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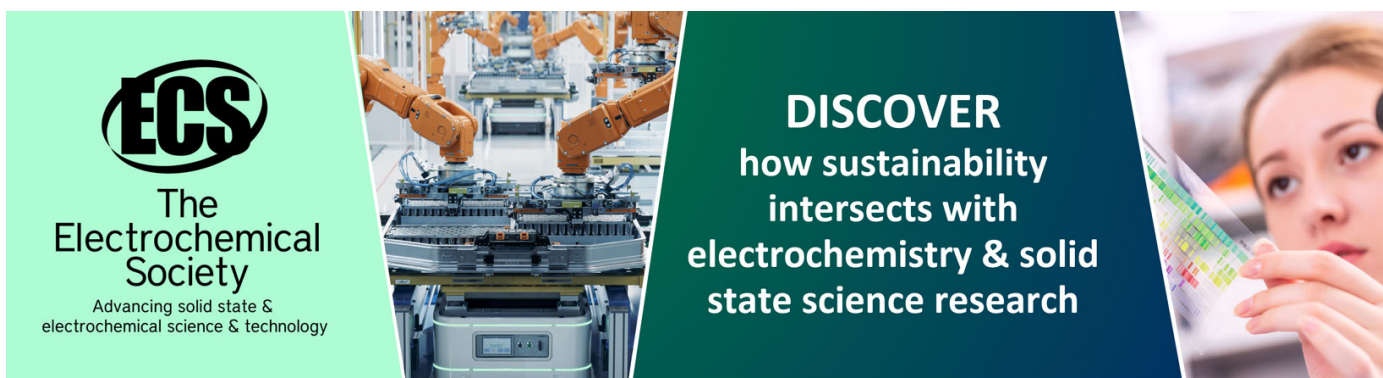
Bioindication of toxicity and mutagenicity of the environment

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Bioindication of toxicity and mutagenicity of the environment

N V Vasilevskaya and A I Sikalyuk

Department of Natural Sciences, Murmansk Arctic State University, 183 038, Captain Egorov Str. 15, Murmansk, Russia

E-mail: n.v.vasilevskaya@gmail.com

Abstract. The article presents the results of studying the impact of industrial emissions of enterprises of the city of Murmansk on the fertility of pollen of *Sorbus gorodkovii* Pojark (Gorodkov's rowan) – an endemic species of East Fennoscandia. A low level of pollen fertility (35 – 52%) was revealed, especially in the vicinity of the local heating plants, operating on fuel oil. In the samples from all sites, a high proportion of sterile pollen grains was noted (48 – 65%). A significant increase of the proportion of sterile pollen of rowan in the vicinity of industrial enterprises of the city of Murmansk indicates the mutagenic effect of their emissions on the male generative system of *S. gorodkovii* and the process of gamete formation.

1. Introduction

Since the beginning of the XXI century, bioindication has become one of the actively developing directions of environmental research. Ecologists are interested not only in information about the level of environmental pollution but also how living organisms react to the influence of xenobiotics [1]. Many of these substances have a mutagenic effect on the biota. In particular, they cause disturbances in microgametogenesis of higher plants, that being expressed in a decrease of pollen fertility, which is described as the ability to pass genes to the offspring [2]. In recent years, genes that regulate male fertility in plants have been actively studied [3]. The pollen quality is influenced by a complex of exogenous and endogenous factors: reproduction method, intraspecific hybridization, cytoplasmic sterility, nuclear male sterility, anthropogenic environmental factors [4]. Environmental stresses (heat, drought, cold, humidity, pollutants) affect the quality of pollen and its viability [5], influencing at all stages of its development [6]. Stressful environmental conditions can cause asynchronous processes during the development of the male gametophyte, affecting the amount of fertile pollen [7]. The suitability of pollen for detecting the phytotoxic and mutagenic effects of pollutants is due to its sensitivity to the exposure of pollutants. It is just in the haploid state that lethal mutations occur during the formation of pollen grains [8].

The studies were carried out in the city of Murmansk, the largest non-freezing port of Russia, situated beyond the Arctic Circle. The city is located in the Atlantic - Arctic zone of temperate climate, in the subzone of the subarctic tundra and forest tundra. The main sources of pollution are: the Murmansk Commercial Sea Port, the plant for thermal treatment of municipal solid waste, the Murmansk heating plants. Over the last decade, the environmental situation has dramatically deteriorated due to an increase of the volume of export oriented open-pile coal handling and utilization of low-quality fuel oil by the Murmansk heating plants. The soils of the city of Murmansk are severely contaminated by heavy metals (Cu, Zn, Ni, V) [9].



For studying the mutagenic effect of industrial emissions on pollen fertility, as a test object was used Gorodkov's rowan (*Sorbus gorodkovii* Pojark), that is a European hypoarctomontane species, one of the few representatives of the genus *Sorbus* L., which grows in the polar latitudes. An endemic of Eastern Fennoscandia, widely spread in green plantations of urbanized territories of the Murmansk region. Palynological studies of *S. gorodkovii* were previously carried out in the towns of Severomorsk and Zapolyarny [10], [11].

The purpose of the research is to study the fertility of pollen of *Sorbus gorodkovii* in the zone of influence of environmentally hazardous facilities of Murmansk and to estimate the toxicity and mutagenicity of the environment

2. Materials and methods

At the end of May 2020, in the city of Murmansk, in the zone of impact of environmentally hazardous facilities, five experimental sites were laid: P1 – Molodezhnaya Str., 170 m from the “Roslyakovo Yuzhnoye” boiler house; P2 – Domostroitel'naya Str., 482 m from the plant for thermal treatment of municipal solid waste; P3 – Portovy proezd Str., 950 m from the industrial site of the Murmansk Commercial Sea Port; P4 – Tralovaya Str., 353 m from the Central Heating Plant; P5 – Baumana Str., 930 m from the South Heating Plant. The control site is located at a 70 km distance from the city of Murmansk, in the Verkhnetulomsky settlement, where there are no environmentally hazardous facilities that have a negative impact on the environment.

At the end of June 2020, during the period of massive bloom of rowan, ten inflorescences were collected from each of the 10 marked trees on the experimental sites, from which five flowers were randomly selected ($N = 50$). The samples were fixed in 40° ethyl alcohol. To determine the presence of cytoplasm in intact pollen, the Pausheva's acetocarmine method [12] was used. Fertile pollen grains are stained, sterile – not (figure 1). Cytological analysis was performed on temporary preparations using light microscopy (magnification 400 times). In each field of view of the microscope, number of fertile and sterile pollen was counted. At least 500 pollen grains were looked through in samples from each experimental site. Sterility index (SI) was calculated, it shows how many times the frequency of the induced level of sterility caused by environmental contamination is higher than the level of spontaneous sterility (the control) [13].

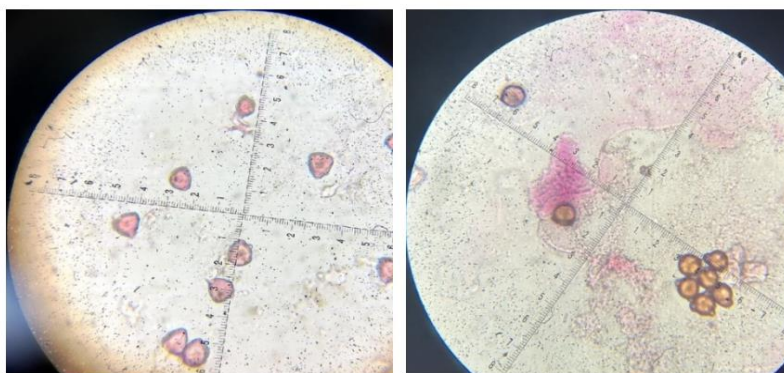


Figure 1. Fertile and sterile pollen grains of *Sorbus gorodkovii* (magnification 400 times).

3. Results and discussion

The studies have shown that the ratio of fertile pollen of *S. gorodkovii* in the control sample (settlement Verkhnetulomsky) is 72%. In the vicinity of the environmentally hazardous industrial facilities in the city of Murmansk, the content of such pollen is much lower and varies from 35 to 52%. The minimum content of fertile pollen grains (35%) was found in the samples from the vicinity of the South Heating Plant, 41% – Central Heating Plant, 45% – the “Roslyakovo Yuzhnoye” boiler house (table 1; figure 2).

The fertility of pollen of *S. gorodkovii* from the experimental sites located in the zone of influence of the incineration plant (P2) and the Murmansk Commercial Sea Port (P3) was reduced by 20% compared to the control (figure 2).

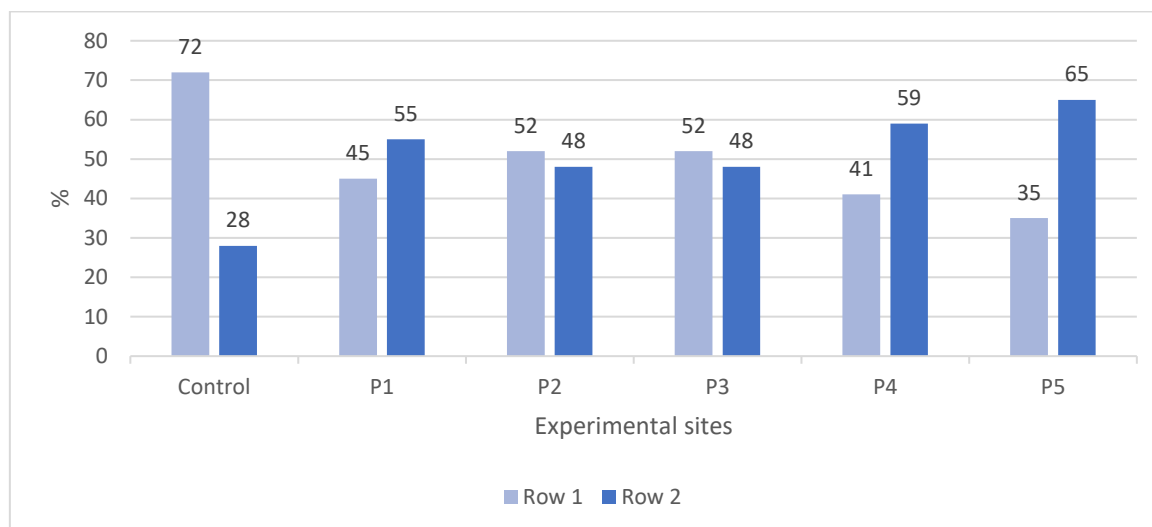


Figure: 2. The ratio of fertile and sterile pollen of *Sorbus gorodkovii* in the vicinity of industrial facilities of the city of Murmansk (in %).

Legend: P1 – the vicinity of the “Roslyakovo Yuzhnoye” boiler house; P2 – surroundings of the incineration plant; P3 – surroundings of the Murmansk Commercial Sea Port; P4 – the vicinity of the Central Heating Plant; P5 – the vicinity of the South Heating Plant. Row 1 - fertile pollen, Row 2 – sterile pollen.

The reproductive system of the rowan reacts to technogenic pollution by an increase in the number of sterile pollen grains. Colorless pollen grains are indicators of sterile (abortive) pollen. In the control sample (Verkhnetulomsky settlement), the proportion of sterile pollen of *S. gorodkovii* is 28%. This hypoarctic species is characteristic of a fairly high spontaneous sterility of the male gametophyte under natural conditions, that having been confirmed by previous studies in Murmansk region [10], [11]. In Murmansk, the highest contents of sterile pollen were found in the vicinity of the South Heating Plant (65%) and the Central Heating Plant operating on fuel oil (59%), as well as the “Roslyakovo Yuzhnoye” coal-fired boiler house (55%) (figure 1; table 1). This sterility of pollen grains is induced by technogenic pollution of the environment. The data obtained are consistent with studies carried out in the city of Severomorsk, where the proportion of such pollen of *S. gorodkovii* increased in the vicinity of the City Boiler House, operating on fuel oil and amounted to 38 – 44% [10].

Sterility index (SI) in the tested samples varies from 1.8 to 2.4 (table 1). It shows how many times the frequency of the induced sterility caused by environmental contamination is higher than the level of spontaneous sterility in the control. The highest values of the index were found in the samples from the vicinity of the Central and South Heating Plants of the city of Murmansk, and the “Roslyakovo Yuzhnoye” Boiler House. The induced sterility of *S. gorodkovii* pollen on these experimental sites is two or more times higher than the spontaneous sterility (in the control).

Sterility of pollen grains can be observed for several reasons: cytoplasmic male sterility, genetic mutations, chemical castration (due to the effect of various chemicals on the plant's reproductive system) [12]. The greatest palynotoxic effect on the male gametophyte of *S. gorodkovii* is exerted by impact of emissions of heating plants of the city of Murmansk operating on fuel oil and coal. This may be due to the presence in the emissions of these enterprises of pollutants with a high degree of mutagenicity and belonging to the hazard classes I and II (benz (a) pyrene, manganese and its compounds (in terms of manganese (IV) oxide), dihydrosulfide (hydrogen sulfide) and others.

Table 1. Content of fertile and sterile pollen of *Sorbus gorodkovii* in the samples from industrial pollution sites, city of Murmansk.

Experimental sites	Control	P1	P2	P3	P4	P5
Total number of pollen	505	500	521	520	509	516
Number of fertile pollen	364	225	270	268	210	182
Number of sterile pollen	141	275	251	252	299	334
Sterility index	-	2.0	1.8	1.8	2.1	2.4

Sterile microgametophyte is characterized by deformation and degeneration of nuclei, cytoplasm, or whole cells. The formation of such pollen in plants is associated with disturbances in meiosis and microsporogenesis [14]. The behavior of chromosomes during meiosis must be under strict genetic regulation in order to facilitate correct separation of chromosomes into daughter cells, while abnormal meiosis inevitably causes abortion of pollen [15]. The highest rates of aborted pollen are associated with the highest rates of meiotic disturbances [16].

4. Conclusion

Pollen sterility caused by abiotic, in particular anthropogenic, stresses is a major environmental problem affecting the reproductive development of many plant species. The high level of pollen sterility in the vicinity of Murmansk enterprises indicates the mutagenic effect of their emissions on the male generative organs of *S. gorodkovii* and the process of gamete formation. The results obtained in this study allow to make a conclusion about the possibility of using Gorodkov's rowan as a test object for bioindication of the mutagenicity of the environment of urbanized territories of the Euro-Arctic region.

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