Palynoindication of the environment in the impact zone of the Apatit mining processing plant with the use of pollen of *Pinus sylvestris* L.

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Abstract. Results of analyzing of pollen of *Pinus sylvestris* in the vicinity of the tailing dump of the Apatit mining processing plant are presented. More than 20 teratomorphs of pollen with various anomalies of development are revealed in the samples, their frequency is very high (80–83%). According to the palynological scale of ecological zoning, the study area can be classified as critically polluted.

1. Introduction
The main goal of ecologically sustainable development is the stability of ecosystems, including under conditions of increasing chemical pollution caused by anthropogenic human activities. The Murmansk region is the most urbanized Arctic territory of the Russian Federation with a developed mining and processing industry. Most extensively mined are copper-nickel, apatite-nepheline, apatite-magnetite, iron and rare metal ores [1]. The Apatit project is one of the largest producers of phosphate raw materials for the production of mineral fertilizers (the world 3rd largest after China and Morocco) based on the Khibiny deposits of apatite-nepheline ores [2]. The mining and processing complex is located in the central part of the Kola Peninsula, in the vicinity of the cities of Kirovsk and Apatity.

The Apatit project includes: 4 mines, concentration plants (ANOF-2 and ANOF-3), a tailing dump and a number of workshops. The ANOF-2 tailing dump is the largest in Russia in terms of area and volume, as well as the level of environmental pollution on the Kola Peninsula. It contains waste products of apatite-nepheline ore mining and processing, which consist of a fine fraction of nepheline sands. Under strong winds, dry sands turn into an aerosol state and form dust clouds that are transported over long distances [3]. The main pollutants entering the environment are strontium, zinc, phosphorus, calcium, sodium, potassium, aluminum, nitrogen and iron containing compounds. Apatite dust also contains a certain amount of natural radionuclides [2]. Due to the accumulated rock refuse in the tailing dumps in the course of the entire period of development and operation of the Khibiny deposits (more than 800 million tons) and the peculiarities of the Arctic nature, the problem of accumulated environmental damage is one of the main environmental problems of the Arctic region, and the Khibiny ore region in particular [4].

Palynoindication is one of the promising and new methods for assessing environmental quality [5]. Plants, under the impact of industrial emissions, produce a large amount of teratomorphic pollen, and the higher the level of environmental stress is, the more developmental anomalies are encountered. Processes of microsporogenesis are studied in various species of conifers exposed to industrial
pollution [6-7], while such studies for the Arctic zone are rather scarce [8-10]. Scots pine is often used as a test object because of its high sensitivity to environmental pollution [8]. The aim of the research is to study the teratomorphism of the pollen of Pinus sylvestris L. in the zone of industrial impact of the Apatit Plant and the palynoindication of environmental quality.

2. Materials and methods
Scotch pine has survived in the natural green spaces of the cities of the Murmansk region. Normally developed pollen grains of P. sylvestris are bisaccate, heteropolar, bilaterally symmetric, distally monoleptomous. The sacci are oblong-elliptical, sharply separated from the pollen grain and shifted to the distal side [5].

The studies were carried out in the vicinity of the apatite-nepheline tailing dump located 7 km from the ANOF-2 industrial site on the shore of the Belaya Bay of the Lake Imandra [11]. In May 2020, in the areas where fragments of taiga were preserved, test plots were laid: P1 - 2 km from the center of the tailing dump of the ANOF-2 concentrating mill in the southeast direction: P2 - Kozlova Str., on the outskirts of the city of Apatity, near the concentrating mill, 9.5 km from the center of the tailing dump. The control site is located in the Verkhnetulomsky settlement, 122.8 km to the north-west. At the end of June, 7 microstrobils with matured pollen were collected from each of five P. sylvestris trees at all sites (N = 35). Male cones were dried and fixed in 40% alcohol solution. The collected material was stored in a refrigerator at 0–4° C. For pollen analysis, microsporophylls from the middle part of microstrobil were used; pollen was stained with Lugol's solution [6]. The studies were carried out using light microscopy with a magnification of 200 times. From each sample, 10 micro preparations of stained pollen were studied. Pollen analysis was carried out on the basis of micrographs taken through the eyepiece of a microscope with a 13 megapixel Xiaomi Redmi Note 5A Prime camera. On the basis of micrographs of each micropreparation (several tens of light fields), 50 pollen grains were described from photographs of good quality. In each sample, 500 pollen grains were analyzed. Palynoindication of the environment was carried out based on the content of normally developed pine pollen in the samples after N. A. Kalashnik [7]. According to this scale of ecological zoning of territories, pollen samples of conditionally clean territories contain more than 90% of normally developed pollen, moderately polluted – 89.4–82.9%, heavily polluted – 82.3 – 75.2%, critically polluted – 68.6–62%.

3. Results
As a result of studies of P. sylvestris, typical and teratomorphic pollen grains were revealed in the tested samples. The highest content of normal pollen is in the control sample from the Verkhnetulomsky settlement (45.4%). In the vicinity of the ANOF-2 tailing dump, normal pollen content is significantly lower and amounts to 20% (P1) and 17% (P2). Content of abnormal P. sylvestris pollen in samples from the experimental sites is very high and varies from 80% to 83% (figure 1).

The control site is located far from the mining centers of the Murmansk region. At the same time, 17 teratomorphs of pollen of P. sylvestris are distinguished in the samples: without sacchi, with reduced sacchi, with a single saccus, two dissimilar sacchi, with their asymmetric arrangement and developmental disorders, lenticular with one saccus, dwarf (including nonsaccate, with sacchi reduction and a single saccus), with exine fissures, with reduced body, giant ones with normally developed sacchi and various pathologies thereof (figures 2 and 3). This is indicative of the long-range atmospheric transport of aerosols from industrial centers and a very high sensitivity of pine to environmental pollution. In samples from the vicinity of the Apatit Plant, a greater number of teratomorphs is found: Tailings dump (P1) – 21, Kozlova Str. (P2) – 22. These samples contain terates similar to the control, but there are also specific developmental anomalies of pollen: with a collar saccus, with large transverse or longitudinal cracks in the center of the body of the pollen grain, polyads.

A large proportion of pine pollen in all samples has pathologies in the development of sacchi: 51.6% in the vicinity of the concentrating mill (Kozlova Str.), 39% in the vicinity of the tailing dump. The most common is pollen with their reduction: in the industrial zone of the Apatit Plant – 16.2% (P1) and
17.8% (P₁), in the control – 18.2% (figure 2). This developmental anomaly is considered the most common in the male pine gametophyte.

![Figure 1](image1.png)

**Figure 1.** The ratio of normal and teratomorphic pollen of *Pinus sylvestris*, in percent.

![Figure 2](image2.png)

**Figure 2.** Ratio of *Pinus sylvestris* pollen groups with pathologies of sacci, in percent.

The samples from the industrial area contain a lot of nonsaccate pollen; its highest content are found at the site P₂ (20.4%), which is several times higher than the control. In smaller quantities, there is monosaccate and dissimilar-sacci pollen, with their asymmetric arrangement, with one lenticular saccus and with a collar hemispherical saccus. These pathologies cannot perform an adaptive function and are indicative of developmental instability under the influence of environmental pollution.

In samples from the vicinity of the town of Apatity, a high content of dwarf pollen of Scotch pine are revealed, including those with various deviations in the development of sacci (with their reduction, monosaccate, nonsaccate). Their total content in the samples: Tailing Dump – 16%, Kozlov Str. – 11.2% (figure 3). The share of small nonsaccate pollen grains is high (6.2 – 8.8%), in most cases they are sterile. Giant pollen is often found, which is 1.5 or more times larger than typical, presumably diploid, including those with various pathologies of sacci: Tailing Dump – 12.8%, Kozlov Str. – 11.8%.

Samples in the zone of industrial impact of the Apatit Plant revealed pollen with large transverse or longitudinal cracks in the center of the body of the pollen grain (figure 3). In the vicinity of the ANOF-2 Tailing Dump, the proportion of such pollen is 8.6%, at Kozlova Str. – 5.8%, it is absent in the control.
Figure 3. Ratio of groups of giant and dwarf pollen of *Pinus sylvestris* including those with saccus pathologies; with a crack in the center of the body, in percent.

4. Discussion
In the palynoteratic complex of the Scots pine in the impact zone of the Apatit Plant, a high content of pollen of normal, dwarf and giant sizes with pathologies of sacci are revealed. Recent studies by infrared microspectroscopy have shown that the spectra of single pollen grains of *P. sylvestris* have relatively high variability due to the bilateral symmetry of bisaccate pollen grains [12]. The reason for this variability is the non-radial symmetry of the pine pollen. G. M. Levkovskaya [13] notes that under geobotanical stresses, nanism of pollen grains and thickening of exine are a general tendency of teratomorphosis. Many authors point to the formation of small pollen grains due to dryness of air under pollution conditions [5-6]. According to optical diffraction tomography, the sacci of *P. sylvestris* are separated from the body of the pollen grain by a very thin exine, while their outer surface consists of a thick but macroporous exine [14]. As a result of water loss, the volume of living pollen grains is reduced, the sacci close up and cover the thinnest part of the exine. These processes lead to a decrease in evaporation and prevent pollen grains from complete desiccation. At the same time, V. N. Bessonova [15] showed that the appearance of dwarf pollen is associated with disturbances in meiosis in which part of the genetic material is lost as a result of damage to the fission spindle or chromosomal mutations. The appearance of giant pollen grains is also associated with disturbances in meiosis as a result of exposure to mutagens of various nature. Such pollen, including those with a huge body and dwarf sacci, and vice versa, was described in the study of natural geobotanical catastrophes [13]. Usually, the size of pollen grains is a stable species trait (more often generic one) and is characterized by very low individual, intrapopulation, and intraspecific geographic variability [16]. That said, sizes of pollen of *P. sylvestris* have low variability in natural populations, in contrast to impact zones.

The distinction of pathomorphosis of *P. sylvestris* in the zone of industrial impact of the Apatit Plant is the frequent occurrence of pollen with large transverse or longitudinal cracks in the center of the body. A similar pathology of pollen of Scotch pine was found near the nuclear power plant in the city of Sosnovy Bor in Leningrad Region [17]. O. F. Dzyuba [17] notes that such teratomorphs, which have scars resembling open or closed slits, indicate the presence of radionuclides and a high level of environmental pollution.

5. Conclusion
The natural polymorphism of pollen of *P. sylvestris* is represented by a fairly large number of teratomorphs, however, the content of abnormal pollen grains in such samples does not exceed 3 – 7% [5]. In the vicinity of the apatite-nepheline tailing dump, pine pollen polymorphism increases to 80 – 83%. The variety of pollen teratomorphs is evidential of high lability of pollen structure. In the technological scheme of mining and processing enterprises, which develop deposits of apatite-nepheline ores, tailings dumps are the most environmentally hazardous facilities [18]. At the same
time, the stored waste belongs to the hazard class V—practically non-hazardous waste [11]. Studies in the vicinity of the ANOF-2 tailing dump have shown that the formation of a large number of genetically abnormal forms of pine pollen and the peculiarities of their teratologies are evidential of a high level of environmental pollution and the impact of radionuclides. According to the palynological scale of ecological zoning by N. A. Kalashnik [7], based on the content of normal pollen of *P. sylvestris*, the territory in the vicinity of the ANOF-2 tailing dump can be classified as critically polluted. For the sustainable development of the Murmansk region, it is necessary to use new technologies for the concentration of apatite-nepheline ores with the minimum possible storage of waste in tailing dumps.

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