1 ml of melting water.

Element	Episode 1		Episode 2	Episode 3	Episode 4	Error, %
	Sample 1	Sample 2				
Al	0.80	0.60	0.05	0.07	0.03	40
Ba	0.020	0.010	0.015	<0.01	0.01	50
Be	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	
B	<0.05	<0.05	<0.05	<0.05	<0.05	
Fe	0.13	0.11	0.03	0.15	0.02	40
Cd	<0.00004	<0.00004	0.004	0.003	0.03	9
Mn	0.007	0.004	0.002	0.005	0.018	24
Cu	0.002	0.001	0.002	0.004	0.002	20
Ni	0.001	0.001	<0.001	<0.001	<0.001	
Pb	0.003	0.002	0.003	0.015	0.002	25
Cr	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Zn	0.01	0.01	0.01	0.015	0.035	65
Si	1.1	1.1	–	–	–	12
Sn	0.002	0.002	<0.002	<0.002	<0.002	60
Ca	4.8	4.8	0.8	1.3	2.9	8
Mg	0.14	0.14	0.04	0.08	0.09	31

Note. "–" means the absence of data.

Table 4. Concentrations of heavy metals in seven samples of snow cover nearby TPP-4 (February 2003) in coarse (C) and fine (F) dust fractions and in hydrosoluble parts of the samples (H), μg/l

Element	Sampling point No.																				
	1			2			3			4			5			6			7		
	C	F	H	C	F	H	C	F	H	C	F	H	C	F	H	C	F	H	C	F	H
B	38	1	51	26	<0.4	56	48	1.3	126	26	1.1	62	24	2.4	37	37	3.5	125	85	1.4	80
Ba	300	9.5	37	390	4.2	32	750	26	82	280	8.4	48	250	12.8	24	600	16	62	1000	5.2	50
Cu	6.5	0.6	8	6.4	0.9	4.8	8.8	0.45	2.7	4.2	1	3	5.7	0.26	2.3	6.5	0.4	2.5	10	<0.1	3
Mg	–	–	1200	–	–	900	–	–	930	–	–	580	–	–	720	–	–	700	–	–	620
Mn	110	3.5	13	140	1.8	18	230	5.6	13	63	1.4	11	73	2.2	8.5	160	4.3	10	300	1.1	12.5
Ni	2.9	0.4	4.7	5.1	0.2	3.3	6.6	0.4	3	1.2	0.2	2.5	3	0.2	3.1	4.6	0.3	<2	10.6	<0.1	6.2
Zn	34	2.2	14	30	1	9	27	1	7	26	1.5	6	16	1.2	3	23	1.8	<2	32	0.5	9.6
Co	1	0.2	–	1.4	<0.1	–	2.4	<0.1	–	<0.6	<0.1	–	1.3	<0.1	–	0.9	0.3	–	2.8	<0.1	–
Cr	0.8	<0.1	–	3.4	0.2	–	4.9	<0.1	–	1.2	0.3	–	2.5	<0.1	–	5.2	<0.1	–	7.6	<0.1	–
Pb	28	0.9	–	36	0.2	–	39	1.5	–	23	1.3	–	18	0.8	–	34	1.6	–	32	0.2	–
Sn	18	2.4	–	15	1.4	–	12.5	1.5	–	7	2	–	19	2.4	–	15	2.4	–	23	1	–

Besides, as was mentioned above, aluminium is the main component of a number of soil-forming minerals, therefore, its presence in a sample can be random.

Actually, modeling of 10-day back trajectories of air masses, bringing snow, has shown that they pass through the Pavlodar, Ekibastuz, Omsk, Nizhnevartovsk, and Surgut town (Fig. 1) which could contribute in the pollution (episode 1). It is possible that the back trajectories of air masses in episodes 2–4, for which no anomalies of element composition were revealed, pass far from acting powerful sources of aerosol pollution. Ten-day air mass back trajectories were modeled with the HYSPLIT-4 program (http://www.arl.noaa.gov/ready/hysplit4.html) for the above-described conditions at the sampling point (54.94°N, 83.23°E) at a height of 500 m.

It is necessary to note, that distinctions in representation of viable microorganisms in fresh snow samples in the considered episodes can be (and most likely) also caused by peculiarities of specific trajectories of air masses, carrying snow. However, the data correlating representation of viable microorganisms in fresh snow samples with a trajectory of air mass, carrying this snow, are lacking at present.

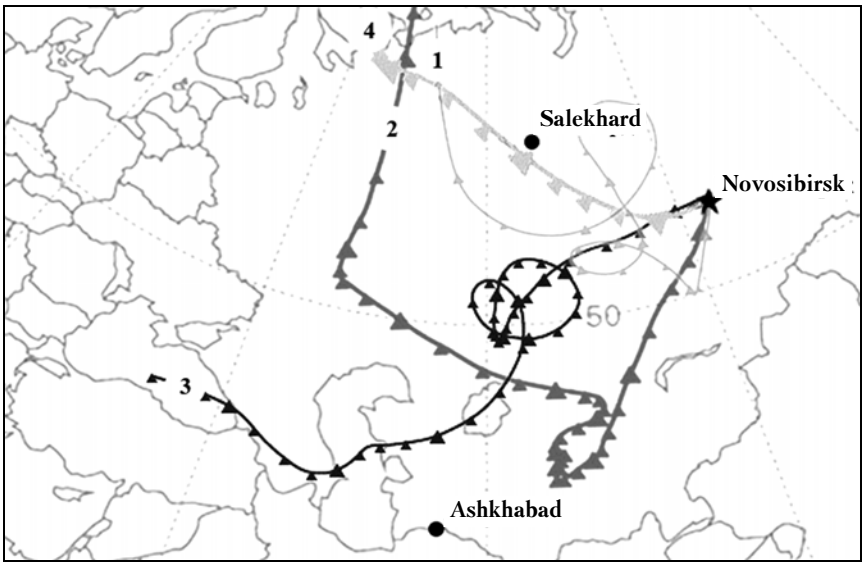

Fig. 1. Back trajectories of air masses, bringing snow in four episodes under study, modeled for a height of 500 m and the sampling mid-time. A trajectory number corresponds to the number of an episode under study.

Conclusion

The conducted investigations have shown that fresh snow samples in each episode differ essentially in concentrations of biochemical and chemical contaminants, presented in them, from samples, taken at the end of winter. In particular, the quantity and combination of microorganisms varies for different samples. These data along with the data on plasmacoagulation, fibrinolytic, hemolytic, and gelatinolytic activities of the studied microorganisms give information about their conventional pathogenicity, i.e. their potential hazard to a human health.

Hence, studying all valuable snowfall episodes for the period of snow cover formation and subtracting pollutant concentrations related to fresh snow, from those observed in snow cover, it is possible to detect the pollutants coming to the snow cover with precipitating atmospheric aerosol. These values can be compared with biological and chemical composition of atmospheric aerosol, integrated over all the winter observation period in the same region.

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