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The Quality of Scots Pine Pollen (*Pinus sylvestris* L.*)* in the Emission Area of JSC «Karabashmed»

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Abstract. The results of the study of the state of mature pollen of Scots pine stands in the zone of action of smoke emissions from plant JSC «Karabashmed» in connection with the level of technogenic impact are presented. It was shown that disturbances in the development of male gametophyte in the zones of technogenic pollution are already realized at the early stages of microspore development and are revealed by the high frequency of small underdeveloped pollen. At the same time, the frequency of small and degenerated pollen was many times higher in the pool of mature pine pollen in the stand under conditions of a low level of pollution than under background conditions. A high frequency of pollen grains with anomalies of air sacs was detected in the stand under background conditions. The results of the study indicate a high sensitivity of the male generative system to technogenic pollution (accumulated in the soil, aerosol, gas) and the possibility of its bioindication in the absence of symptoms of damage to the assimilation apparatus. It was found that most of the pine trees growing on the soil of fine-stone-sandy composition correspond to the categories of weakened and dying ones, which makes it possible to recognize the conditions of the man-made land as extremely unfavorable for the growth and development of pine. The mature pine pollen of these stands is characterized by the peculiar features that distinguish it from the pollen of other stands. The conclusion is made about the negative effect of smoke emissions from the JSC «Karabashmed» plant on the condition of pine plantations and the male generative system of pine, even at a considerable distance from the source of emissions.

INTRODUCTION

Scots pine (*Pinus sylvestris* L.) (hereinafter referred to as pine), due to its wide ecological amplitude, is capable of growing in a variety of environmental conditions. However, the reproductive capabilities of pine in extreme conditions for the species are limited, which is due to the high vulnerability of the generative system to the action of unfavorable factors. Many studies have shown the negative impact of natural (climatic and meteorological) factors on pine reproduction [1, 2]. Technogenic pollution can also affect the qualitative and quantitative parameters of pine seeds and pollen [3–6]. The combined effect of atmospheric technogenic pollution and unfavorable soil nutrition on the development of the pine generative system has been little studied.

The ecosystems of the environs of the city of Karabash have repeatedly been the object of scientific research [7–9]. Scientific interest in these areas is due to the need to study the biological consequences of more than a century of exposure to toxic smoke emissions on biota and human health and factors that led to the destruction of natural objects, the loss of their quantitative and qualitative parameters, as well as the need to find ways to resolve the most severe environmental crisis in ongoing technogenic pollution.

The main source of technogenic pollution of the environs of Karabash is the copper production enterprises. Copper smelting was founded on the territory of the present-day Karabash city in 1837. The current enterprise - JSC «Karabashmed» under the management of the Russian Copper Company – was launched in 1910. The technologies and equipment used at the plant, advanced at the time of the enterprise opening, have not been changed fundamentally

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and have not met the requirements of environmental safety and the conditions of industrial sanitation for many decades of work.

For 1 ton of blister copper smelted at the enterprise before 1974, there were more than 7 tons of atmospheric emissions [10]. The plant operated without tailing dumps until 1957, dumping all waste into the floodplain of the river Sak-Elga, which led to significant pollution of water bodies in the study area [11].

The volume of smoke emissions from the plant amounted to 12 million tons of mainly sulfur-containing substances by 1996; annually (until 1989) 180 thousand tons were emitted into the environment [10].

At the beginning of the XXI century the enterprise held gradual production modernization, due to which emission volume and the degree of technogenic impact of the enterprise on the environment were reduced. However, more than a century of the enterprise's impact on the natural environment has led to the degradation of natural ecosystems and the destruction of vegetation and soil cover in the areas closest to the enterprise.

Natural restoration of vegetation cover in such areas is difficult, since the soil cover is oversaturated with air pollutants or degraded. The reforestation processes are also hampered by the lack of seed material sources due to the shift, for many kilometers, of the boundaries of the natural ecosystem.

Measures to rehabilitate and restore forest ecosystems have been conducted in the environs of Karabash since the second half of the twentieth century. At present, many cultures have entered the reproductive stage of ontogeny. Are they capable of becoming a source of pollen and seed and, therefore, the basis for further reforestation, including on polluted and disturbed soils? The answer to this question can be provided by studying all aspects of arborous forest-forming plant species reproduction.

Objective of our work was to study the state of the male generative system of Scots pine stands growing on zonal soils and fill soil under conditions of different levels of atmospheric technogenic pollution by JSC «Karabashmed». The stage of pollen formation is a critical link in pine reproduction, since pine is renewed only by seed, and pollination and fertilization of pine ovules are essential conditions for seed formation.

MATERIAL AND METHODS

The Karabash copper smelting plant is located in the Simonovskaya valley – a natural low area surrounded by a chain of mountains and hills, the relative height of which does not exceed 200–250 m. The Karabash region is referred to as the forest zone of the Ural mountain-forest forest-growing region [12]. The climate is continental. The average long-term temperature in January is -16.3 ºC, in July – +15.7 ºC. Snow falls in October-November and lasts until April [9]. Westerly, south- and northwestern wind directions prevail. The soils are diverse: brown mountain-forest, brown forest, podzolized gleyic, gray mountain-forest, mountain-podzol types. The area is characterized by high rockiness and low-profile thickness.

The objects of the study were the Scots pine cultures of the 3rd age class growing in the environs at various distances from Karabash. Sample areas (SA) were established in the pine planting. SA 11-2, SA 21 and SA 20 were established in pine stands growing on zonal soils at a distance of 8, 13 and 19 km from the main source of technogenic pollution in the eastern, southeastern and southern directions from the enterprise. SA 28p and SA 11-1p were composed of pine cultures on fill soil of fine-stony-sandy composition at a distance of 4 and 8 km in the eastern and southeastern directions.

The level of technogenic pollution of the study area was determined by the accumulation of pollutants in the snow cover [13]. When determining the level of technogenic load on planting, the state of forest stands was taken into account according to a set of indicators: defoliation of crowns, the lifespan of needles, its length, the presence of chlorosis and necrosis, the presence and degree of decomposition of forest litter [14].

Pine pollen was collected before dusting separately from each model tree of all studied stands. Pollen fertility and viability were determined using traditional methods [15, 16]. Pollen microscopy was performed on an AxioScope.A1 microscope (Zeiss) with an AxioCam MR5 camera using the AxioVision Rel 4.8 image processing software. Methods of descriptive and multivariate statistics were used for statistical data processing.

RESULTS AND DISCUSSION

The condition of 82 and 96% of trees on SA 21 and SA 20 was assessed as background (the trees had no signs of significant damage). In SA 11-2, at a distance of 8 km from the source, 59% of trees did not show signs of crown damage, the share of weakened trees was 22%, severely weakened and drying out – 15 and 4%, respectively. In SA 11-1p, only 20% of trees are in the background category. No trees of the background category were found on SA 28p.

More than 65% of living pine trees in SA 28p were defined as the 4th class of life state (drying out). The soil cover and herbaceous vegetation in both stands on the fill soil are practically absent.

SA 11-1p is located ~ 100 m away from SA 11-2. Both areas are characterized by equal levels of atmospheric technogenic pollution, but different conditions of root nutrition. Significant differences revealed in the state of the plantings under study and the pine assimilation apparatus indicate an extremely unfavorable effect of the fill soil conditions on the growth and development of pine trees in SA 11-1p and SA 28p.

The level of technogenic pollution was determined by the content of pollutants in the snow cover at the snow sampling points (SSP) and at the site. In accordance with the set of atmospheric technogenic pollution indicators and the state of tree vegetation, the following zones were identified: a very high level of technogenic load – SSP 18 and 17, located at a distance of 1.2 and 2.7 km from the enterprise in the eastern and southwestern directions, respectively, where there is no arborous vegetation; strong – SA 28, located at a distance of 4 km; middle – SA 11, 8 km; weak – SA 21, 13 km; background – SA 20, 19 km from the source.

At a short distance from the production plant, the main air pollutant was determined to be a fraction of water-insoluble substances (suspended substances), while at a distance of 4, 8, and 13 km – a fine-dispersed fraction of water-soluble substances (dry residue). The finely dispersed fraction of smoke emissions can be transported over considerable distances in the atmosphere; Substances then settle and accumulate in all components of the environment in the form of mobile water-soluble forms with high biological activity [17].

With regards to the quality of pine pollen in the studied stands, the values of the fertility index of all forest stands pine pollen varied in the range of 87.0–93.7% (Figure); there are no significant differences between SA for this indicator.

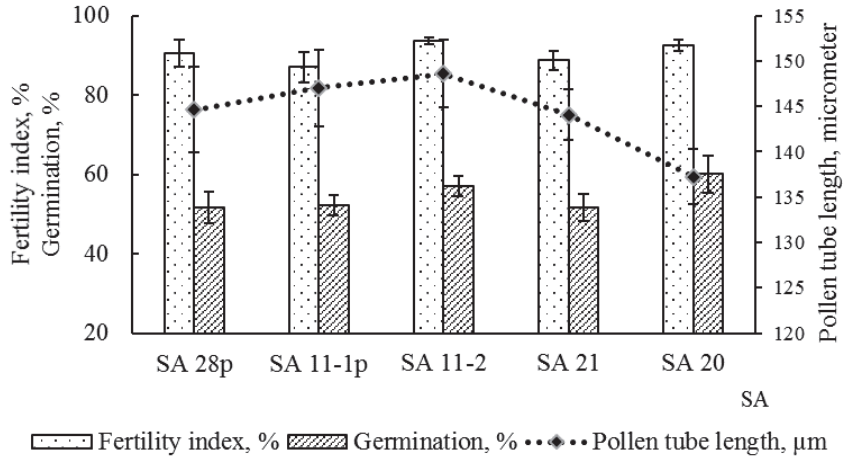

FIGURE. Indicators of fertility and viability of pine pollen

Pine pollen from different stands, although having close values of the fertility index, is characterized by the various spectra of anomalies. Thus, in the background tree stand, the main contribution to the sterility of pollen is made by pollen grains of a size typical for mature pollen with cytological disturbances (pycnosis, plasmolysis, etc.); they account for 73% of pollen grains with anomalies. Pollen with cytological abnormalities is rarely detected at the first stage of gametogenesis before the start of protallial divisions [16]. Thus, microspores of the pine of the background stand have a high viability and successfully pass the first stages of microgametogenesis. Signs of degeneration of the cytoplasm and nuclei of such pollen grains appear later, at protallial division stages. For the background stand, we note a relatively higher frequency of pollen with air sacs anomalies, which exceeds the same indicator for other stands by 1.4–2.6 times.

In tree stands in zones of technogenic pollution, the main contribution to sterility (55–68%) is made by small underdeveloped pollen. The frequency of occurrence of small underdeveloped pollen in the zones of technogenic pollution is 1.2–6.5 times higher than in the background forest stand. The frequency of small pollen in the pool of mature pollen reflects the frequency of early realization of disorders in the male generative system.

The content of reserve substances is another important physiological characteristic of mature pine pollen grain. No differences were found between the studied stands in terms of lipid content in mature pine pollen. The starch content

in the pollen of the background stand is relatively lower than under conditions of technogenic pollution ($p < 0.05$). This pattern reflects both the average level of starch content in mature pine pollen and the proportion of pollen grains with starch and especially high (3–4 points) level of starch accumulation.

To determine the functional usefulness, pollen was germinated on a nutrient medium. On the third day of the experiment, the pollen from the background stand pine had higher values of germination but formed pollen tubes which are marginally shorter than that in pollen from the technogenic pollution zones. However, the pollen tubes of pine pollen from the contaminated areas were characterized by a relatively high frequency of swelling and branching – signs that characterize the quality of the pollen tubes of pollen and pollen themselves. The frequency of occurrence of pollen tubes with developmental anomalies in the zones of technogenic pollution is 9–38% higher than in the background stand.

Thus, the pollen of the background stand, in comparison with the pollen from the technogenic pollution zones, has an average population value of the fertility index, a higher value of the germination index with a significantly lower level of starch content in pollen. The pollen of the background stand is much less likely to form pollen tubes with developmental disorders than pollen from the zones of technogenic pollution. The relatively high occurrence of pollen with air sacs anomalies in the stand under background conditions indicates the unfavorable growing conditions of ecological stands, which do not have a significant effect on the pine assimilation apparatus but are manifested in developmental disorders of the male gametophyte.

The following are possible reasons. First, the smoke emissions from the smelting of copper include the gaseous fraction (mainly SO_2), which accounts for 65–90% of the volume of emissions into the atmosphere [11]. The gas fraction is transported over long distances; its chronic effects were revealed by chlorosis of leaves and needles in all SA.

Secondly, the environs of Karabash are classified as territories with huge accumulated environmental damage. The concentration of mercury in the soil exceeds the maximum permissible concentration by 2 times, arsenic – 279 times, copper – 368 times, lead – 300 times [18], cadmium – 5.2 times, zinc – 3.8 times [19].

The colossal level of technogenic soil pollution of the research area, formed over many decades of the enterprise's operation and the atmosphere gas pollution cause the accumulation of pollutants in plant tissues and damage to the vegetative and generative systems of plant stands, including those remote from the emissions source.

The discriminant analysis made it possible to assess the level of differences between pine stands by a set of indicators in mature pollen. Tree stands in SA 11-2, SA 21 and SA 20, growing on zonal soils, along the gradient of aerosol atmospheric technogenic pollution southeast and south of the source of smoke emissions, were compared. Reliably significant differences were revealed between them in terms of pollen indicators set ($p < 0.05$).

The results of the forest stands comparison in SA 11-1p and SA 28p, growing on fill soil under similar conditions of root nutrition but differ in the level of atmospheric technogenic pollution, as well as tree stands SA 11-1p and SA 11-2, growing at equal atmospheric pollution conditions but in different soil conditions, are of particular interest. The results of the discriminant analysis indicate a significant similarity between the forest stands of SA 28p and SA 11-1p ($p = 0.626$), as well as significant differences between the stands of SA 11-1p and SA 11-2 ($p < 0.05$) in terms of male generative system.

CONCLUSIONS

A vast zone of technogenic pollution has been formed in the environs of Karabash, due to the long-term effect of smoke emissions from JSC «Karabashmed». The state of the vegetation cover as a whole is in accordance with the identified zones of technogenic pollution, on the basis of the snow water composition analysis. There is no vegetation cover in the under-plume zone of the enterprise. There are no pine stands at a distance of less than 3.5 km from the source of smoke emissions. A zone of chronic technogenic pollution is formed at a distance of 8 km in the southeast direction, which is characterized by the presence of arborous vegetation, including pine stands, with symptoms of chronic and acute damage to the assimilation apparatus and disturbance of the generative sphere.

We note a significant similarity in the life state of forest stands at a distance of 13 km (low level of pollution) and 19 km (background level) from the source, while differences in the content snow cover pollutants varies from 1.3 to 4.5 times (depending on fraction). The frequency of occurrence of small and degenerated pollen is 4.8 and 1.5 times higher respectively, than under background conditions, in the pool of mature pollen of the stand at a distance of 13 km.

The snow cover pollution degree by air pollutants is 15 times lower at a distance of 19 km from the enterprise than in the territories closest to the emission source. However, the male generative system state of pine under conditions

of the background level of atmospheric pollution with aerosols indicates an ecologically unfavorable condition of the environment, possibly the effect of the gas fraction of smoke emissions and soil pollution.

Mature pine pollen is characterized by equal values of the fertility index in both zones of technogenic pollution and background conditions, relatively low values of the germination index, and significantly higher values of starch accumulation and the frequency of occurrence of pollen tubes with developmental disorders was observed for zones of technogenic pollution in comparison with background conditions. Disturbances in the development of the male gametophyte are already realized in the early stages of microspore development and are revealed by the high occurrence frequency of small underdeveloped pollen. The results of the study indicate a high male generative system sensitivity to technogenic pollution (accumulated soil, aerosol, gas) and the possibility of its bioindication in the absence of symptoms of assimilation apparatus damage.

Most of the trees in pine stands, growing on a soil of fine-stone-sandy composition, correspond to the categories of weakened and drying out, which makes it possible to recognize the conditions of fill soil as extremely unfavorable for the growth and development of pine stands. The mature pine pollen of these stands is characterized by distinctive features distinguishing it from the mature pollen of other stands.

In general, it should be recognized that long-term technogenic pollution of all environmental components with smoke emissions from the JSC «Karabashmed» has a negative impact on the state of pine plantings and reveals itself in toxic and mutagenic effects on the male generative system even at a considerable distance from the emission source.

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