Conference paper

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Snow samples from settlements of the Murmansk region-genotoxic effects in Drosophila germ cells

https://doi.org/10.1515/pac-2021-0305

Abstract: The *Drosophila melanogaster* organism is highly sensitive Biotester to mutagens or mutagenic carcinogens and can reveal mutations in both somatic and germ cells. The results of assessing the genotoxicity of snow samples from different inhabiled localities of Murmansk region – a region of with increased anthropogenic load – are shown in the article. In addition to the detection of genotoxic effects in different settlements, a highly significant correlation with the content of a number of metal ions in the samples, which makes the obtained results especially relevant.

Keywords: Aerosols; atmospheric precipitation; chemistry and climate; dominant lethal mutations; *Drosophila melanogaster*; genotoxicity; germ cells; melted snow samples; snow cover.

Introduction

Snow cover is one of the most effective indicators of the environmental pollution at the expense of sorption of pollutants during winter, which is especially revelant for the towns of Murmansk region, in which there are different industrial enterprises and snow cover remains for a long time [1].

The counting method applied to dominant lethal mutations (DLM) in germ cells of male Drosophila is highly sensitive enabling to assess genotoxicity and toxic effects of practically all the types of activities in a relatively short period of time [2, 3]. Unfortunately, the method is rarely applied in practice of environmental and environmental and hygienic research, although the results obtained by the method are in demand and enable to assess genotoxic load on the population.

The research objective

The research objective is to study the genotoxic effects of the total snow samples taken from different settlements of Murmansk region by induction of dominant lethal mutations (DLM) in germ cells of *Drosophila melanogaster*.

Article note: Snow cover, atmospheric precipitation, aerosols: chemistry and climate: reports of the III Baikal international scientific conference endorsed by IUPAC (March 23–27, 2020).

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Materials and methods

The total snow samples were taken in four settlements of Murmansk region (Table 1) in April, 2019 (GOST 17.1.5.05-85) not closer than 250 m from motor roads. The samples were melted at room temperature, water was filtered, a quantitative chemical analysis was performed and the samples were placed in plastic containers into household refrigerators. The samples were coded before the experiment; the samples were decoded only after the experiment was performed.

D. melanogaster flies of the D-32 line were used in the experiment. Flies were kept at a room temperature and to a standard diet [4]. All the experiments were carried out in one repetition in accordance with the guidelines [5].

In order to study the effects of each melted snow sample, 55 male flies 3–4 days old were used in the experiment. The exposure lasted for 2 h followed by crossing with 110 virgin female flies of the same age. Distilled water was used as negative control, and 0.1 % water solution of diethyl nitrosamine (DENA), being a standard mutagen, was used as positive control. In addition, 5 % sucrose was added to water samples and solutions to make them more attractive for the exposed male flies.

Female flies were separated from male flies after crossing and placed into plastic houses. The bottom of each house was a replaceable plastic cuvette for egg laying, with "hungry" feed in it.

In total, the experiment made allowance for six egg layings for each snow sample. The cuvettes were replaced every 12 h; the number of eggs laid was counted and placed into wet containers for 48 h.

Then the undeveloped eggs found in cuvettes were counted. The eggs that did not change their appearance were recorded as early embryon lethalities (EEL). If the eggs were of all the shades of yellow or brown colour, they were recorded as late embryon lethalities (LEL).

The number of early embryon lethalities is indicative of embryon lethality at early stages of development mainly due to non-genetic causes.

Late embryon lethalities show the true number of mutations that occur at late stages of embryon development and result in its lethality.

The exposure effect on the male fly fertility was assessed (%) by correlation of the laid eggs number in the experimental and control groups.

The statistical analysis was made using criterion X^2, and the correlation analysis was made by the Spearmen criterion. The differences at the level of $p \leq 0.05$ were considered to be significant.

Table 1: The number of dominant lethal mutations detected in male *D. melanogaster* exposed to snow samples taken from different sites of the town.

Code	Sampling site	Male fly fertility		Toxic effects			Genotoxic effects		
		Eggs laid	% of control	The number of EEL	EEL, %	X^2	The number of LEL	LEL, %	X^2
1	Apatity, Academgorodok (KSC campus)	1384	118	47	0.77	**12.93**	14	0.23	**3.21**
2	Apatity, Voinov Internatsionalistov St.	1852	158	77	0.59	**7.23**	54	0.41	**3.36**
3	Apatity, Zhemchuzhnaya St.	1558	133	46	0.52	**18.32**	43	0.48	**2.29**
4	Settl. Umba, north	1280	109	75	0.77	**2.37**	31	0.23	1.30
5	Settl. Umba, south	469	40	75	0.5	**12.37**	10	0.5	0.11
6	Settl.Lovozero, north	1081	92	50	0.77	**3.48**	15	0.23	0.68
7	**Settl. Umba, south**	863	74	36	0.8	**4.98**	9	0.2	**2.10**
8	**v. Revda, north**	932	80	208	0.97	**110.93**	7	0.03	**2.59**
9	v. Revda, south	1754	150	38	0.58	**34.16**	27	0.42	0.47
10	Control distil water)	1171		75	0.78		21	0.22	
11	DENA (0.2 %)	1006		114	0.81	**16.57**	26	0.19	**2.53**

The significant differences from the control are in bold.

Results

The study results are shown in Table 1. A significant decrease in male fly fertility is observed in the sample from the locality in the southern area of the settlement of Umba ($p = 0.004$). The male fly exposure in all the samples didn't result in a significant increase in their fertility.

The number of early embryon lethality significantly exceeded the control level in all the snow samples taken in the area of the town of Apatity (which may be due to de-icing mixtures and because of the tailing ponds of mining and processing companies), as well as in the northern area of the settlement of Umba, and in all the sampling sites within the village of Revda.

It may be due to the activity of Lovozersky mining and processing company – located not far from sampling sites – that deals with rare metal ores mining and processing. The number of early embryon lethalities with relation to the control one, which was observed in all the other sites of snow sampling, is indicative of sperm cell lethality occurring before fertilization and, hence, of the evident toxic effects of the pollutants contained in the snow samples.

In samples taken at different locations in Apatity, all sampling points of the settlement Revda and the north of the village of Umba the number of late embrion lethalities (LEL) exceeded the level of the control that indicates the presence of the mutagenic effect of the components contained in the snow samples.

The chemical analysis of the snow samples has revealed the presence of the ions, such as SO_4^{-2}, Cl^-, NO_3^-, NH_4^+, Si^{+4}, K^+, Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, Sr^{2+}, Cd^{2+}, Pb^{2+}, V^{3+}.

The increased content of ions Sr^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} in snow samples taken in Apatity compared to that in other settlements may be due to the presence of tailings particles transferred by wind from the tailing ponds of the Apatit mining and processing company.

In addition, high content of NH_4^+, NO_3^-, and $N_{общ.}$ was detected in all the snow samples taken in Apatity.

The higher, if compared to other localities, content of V^{3+}, Ni^{2+} and Fe^{3+} is found in snow samples from the southern area of Lovozero, which may be due to the activity of Lovozersky mining and processing company located near the settlement.

The correlation analysis has revealed the significant correlation between some pollutants detected in snow samples and the dominant lethal mutations (DLM) indicators (Table 2).

As one can see the correlation analysis has revealed the dependence of male flies fertility on the content of ions NH_4^+, $N_{общ}$, Si^{+4}, Fe^{+3}, Cu^{+2}, $Ni^{+}2$, V^{+3} detected in snow samples.

It may be due to a great content of iron in water, as well as to the discharge of municipal and industrial water not purified or purified insufficiently.

The control of chemical soil pollution by a number of metal ions is carried out in Murmansk region because there are a number of mining and processing operations within the region.

According to the death rates and the share of neglected cases of malignant neoplasm (MNP), the most unfavourable situation is observed in the towns of Murmansk, Apatity, Kirovsk, Monchegorsk, the Kandalaksha and Kola areas. According to the data of the Ministry of Health of the Russian Federation in Murmansk region, the number of neglected cases of malignant neoplasm (MNP) is permanently increasing. For instance, in 2018, 3471 people died, that equals to 0.46 % of the population.

Based on the experimental data, it can be assumed that toxic and genotoxic effects produced by snow samples enable to reveal not only unfavorable state of the environment in specific areas of the region but also

Table 2: Some correlations between the indicators in DLM testing and the results of the chemical analysis of snow samples.

	pH	NH_4^+, mkg N/l	N_{total}, mkg N/l	Si^{+4}, mg/l	Fe^{+3}, mkg/l	Cu^{+2}, mkg/l	Ni^{+2}, mkg/l	V^{+3}, mkg/l
Fertility	0.650	**0.867**	**0.717**	0.202	**−0.717**	**0.783**	−0.583	**−0.717**
% EEL	**−0.667**	**−0.783**	**−0.733**	−0.123	0.350	−0.583	0.367	0.583
% LEL	0.383	0.250	0.300	**0.782**	−0.433	0.250	**−0.767**	−0.567

Correlations significant at the level of $p \leq 0.05$ are in bold type.

predict the diseases, one of the sources of which is a genotoxic effect of the environmental components. So the research of snow melted water in other towns of Murmansk region needs to be carried out to see an overall pattern of pollution in the region.

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