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Fractional Composition and Toxicity Coal–Rock of PM_{10}-$PM_{0.1}$ Dust near an Opencast Coal Mining Area and Coal-Fired Power Station

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Abstract: This study is aimed at elucidating the fractional composition, volume and toxicity of dust that is deposited in the snow cover for the period of snow accumulation at different distances from coal mines and a coal-fired power station in the Kemerovo region (Russia). During the filtration process, fractions of 10–0.1 μm and less than 0.1 μm were isolated and weighed. Light microscopy was used to estimate the size of dust particles in the 10–0.1 μm fraction. We found that the total volume and fractional composition of dust has no significant trend to change in the research space. The dust contamination is associated mainly with PM_2 particles. Genotoxic tests on cell lines A549 and MRC-5 with different concentrations of dust showed high toxicity (including control points). Taking into account the fact that an increase in the concentration of PM leads to intensification in the toxicity of dust, we can determine that the territory within the studied boundaries is dangerous for the population. Our study is important for understanding the processes of formation, toxicity, transport and sedimentation in the snow cover from dust generated in the process of coal mining and the operation of a coal-fired power station.

Keywords: open-pit coal mines; $PM_{0.1}$; PM_{10}; particulate matter (PM); coal–rock dust; fractional composition of dust; DNA damage; MRC-5; A549

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1. Introduction

Particulate matter (PM) is an important air pollutant that has significant influence on people all over the world. Studying the fractional composition, migration, concentration, sources and toxicity of PM is substantial for formulating and evaluating air safety from the standpoint of sustainability. PM is a major problem in many countries, especially those with developed industries and road transportation infrastructure, but with insufficient efforts for atmosphere protection [1,2].

PM is an air pollutant emanated from both natural [3,4] and anthropogenic sources [5–7]. The extractive and energy industries stand out among the latter sources, and produce a significant PM volume [8,9]. Previous studies have shown that these plants emit significant amounts of various pollutants, including PM, and thereby change the dust load in the surrounding atmosphere [10–13]. Various models have demonstrated the influence of coal mining on the growth of PM extending up to 200 km [11] and [14]. Mining and supporting transporting processes generate a sufficient amount of rock, coal and mixed dust. PM spreading around open-pit coal mines depends on their design, local meteorological

condition [15] and surrounding landscape. Proximity of homes to mining area, hauling and transportation ways can be determined by a landscape that leads to chronic residential exposure [16]. Sometimes the coal traffic process can induce a higher PM emission than materials storage [17]. Coal dust contains a substantial amount of harmful elements including Pb, Cr, Cd, Ni, Cu, Co and Zn [18]. Cd, Cr and Pb have been identified as coal and oil combustion contributors. Coal dust has been suggested as a significant hazard and cancer risk near mining areas [19]. Toxicity is also attributed to some rock derivate as secondary crystallized minerals from mine drainage [20]. In this connection, this problem is not local for settlements located around coal mining. Thus, we can talk about trans-regional pollution as a result of mining activities. However, it is generally accepted that the industrial impact on air quality decreases with distance from the polluting object.

It is established that atmospheric pollution with PM has serious annual, seasonal and daily differences [21–24]. In addition, the change in dust emissions from the enterprise is associated with its mode of operation, transport and other factors [25,26]. To overcome the influence, the dust load on the territory can be assessed by using snow. Snow cover is an accumulate PM reservoir. According to previous studies, the winter season has the most polluted atmosphere due to a decrease in wind speed, precipitation and other reasons [27,28]. Earlier results were obtained showing that the winter season has the most polluted atmosphere due to a decrease in wind speed, precipitation and other reasons [29–33].

Adverse health effects have been well documented in research studies. Publications have reported the potential effect of atmospheric PM on respiratory morbidity [34,35], premature mortality and cardiovascular disease among others [36–39]. In particular, scientific papers have presented data on an increase in health risks, including carcinogenicity, for populations living near a coal mine [19,40–42]. Adverse cytotoxic and genotoxic effects have also been recorded in these territories [43], and other areas with high levels of PM [44]. It is important to investigate the PM which transfer a significant distance from industrial enterprises (coal-fired power station, open-pit coal mines and other) to which resident population groups can be exposed.

The toxic effects are widely attributed to inhalable particulate matter <10 μm including urban and traffic emission [45–47]. Metals, PAHs and other organic substances absorbed on particle surface may increase the oxidative damage to DNA. Exposure to coal and coal fly ash particles generated in experiments by coal combustion is toxic to various cell types. A higher level of DNA damage and micronucleus frequency in V79 cells has been shown [43,48], with highest toxicity in particles generated by complete combustion at 700 °C [49]. Direct exposure of airways tissue by coal/ash/oil fuel particles demonstrated a significant DNA damage obtained by comet assay [50]. Genotoxic damage also supposed for native PM and other coal mining pollution components during occupational exposure [41,51,52] and in a wildlife population [53]. Evaluation of the volume and fractional composition, therefore, is a significant parameter in the assessment of health effects, since differently sized PMs penetrate different areas of the human respiratory tract. There are numerous adverse effects associated with PM including respiratory illness [54]. Respirable coal dust particles less than 4 μm cause unacceptable lung tissue damage provided with oxidative stress [55]. Coal dust PM was found to produce oxidative damage in vitro in lung cells [48,56]. This could potentially lead to distinctive health consequences for various particle size ranges [57].

This study focuses on open-pit coal mines and a coal-fired power station, which is an anthropogenic source of PM in the Kemerovo region. We attempted to identify the fractional composition and volume of PM in the snow outside the sanitary protection zone (established 0.5–1 km in Russia) and at different distances around open-pit coal mines and a coal-fired power station. For the study area, emission, transportation and sedimentation of dust particles occur under winter conditions for a significant amount of time per year. After sampling, genotoxic tests were carried out on A549 and MRC-5 cell lines. Additionally, the study of dust at a great distance from its source is important for assessing the epidemiological safety of the territory of most settlements of Kuzbass and similar territories.

Thus, our study is aimed at elucidating the fractional composition, volume and toxicity of dust that is deposited in the snow cover in winter at different distances from coal mines and a coal-fired thermal power plant. These results may be important for areas with similar climatic conditions in the world [58]. Earlier work has focused on the evaluation of larger fractions, which have less impact on human health and have less transport capacity [59].

2. Materials and Methods

2.1. Study Location

Figure 1 shows a geographical map indicating the anthropogenic sources of coal–rock dust, including our research area (open-pit coal mines and coal-fired power station). The study area belongs to a sharply continental climate zone.

The average annual rainfall is 300–400 mm, and is maximal during summer (July). For this area, the average annual wind speed is 2–3 m/s. In general, the Kuznetsk coal basin is characterized by a significant number of windless days. According to the wind rose, south and southwesterly winds prevail in the open area. Usually, the snow cover lasts for 5 months, October/November–March with a thickness of 0.3–1.5 m, depending on the local landforms and landscapes. In the year of the study, the snow cover began to form at the end of October, and became stable in the first 10 days of November.

The landscapes are represented by dry steppes, with rare shrubs and woody vegetation along the rivers. In addition, vegetation is found in wind-breaks planted by humans to reduce soil erosion. The soil is predominantly leached black soil.

The relief of the area is represented by trough-shaped river valleys, which have gentle watershed spaces. The average height of the terrain is approximately 240–260 m, and the difference corresponds to an interval of 200–360 m. The highest of the terrain are fixed in the area of the observation point C. The relief has been markedly transformed because of human activities, the primary contributor being mining. Anthropogenic landforms are associated with open-pit coal mining, overburden deposition and reclaimed land.

The study area is located within the western Salair and Central parts of the Kuznetsk coal basin. Rocks are represented by rhythmically bedded deposits containing sandstone, siltstone, mudstone and coal. These rocks belong to the Carboniferous and Permian systems. Paleozoic rocks are covered with quaternary loose deposits, which are mainly in the form of loams.

In this case, anthropogenic sources of dust arose from coal mining, transportation and storage of rocks in dumps. In addition, the engines of the operating equipment may also be contributing sources. Additionally, a coal-fired power station located in the vicinity of the city of Belovo is involved in dust formation. Case sample points were located near the open-pit coal mines (“Bachatskiy”, “Novobachatskiy”, “Permyakovsky”) and the coal-fired power station “Belovskaya”. These open-pit coal mines overall coal production is approximately 15 million tons per year. “Belovskaya” power station utilizes about 2.8 million tons of coal per year for power and heat production. Control PM samples collected near the Kuzbasskiy (K1) and Krasnoe (K2) villages, which are placed at least a 15 km distance from the industrial enterprises (open-pit coal mines, coal-fired power stations and other).

2.2. Snow Sampling Process

Particle samples were collected from the territory Salair and Central parts of the Kuznetsk coal basin at the second decade of March 2020 (corresponds to the end of the snow accumulation period end of the snow accumulation period) (Table 1). The snow was sampled from six territories (3 near open-pit coal mines, 1 coal-fired power station and 2 control areas). Within the case territories, snow was sampled at a distance of 500–3700 m from the source of dust (open-pit coal mines, coal-fired power station) with a step between sampling points of 500 m. The sampling lines were located parallel (B,C,D) and in some cases, perpendicular (A) to the dominant wind direction, which was determined from the data of meteorological stations and using snow pollution information obtained from Google

Maps. In all cases, the first sampling points were determined by the local conditions. Water and river barriers, fences and other objects have led to some difference in the distances between the study areas.

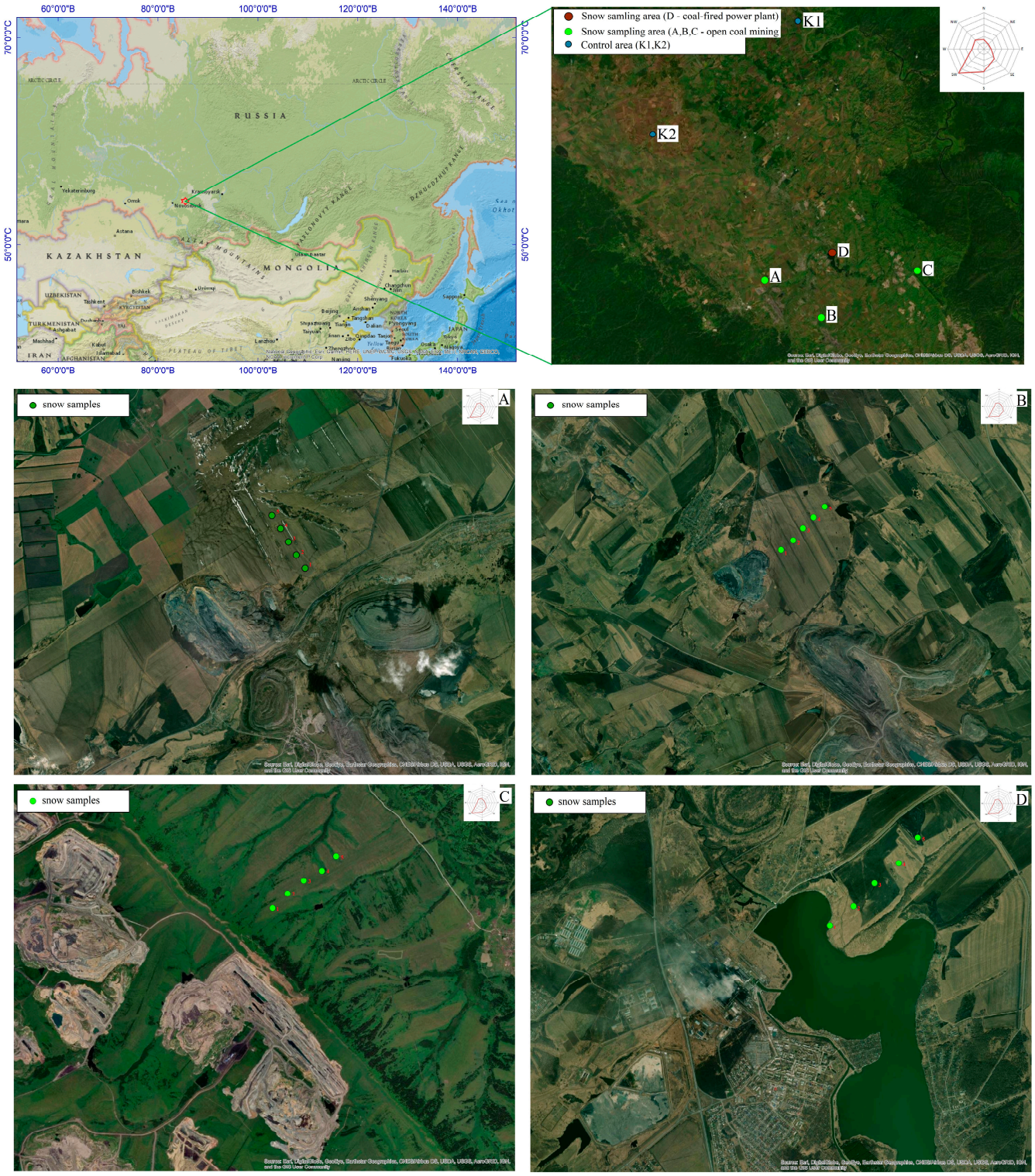


Figure 1. Location of sample collecting to case (coal-fired power station and open pit mines) and control points. Note: Case ((**A**)—Open-pit mine "Bachatskiy", (**B**)—Open-pit mine "Novobachatskiy", (**C**)—Open-pit mine "Permyakovsky", (**D**)—Coal-fired power station "Belovskaya"), Control (K1—Near v. Kuzbasskiy, K2—Near v. Krasnoe).

Table 1. Characteristic of PM collection points.

Samples Area	Symbol	Distance from the First and Other Observation Points to the Industrial Enterprises, m				
		1	2	3	4	5
Open-pit mine "Bachatskiy"	A	1250	1300	1480	1800	2180
Open-pit mine "Novobachatskiy"	B	500	1000	1500	2000	2500
Open-pit mine "Permyakovsky"	C	1250	1750	2250	2750	3250
Coal-fired power station "Belovskaya"	D	1700	2200	2700	3200	3700
Near v. Kuzbasskiy	K1	>15,000				
Near v. Krasnoe	K2	>25,000				

The snow was collected in plastic barrels with a tightly sealed lid and a total volume of 50 L. Samples were obtained at total capacity by snow sampling devices made of chemically resistant polymer material. To reduce the ingress of soil particles into the sample during the collection of snow, the above-soil layer of approximately 5 cm thickness was not obtained. Sampling was processed according to the snow survey method on a 5 × 5 m plot (GOST 17.1.5.05-85). From each sampling point, snow samples were collected, and mixed in the laboratory after thawing in equal proportion. Then 1 L of liquid sample was used for sampling and subsequently frozen.

Thawed samples were subjected to sequential filtration. We used 10, 2.5 and 0.1 μm membrane nylon filters (CVS, Sanford, FL, USA) and Sterifil vacuum system (Merck KGaA, Darmstadt, Germany) as described early [60].

The last step was to obtain a suspension of $PM_{0.1}$ by partial concentration using an Eppendorf Concentrator plus vacuum rotary concentrator (Eppendorf, Hamburg, Germany).

Finally, PM suspension was dissolved in Hanks' solution (Biolot, Saint Petersburg, Russia) to the final concentration of 10 mg/mL and disinfected in Elmasonic S30H ultrasonic bath (Elma, Singen, Germany) for 30 min. A positive control sample, a suspension of ALEX nanosized aluminum powder (Advanced Powder Technologies, Tomsk, Russia), was autoclaved 121 °C for 30 min and processed similarly.

2.3. Statistical Analysis

The Statistica 12.0 software package (StatSoft, Tulsa, OK, USA) was used for statistical analyses. The mean values and standard deviations were calculated for each parameter. Comet assay parameters, PM particle size and area were submitted to the Kolmogorov–Smirnov test to verify normality. Comparisons among groups with different PM type and concentration were performed by Kruskal–Wallis rank test. Alpha level $p = 0.05$ was considered statistically significant.

2.4. Light Microscopy

For optical microscopic images, a preliminary filtered PM suspension and deionized water (10 μL) were resuspended in 50 μL of isopropyl alcohol (Vekton, St. Petersburg, Russia). The dust suspension was dropped from a height of approximately 1 cm onto clean glass slides and three drops were applied to one slide. Light microscopy images were acquired with Altami Lum1 microscope (Altami, Saint Petersburg, Russia) with 100 × 10 immersion objective. Particle counting was conducted under a light microscope Altami LUM 1 (Altami, St. Petersburg, Russia) at 1000× magnification under a 100× objective (Altami Plan ICCOS PL 100×/1.25) in the oil immersion phase. We used an Altami MTR3CCD06000KMA camera (Altami, St. Petersburg, Russia). The area, perimeter,

length, width and ellipticity of the particles were measured using JMicroVision v 1.3.3 (Switzerland).

2.5. Cell Cultivation and Exposure

MRC-5 human embryonic lung fibroblasts and A549 adenocarcinomic human alveolar basal epithelial cells were used for the exposure by PM samples. Cell cultures were obtained from the State Scientific Center of Virology and Biotechnology "Vector" (Novosibirsk, Russia) collection. Cells were cultured in a 5% CO_2 humidified atmosphere. MRC-5 were grown in Eagle's MEM nutrient medium (Biolot, Saint Petersburg, Russia), with 10% fetal calf serum (Biolot, Saint Petersburg, Russia), non-basic amino acids solution (1%) and 50 IU/mL penicillin–streptomycin (Paneco, Moscow, Russia). A549 were grown in F-12 K medium (Corning, Manassas, VA, USA) with 10% fetal calf serum (Biolot, Saint Petersburg, Russia) and 50 IU/mL penicillin–streptomycin (Paneco, Moscow, Russia). Cell cultures were grown in 75 cm^2 culture flasks (Eppendorf, Hamburg, Germany). For experiments, cells were seeded into six-well culture plates (Eppendorf, Hamburg, Germany), harvested and counted as described previously [60].

Three replicates were cultured for each sample type. In addition, we used three control samples ("negative control", C−), positive ("positive control", C+) and dilution control ("dilution control", Cd).

2.6. Single-Cell Gel Electrophoresis (Comet Assay)

The single-cell gel electrophoresis (comet assay) was performed according to the protocol by Singh et al. [61] with slight modification. 30 µL of cells suspension were gently mixed with 100 µL of 1% low-melting-point agarose (AppliChem, Council Bluffs, IA, USA) at 39 °C. Then, 50 µL of cell agarose suspension was placed on a slide (Deltalab, Barcelona, Spain) pre-layered with 1% normal agarose (AppliChem, Council Bluffs, IA, USA) and covered with a coverslip (Deltalab, Barcelona, Spain). Slides were cooled on ice for 5 min and placed in a lysis solution (pH 10; 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris base, 1% Triton X-100, 10% DMSO, (AppliChem, Council Bluffs, IA, USA) overnight (16 h) at 4 °C. Then, the slides were subjected to alkaline electrophoresis in a horizontal electrophoresis chamber (Bio-Rad, Hercules, CA, USA) in the dark conditions at 4 °C for 20 min at 0.8 V/cm and 300 mA. Electrophoresis buffer contains 300 mM NaOH, 1 mM Na_2EDTA (pH > 13). After electrophoresis, slides were washed twice in PBS buffer (pH 7.4) and single in 76% ethanol for 5 min, per step, dried in the dark for 1 h and stained with SYBRGreen X100 (Sigma, Steinheim, Germany). Comet images were obtained under a Carl Zeiss fluorescence microscope (200× magnification). From each sample, 300 comets were counted from three slides in equal proportion. CASP 1.2.3beta2 software (CaspLab) was used to obtain the tail intensity (TI, the percentage of DNA in the comet's tail) and OTM (Olive tail moment) [62].

3. Results and Discussion

3.1. Characteristics of Dust Pollution of Snow Collection Points

Table 2 shows the results of the PM contents obtained by the fractional separation. The dust accumulation rate during snow accumulation was also calculated. Most of the collective body of evidence used filter-collected PM or crushed (burned) coal/rock samples. Our samples were collected from snow samples in a distance (500–1700 m) from the PM-generating object. Snow accumulated for about 5 months prior to sampling. Thus, we suggest such samples provide a good representation of PM, and it can also be sufficiently chemical modified. However, this did not exclude a further distribution of these particles and mixing, for example, with disturbed soil concretions and exposure to the local population.

Table 2. Characteristics of dust pollution of snow collection points.

PM Collection Area	Point Number	Snow Samples					
		Total for the Period of Snow Accumulation, μg/cm^2			Accumulation Rate, μg/cm^2·day		
		≤PM_{10}	≤PM_{10} > $PM_{0.1}$	≤$PM_{0.1}$	≤PM_{10}	≤PM_{10} > $PM_{0.1}$	≤$PM_{0.1}$
A	1	536.5	263.2	273.3	4.79	2.35	2.44
	2	331.58	88.6	242.98	2.961	0.791	2.17
	3	157.8	63.2	94.6	1.409	0.564	0.845
	4	154.1	49.1	105	1.376	0.438	0.938
	5	153.3	53.2	100.1	1.369	0.475	0.894
	Av.1–5	266.656	103.46	163.196	2.381	0.924	1.457
B	1	472.96	114.11	358.85	3.975	0.959	3.016
	2	190.26	40.77	149.49	1.599	0.343	1.256
	3	207.96	82.83	125.13	1.748	0.696	1.052
	4	224.11	101.80	122.31	1.883	0.856	1.028
	5	197.57	39.49	158.08	1.660	0.332	1.328
	Av.1–5	258.572	75.8	182.772	2.173	0.637	1.536
C	1	407.06	276.03	131.03	3.107	2.107	1
	2	152.69	62.82	89.87	1.166	0.480	0.686
	3	291.15	91.92	199.23	2.223	0.702	1.521
	4	101.67	15.13	86.54	0.767	0.116	0.661
	5	400.26	187.44	212.82	3.055	1.431	1.625
	Av.1–5	270.566	126.668	143.898	2.065	0.967	1.099
D	1	608.21	213.21	395	5.069	1.777	3.292
	2	265	41.03	223.97	2.208	0.342	1.866
	3	368.21	71.54	296.67	3.068	0.596	2.472
	4	187.82	59.23	128.59	1.565	0.494	1.072
	5	199.49	21.03	178.46	1.662	0.175	1.487
	Av.1–5	325.746	81.208	244.538	2.715	0.677	2.038
K1		126.41	16.67	109.74	1.149	0.152	0.998
K2		125.58	38.85	86.73	1.144	0.353	0.789

Crystalline particles found in the samples were represented by amorphous silicate phases, polycrystalline quartz and calcite (except A sample). Besides this, it was found that amorphous carbon and quartz—which had particles bigger than 100 nm—disappeared in $PM_{0.1}$ samples compared to PM_{10} samples. The chemical composition of our samples has been previously presented in detail [60]. PAHs also were identified in water samples obtained from snow. The highest PAHs concentration was found in A sample "Open-pit mine "Bachatskiy".

Based on the results of our work, we recorded an increase in snow cover average pollution between sampling points of $PM_{0.1}$ fraction near a coal-fired power station compared to open-pit coal mines. Moreover, according to our data, in general, higher snow pollution was found near the coal-fired power station than near the open-pit coal mines. The results of the control measurements showed a cleaner snow cover. The content of dust in the snow in the vicinity of the studied enterprises amounted to being two to three times higher than in the control areas. At almost all of the research points, the weight and accumulation rate of $PM_{0.1}$ was higher than that of $PM_{0.1}$–PM_{10} (Figure 2).

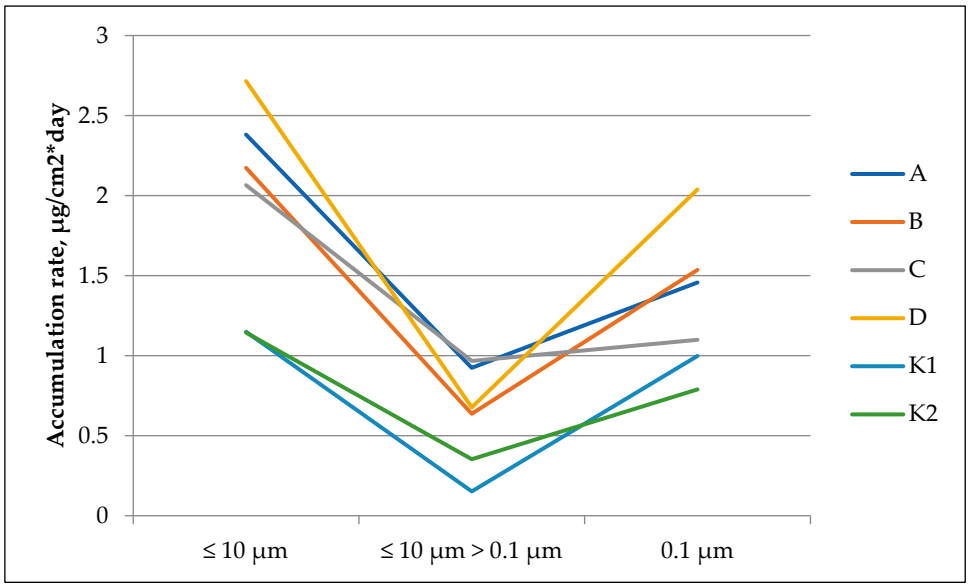


Figure 2. Accumulation rate near dust sources and control territory.

We recorded an increase in the amount of the smaller sized dust fraction in snow cover at almost all research points. This may be associated with the better migration ability of this fraction in the atmosphere [63], better removal from the air by precipitation (snow), and better ability to accumulate efficiently in the snow cover, among other fac-tors. This circumstance should be taken into account in monitoring and assessing risks to public health, since, as is known, the toxicity of this fraction is significantly higher than that of large fragments [55]. Near the open-pit coal mine coal pits (distance from 500 to 3250 m), a comparable average value of PM_{10} dust content in snow was recorded between five observation points. In addition to this, at all the closest observation sampling points, PM_{10} was higher than all the others. This was the same for a coal-fired power station. We did not observe a regular decrease in the total dust volume with distance from the source of dust (open-pit coal mines, coal-fired power stations), with the exception of points one and two in all cases. This statement is also applicable to the gradual decrease in the amount of dust from PM_{10} to $PM_{0.1}$. Thus, according to the analysis of snow, we did not see a significant decrease in pollution with distance from the source of dust, which is typical in the study of dust air pollution [59]. This result needs an additional study with a more regular network of observations in different wind directions. In our opinion, the fact of a significant excess of the dust content of less than 10 µm and 0.1 µm at such a distance from the dust source cannot be left without attention in relation to the control areas (the excess is more than two to three times, at some observation points and five times).

In coal mining areas, the main source of PAHs is coal mining and combustion, which is confirmed by previous studies [64,65], including for Kuzbass [60]. Previously, the chemical composition of dust particles was studied for these territories, so quartz (SiO_2), calcite ($CaCO_3$) and carbon were found in the PM_{10} fraction, and calcite ($CaCO_3$), gypsum ($CaSO_4*2H_2O$), halite (NaCl) and sylvan (KCl) were found in the $PM_{0.1}$ particles. Sr, Cu, Ti, Zn, Mn and Pt were found in particles of 10 µm, and Sr, Cu, Zn, Hf, Al, Mn and Rn were found in particles of 0.1 µm [60]. PAH emissions can be associated with spontaneous combustion of coal in outdoor storage conditions and coal combustion in homes and coal-fired power stations [66].

3.2. Fractional Composition by Light Microscopy

The research results of light microscopy are shown in Figure 3.

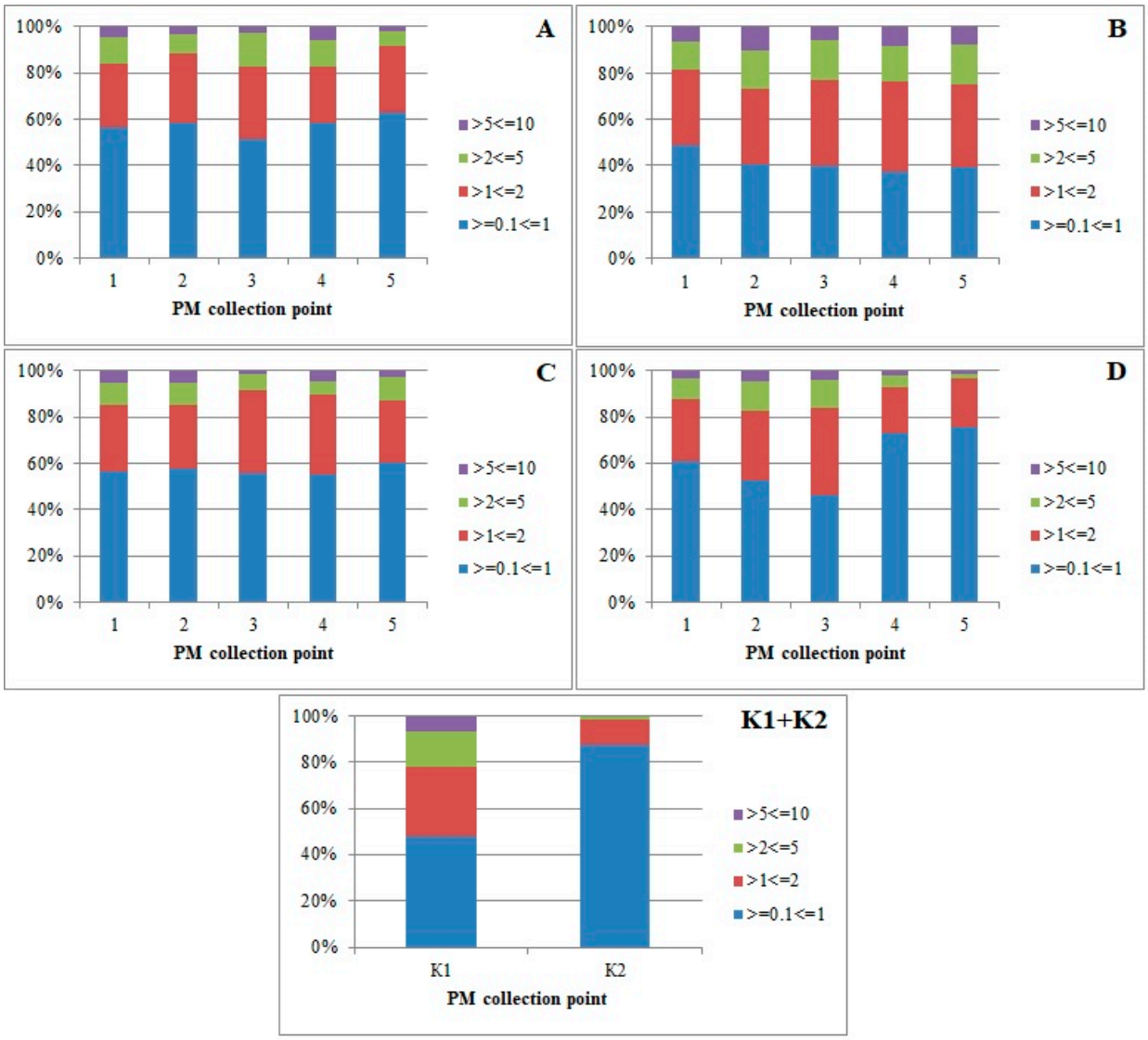


Figure 3. Fractional composition by light microscopy for samples area. Note: Case ((**A**)—Open-pit mine "Bachatskiy", (**B**)—Open-pit mine "Novobachatskiy", (**C**)—Open-pit mine "Permyakovsky", (**D**)—Coal-fired power station "Belovskaya"), Control (K1—Near v. Kuzbasskiy, K2—Near v. Krasnoe).

According to our results, for almost all observation points (including control areas), the fractional composition consisted of approximately 80% of dust in size from 0.1 μm to 2 μm. In addition, according to the previously given data in Table 2, we noted the excess of the $PM_{0.1}$ fraction over the other fraction. Thus, almost all extracted PM_{10} dust consisted of fragments with a dimension of 2 μm or less. Apparently, it is this dust fraction that is able to migrate over significant distances (up to 3700 m) in winter around open-pit coal mines and coal-fired power stations. In addition, it should be carefully studied in relation to toxicity to the population. The industrial specialization of most of the settlements of the Kuznetsk coal basin and the proximity of quarries and mines to residential areas increase the relevance of these studies. This is extremely important because large amounts of toxic particles are released during mining and power generation [25,67]. The sanitary protection zone of 1 km regulated by law in Russia for open-pit coal mines apparently does not provide the necessary level of protection of the population from dust pollution, especially associated with particles less than 2 μm. These particles are a great danger to humans, as stated in previous studies [68]. It should be noted that the fractional composition of dust for the control areas was very similar to the areas near the coal pits and power plants. For K1, a

slightly coarser fraction was noted, and this is probably due to a closer location to large settlements and highways.

The comparison of dust fractions between areas of snow cover study (Figure 4) shows that even if there is an equal dust load, dust dimensions can be different and this must be taken into account due to its different toxicity to humans.

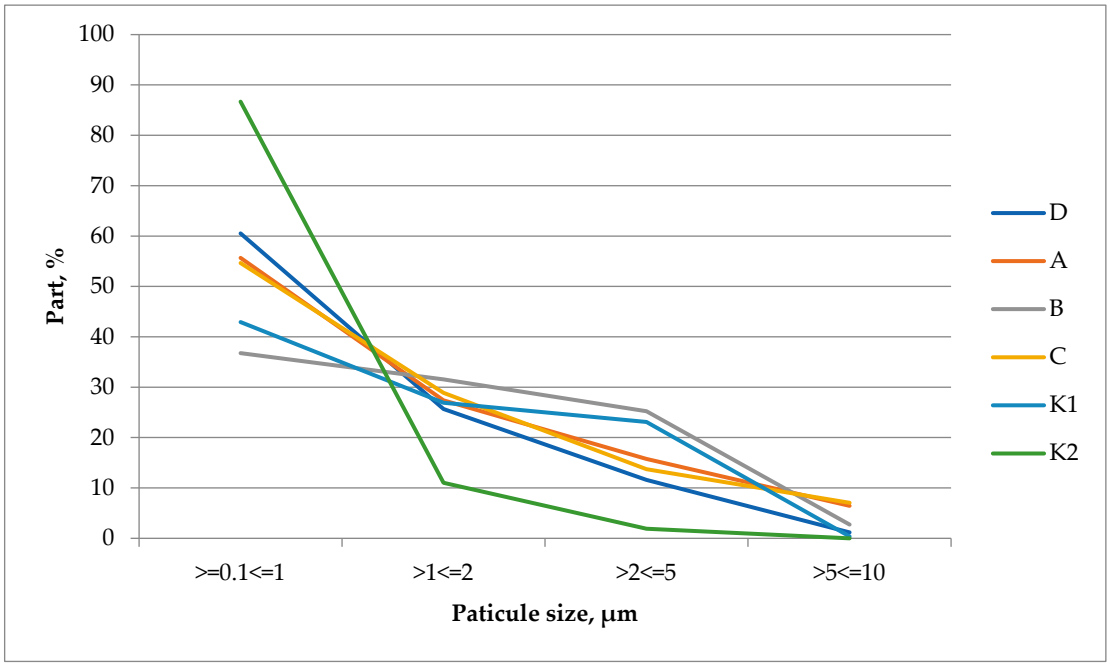


Figure 4. Comparison fractional composition by light microscopy for samples area.

The fractional composition of dust particles, apparently, can be associated not only with the proximity of the source of pollution, but also with the possibility of the territory to sedimentation of dust of one dimension or another. In addition, the process of secondary transport and secondary sedimentation of snow, already together with dust particles, interferes with the results of dust distribution in space. However, this requires more detailed studies and does not exclude the existence of a danger of this dust for the population even at considerable distances from its source.

In addition, due to the lack of self-purification of the atmosphere at these distances from dust particles, it is worth exploring the possibilities of protection with the help of various plants that are able to purify the air from fine fractions.

3.3. DNA Comet Assay

The comet assay results (TI, OTM) of suspension of snow-collected PM samples (A–D, K1, K2) are presented for MRC-5 (Figure 5) and A549 cells (Figure 6). In MRC-5 cells, the positive dose–response effect for TI was observed for all samples with $PM_{0.1}$ and for A, B, C and K1 samples with PM_{10}. The TI and OTM values obtained for $PM_{0.1}$ were significantly higher than PM_{10} for D and K2 points and equal for C and K1 points. The highest damage response was observed for D and K2 points (power plant and control territory two) both for $PM_{0.1}$ and C point (open-pit mine "Permyakovsky") for PM_{10}.

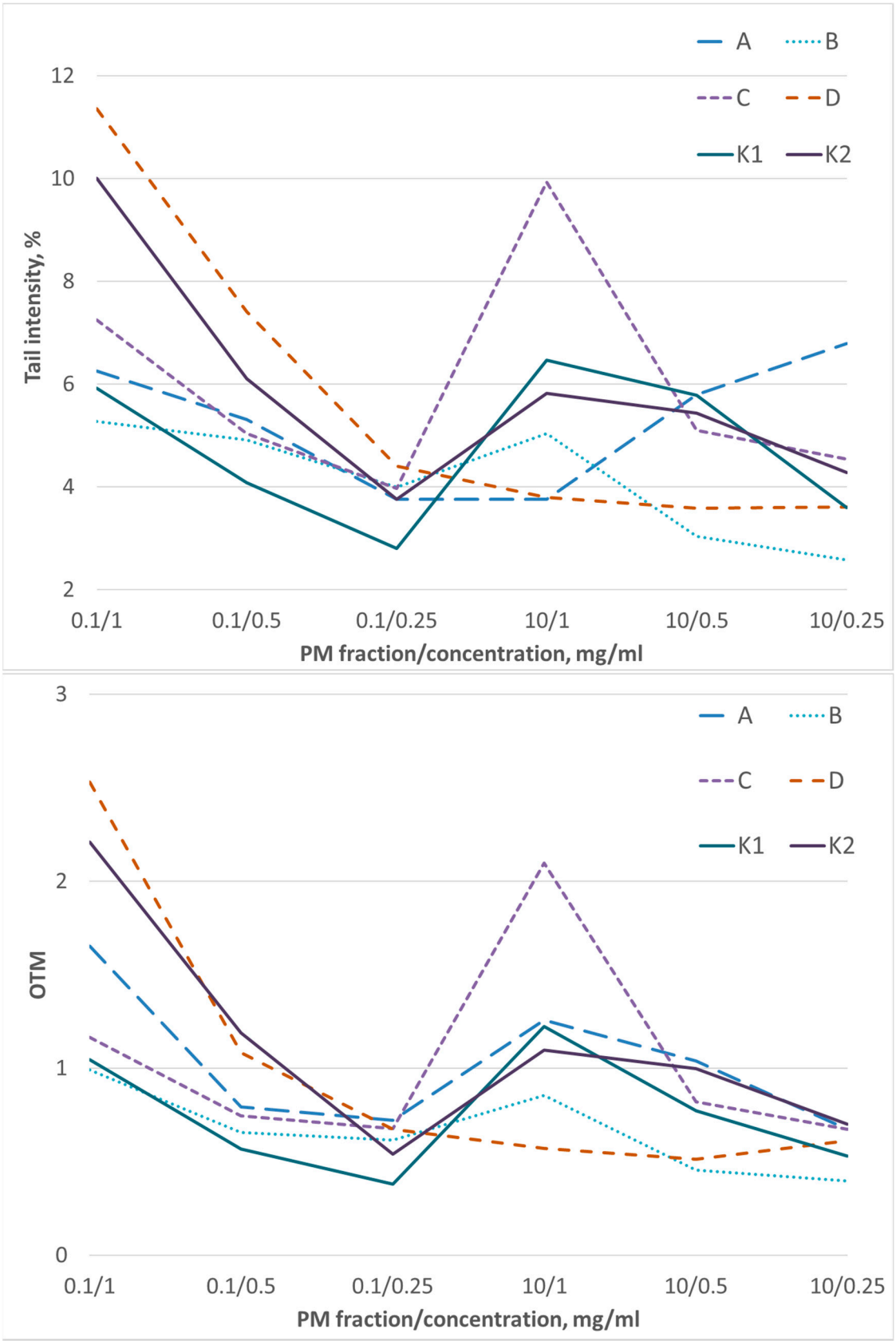


Figure 5. PM induced DNA damage in MRC-5 cells (samples named as PM fraction first 0.1 or 10 for $PM_{0.1}$ and PM_{10} and concentration follows 1, 0.5 or 0.25 mg/mL).

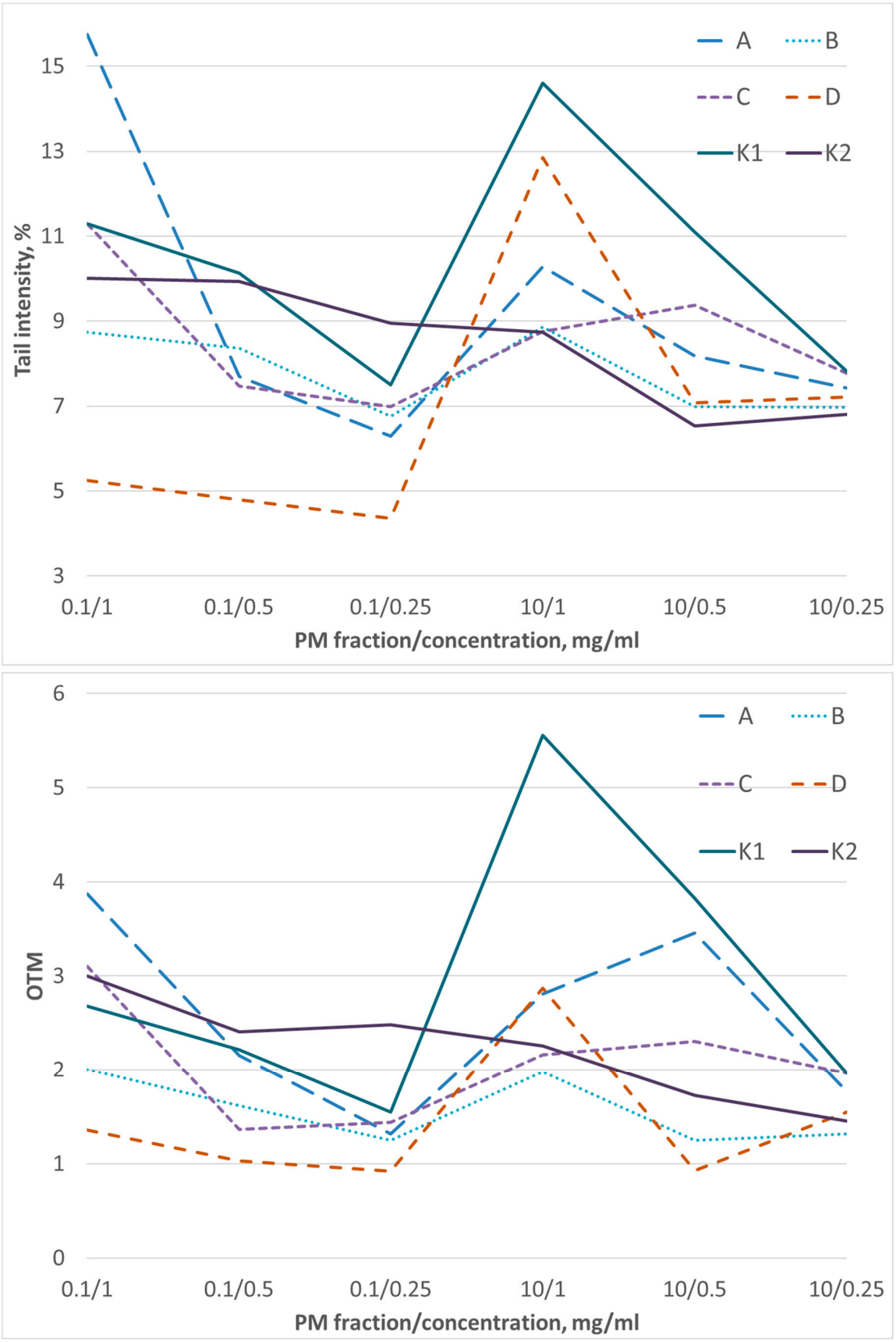


Figure 6. PM induced DNA damage in A549 cells (samples named as PM fraction first 0.1 or 10 for $PM_{0.1}$ and PM_{10} and concentration follows 1, 0.5 or 0.25 mg/mL).

In A549 cells, the positive dose–response effect was observed for A, B, D, K1 and K2 for $PM_{0.1}$ and PM_{10} and C point only for PM_{10}. DNA damage values obtained for $PM_{0.1}$ were significantly higher in comparison to PM_{10} for A and K2 points, significantly lower for D and K1 and equal for B and C points. The highest damage response was observed for A point $PM_{0.1}$ and D, K1 points PM_{10}. All DNA damage values were significantly lower in comparison with the negative and dilution controls both for MRC-5 and A549 cells, whereas the positive controls exposed by nanoparticles showed a highest damage level (Table 3).

Table 3. DNA damage in control samples.

		TI, %	OTM
MRC-5	C−	2.063945	0.268355
	C+	22.01629	19.43845
	Cd	2.757954	0.358818
A549	C−	4.021566	0.815793
	C+	23.67538	12.36745
	Cd	4.269289	0.903633

Note: C−—negative control (growth medium), C+—positive control ($Al(OH)_3$ nanoparticles), Cd—dilution control (Hanks' solution).

As shown previously for the same PM samples in MRC-5 cells, the high damage response in the micronucleus test was obtained for A, D and K2 samples exposed by $PM_{0.1}$, with the highest effect in $PM_{0.1}$ A sample [60]. Additionally, RICC (relative increase in cell count) values in the samples exposed by $PM_{0.1}$ were significantly lower than PM_{10} samples. This reflects a lower proliferation activity in $PM_{0.1}$ exposed MRC-5 cells. D, K2 and, partly, A samples, showed a common tendency of the nanoparticles' increased toxicity compared with the microparticles, for both the comet and micronucleus assays for MRC-5. The high PM_{10} damage for A549 cells in the D point coal-fired power station "Belovskaya" can be interpreted as a result of increased level of coal ash products in this sample.

Increased DNA damage level in the majority of samples can reflect a large surface area, providing oxidative species producing and absorption properties. The assumption of a pro-inflammatory role and overall health risk from nanoparticles is supported by a number of studies [68–70].

A relatively high level of DNA damage obtained in non-mining points (K1, K2) shows that toxicity effect can be produced by a widespread PM from common soils if exposure reaches a proper intensity. Taking into account the fact that an increase in the concentration of PM leads to intensification in the toxicity of dust, we can determine that the territory within the studied boundaries near coal pits and a coal-fired power station is dangerous for the population. The concentration of dust there significantly exceeds the control areas. It is also obvious from our data that the toxicity of dust is determined not only by the fractional composition, but also probably by the elemental composition.

4. Conclusions

Our results recorded a large volume of dust PM_{10} and $PM_{0.1}$ in the snow cover at all observation points outside the sanitary protection zone of the coal mining enterprises and coal-fired power station. At almost all of the research points, the weight and accumulation rate of $PM_{0.1}$ was higher than that of PM_{10}. We found that the total volume and fractional composition of dust has no significant trend to change in research space. According to our results, for almost all observation points (including control areas), the fractional composition of PM_{10} consists of approximately 80% dust ranging in size from 0.1 micron to 2 microns. Thus, dust contamination is associated mainly with PM_2 particles. Apparently, it is this dust fraction that is able to migrate over significant distances (up to 3700 m) in winter around open-pit coal mines and a coal-fired power station. We also note that dust pollution of the snow cover and, accordingly, the air, is increasing due to mining activities

and coal-fired power stations. The volume of dust around such objects is two to three times higher than in the control areas. Genotoxic tests on cell lines A549 and MRC-5 showed high toxicity of dust particles selected from the snow cover. A relatively high level of DNA damage obtained in control points shows that the toxicity effect can be produced by a widespread PM from common soils if exposure reaches a proper intensity. Taking into account the fact that an increase in the concentration of PM leads to intensification in the toxicity of dust, we can determine that the territory within the studied boundaries near coal pits and a coal-fired power station is dangerous for the population. It is also obvious from our data that the toxicity of dust is determined not only by the fractional composition, but also probably by the elemental composition.

Our study demonstrates that long distances are not able to protect against fine and ultrafine dust fractions generated in industrial plants (coal mines and coal-fired power stations). Previous works have shown that particle size is inversely related to the extent of human hazard. In our research, pollution is associated with PM_2 dust particles, which, most likely, will determine the incidence of the resident population at these distances. Due to the high penetrating power of these dust particles into the human body, the population will be significantly affected by this exposure.

Our study is important for understanding the processes of formation, toxicity, transport and sedimentation in the snow cover of dust generated in the process of coal mining and the operation of a coal-fired power station. Coal and coal-fired power stations continue to play a significant role in the production of energy in the world, and their impact on the resident population remains very relevant.

However, our study is limited to the winter season and local climatic conditions. It is also important to study the processes of dust transport and sedimentation in other seasons of the year and in wind directions for a given area. Further research should thus focus on determining the volume, chemical and fractional composition of dust arising from different directions of wind flow from the coal enterprise and its related infrastructure.

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