

# Teratomorphism of *Pinus sylvestris* L. pollen as the bioindicator of atmospheric air pollution of the Subarctic industrial city

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**Abstract.** The article presents the results of the palynological analysis of *Pinus sylvestris* L. pollen of the industrial city of Apatity, located above the Arctic Circle (Murmansk region, Russia). The city is in the impact zone of the Apatit mining and processing complex, which is one of the world's leaders of producing of phosphate raw materials for mineral fertilizers. Four experimental sites were laid in the city of Apatity and its vicinity. At the end of June microstrobiles with mature pollen were collected and fixed in a 40% alcohol solution. The studies were carried out by light microscopy. As the result, 23 types of teratomorphic pollen were identified. The content of teratomorphic pollen in the samples is very high (80.4–90.4%). The pollen grains of normal, dwarf and giant sizes with various anomalies of sacchi are most common. The formation of a large quantity of genetically abnormal forms of *P. sylvestris* pollen and the peculiarities of its teratology indicate high level of environmental pollution of the city of Apatity by toxic compounds. The pollen of *P. sylvestris* can be used as biomonitor for detecting of the phytotoxic and mutagenic effects of pollutants.

## 1 Introduction

Ecological palynology is a new direction of environmental research that has been actively developing in recent decades. Previously the pollen analysis was used for paleogeographic and paleoecological reconstructions, recently this method is used to assess the quality of the environment [1]. Pollen is a good biomonitor for the capture of heavy metals and other pollutants [2, 3], for study of the developmental anomalies and viability of pollen grains [4, 5], their allergenicity [6–8]. Pollen of various plant species is using for bioindication of atmospheric air quality in the urbanized territories with high transport load [9–11] and industrial impact zones [12–14]. Cytological abnormalities in plants can be a useful monitoring method for the recognition of chemicals present in the environment that might cause a genetic threat [15].

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Spectrum of studied plant species is very large, from ornamental plants to different trees, the pollen of which being known as allergenic or not [6]. Under the influence of industrial emissions, plants produce a lot of teratomorphic and sterile pollen grains. It is known, then higher the level of environmental pollution, than higher the quantity of pollen with anomalous of morphological structure [1, 3, 14]. A lot of teratomorphic pollen grains with developmental pathologies (from 45 to 100 %) are recorded in areas with a high level of gas pollution from transport emissions, the presence of many industrial enterprises [17]. The proportion of natural polymorphism of pollen grains of plants in favorable environment usually does not exceed 5–10, less often 20% [1]. Studies of microsporogenesis processes of various conifer species exposed of industrial pollution are of especially relevance because their pollen are most sensitive [12, 13]. *Pinus sylvestris* L. is often used as the test object to determine the level of air pollution, since under conditions of environmental stress, it exhibits an increase of pollen teratomorphism [1, 14]. The most common anomalies of *P. sylvestris* pollen grains are including the reduction of sacci, an increase of the number of saccus to 3–4, fusion of sacci, giant pollen (1.5–2.5 times larger than average), dwarf pollen, and some others [18].

The purpose of the study is to research the teratomorphism of *Pinus sylvestris* L. pollen under industrial pollution of the city of Apatity.

## 2 Material and methods

The object of the study is Scots pine (*Pinus sylvestris* L.), which is widely spread in the cities of the Murmansk region. In the city of Apatity, *P. sylvestris* mainly grows on the outskirts of the city as part of spruce and pine forests with an admixture of birch. Pine pollination in the Murmansk region begins in late June – early July. Pollen grains of Scots pine have two sacci, heteropolar, bilaterally symmetrical. The sacci are oblong-elliptical in shape, sharply separated from the pollen grain and shifted to the distal side [1].

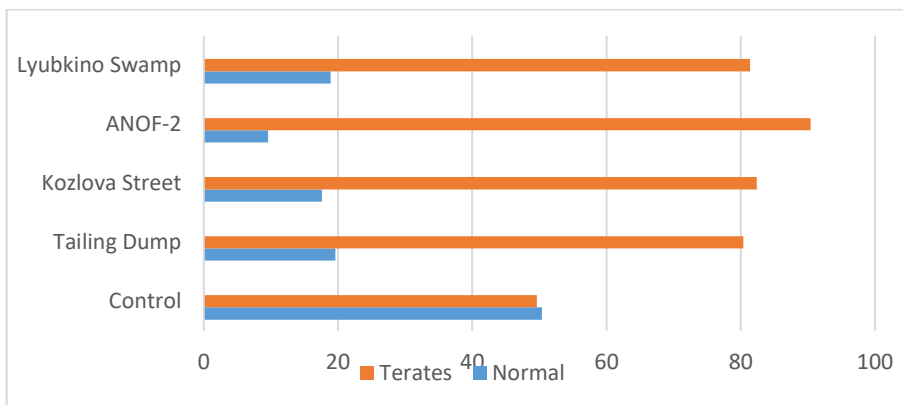
The studies were conducted in the city of Apatity, which is located beyond the Arctic Circle, in the foothills of the Khibiny Mountains (67°34'00" N, 33°24'00" E). The city is in the impact zone of the Apatity Plant, one of the world's leading producers of phosphate raw materials for mineral fertilizers [19]. The mining and processing complex includes mines, processing plants (ANOF-2 and ANOF-3), the tailing dump and several workshops. Their emissions contain strontium, zinc, phosphorus, calcium, sodium, potassium, aluminum, nitrogen compounds and iron. Apatite dust contains a certain number of natural radionuclides [19]. Wastes of the enrichment of apatite-nepheline ores are stored in the tailing dump located on the shore of the Belaya Bay of Lake Imandra, in 9 km from the city of Apatity. The wastes consist of the fraction of finely dispersed nepheline sands, which, when strong winds occur, turn into an aerosol state, forming dust clouds, and are carried over long distances. The atmospheric air of the city of Apatity is heavily polluted by dust, which contains toxic compounds. The strontium content is 4 times higher than background values, copper – 2 times, phosphates – 10 times, sulfates and fluorides – 3 times [20].

In May 2020, four experimental sites were established in the city of Apatity and its vicinity: "Tailing Dump" – the forest area in 2 km from the center of the tailing dump; "ANOF-2" – the forest area in 1 km from ANOF-2; "Kozlova Street" – the forest park on the northern outskirts of the city of Apatity, in 3.8 km from the center of ANOF-2 and 35 m from the railway; "Lyubkino Swamp" – the forest park in the south-eastern part of the city of Apatity, in 5.7 km from the center of the ANOF-2. The control site was in the settlement of Verkhnetulomsky, 128 km from the city of Apatity. There are no environmentally dangerous industrial facilities on the territory of the settlement. At the end of June, during the period of mass pollination of Scots pine, 7 microstrobiles with ripe pollen were collected from 5 trees from the each of test sites. The male cones were dried and fixed in a 40% alcohol solution. For palynological analysis were used the microsporophylls from middle part of

microstrobiles. The pollen was stained by Lugol's solution. The studies were conducted using light microscopy with a magnification of 200 times. Palynological study was conducted using microphotographs, which were done through the eyepiece of a microscope by the 13-megapixel camera Xiaomi Redmi Note 5A Prime. 550 pollen grains were described in the samples of each of the experimental sites. Teratomorphs were identified according to O. F. Dzyuba [1], the amount and percentage of normal and teratomorphic pollen in the samples were determined.

### 3 Results and discussion

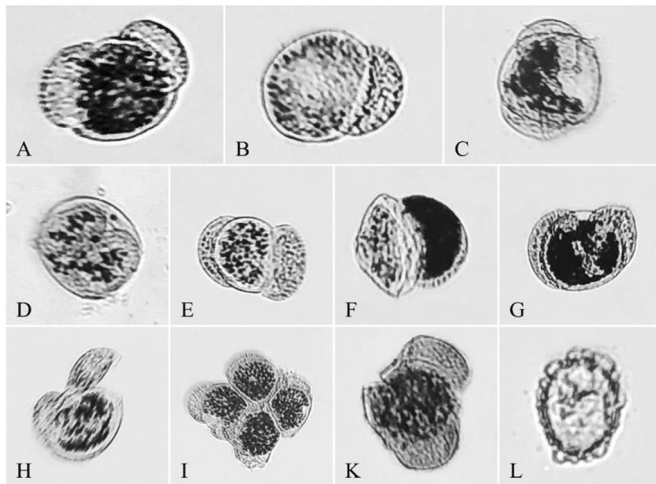
As a result of palynomorphological studies, it was found that the largest amount of normal pollen of *P. sylvestris* is contained in the control samples (figure 1). In the city of Apatity and its vicinity, the proportion of normal pollen is very low and varies from 9.6% (ANOF-2) to 19.6% (Tailing Dump). In all samples, were found anomalous pollen grains of Scots pine. Teratomorphic pine pollen differ from normal ones by size, shape, number of saccus and other developmental disorders [16]. 23 types of teratomorphic pollen are identified (figure 2): without sacci, with their reduction, with a single saccus, with two dissimilar sacci, with their asymmetric arrangement and developmental disorders, with one fusion lenticular or collar saccus, dwarf (including without sacci, with their reduction, with a single saccus, with a loop-like overgrown exine), giant with normally developed sacci or with their pathologies (with a single saccus, without sacci, with two dissimilar sacci, with their reduction, with a collar saccus and with exine disorders), with exine ruptures, with reduced body, with large transverse or longitudinal cracks in the center of the pollen body, polyads. The content of teratomorphic pollen in the samples of the experimental sites of the city of Apatity is very high (80.4–90.4%) (figure 1). The largest amount of abnormal pollen is found in the samples from the vicinity of ANOF-2 (90.4%) and on Kozlova Street (82.4%). According to environmental monitoring conducted by scientists from the Moscow State University after M. V. Lomonosov, the city of Apatity, and especially its north-east and east districts are under the influence of activities of ANOF-2 and dusting of the tailing dump [20].



**Fig. 1.** The ratio of normal and teratomorphic pollen of *Pinus sylvestris* in the city of Apatity and its vicinity (%).

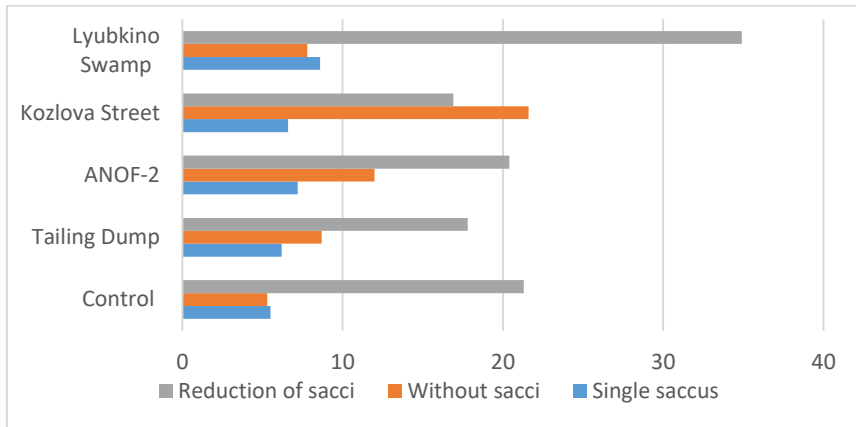
The pollen grains of normal size with pathologies of sacci are most common: with reduction of sacci, without sacci, single-saccus, multi-saccus, with their asymmetrical arrangement, with developmental disorders, with fused saccus (lenticular and collar-like) (figure 2; figure 3). The high total share of pollen with such pathologies is found in the

samples of the experimental sites “Lyubkino Swamp” (59.6%), ANOF-2 (53%), and Kozlova Street (52%). The reduction of pollen sacci (16.9–34.9 %) is most common (figure 2 C; figure 3). This anomaly of development is most characteristic of the male gametophyte of Scots pine [18]. The samples contain a lot of pollen without sacci (7.8–21.6%), especially on Kozlova Street (figure 2 D; figure 3). The content of the pollen with single saccus is significantly lower (6–8.6%) (figure 2 B; figure 3). The pollen grains with two dissimilar sacci (0.6–3.8%) (figure 2 E), with their asymmetrical arrangement (1.8–4.0%), with fusion of sacci to one lenticular saccus (figure 2 F) (0.7–1.5%) are less. In all samples pollen with a collar-like saccus (figure 2 G) is found (0.6–3.3%). Near the ANOF-2 and on the Lyubkino Swamp its content reached maximum and is absent in the control sample. Such teratomorphic pollen is usual only in the conditions of very strong environmental pollution [12]. The pollen of *P. sylvestris* with pathologies of the sacci is excluded of the participation in pollination, because sacci, primarily, are the balancer during pollination [21].



**Fig. 2.** Teratomorphs of pollen of *Pinus sylvestris* in the city of Apatity: A – normal, B – with a single saccus, C – with reduced sacci, D – without sacci, E – with two dissimilar sacci, F – with one lenticular saccus, G – with collar-like saccus, H – with disorders of saccus, I – polyads, K – with crack in the body, L – dwarf with loop-like growths on the exine

The giant pollen is often, 1.5 times larger than the typical one, presumably diploid, including with anomalies in the development of sacci and exine (single-sacci, without saccus, with two dissimilar sacci, with their reduction, with the collar-like saccus, with exine disorders). The highest total content of giant pollen with various pathologies is found in the samples of the test sites Tailing Dump (11.8%), Kozlova Street (10.9%), ANOF-2 (9.1%).



**Fig. 3.** The ratio of pollen of *Pinus sylvestris* with pathologies of sacci (%).

The samples contained a lot of dwarf pollen (10.8–17.3%), including with anomalies of sacci (with their reduction, without sacci, single-saccus), its total content is maximum near the Tailing Dump (17.3%), ANOF-2 (15.9%). The proportion of dwarf pollen grains without sacci is especially high (7.3–13.1%), in most cases it is sterile. The nano-pollen with loop-shaped growth of exine (figure 2 L) is found in the samples of the sites Tailing Dump, ANOF-2, and Lyubkino Swamp (0.4–1.6%), it is also sterile. A similar pollen was discovered near the Leningrad Nuclear Power Plant and in the city of Sosnovy Bor [22].

Pollen grains with large transverse or longitudinal cracks (open or closed) in the center of the body (figure 2 K) are the strike feature of the pathomorphism of *P. sylvestris* pollen in the impact zone of the Apatity Plant. Their maximum content is found in the samples of the Tailing Dump (8.2%) and ANOF-2 (6.6%), they are absent in the control. The pollen grains with disturbances of the exine (1.1–5.1%) and a reduced body (0.4–1.1%) are also presence. The polyads (figure 2 I) are found in the samples of Tailing Dump and Kozlova Street (0.2–0.4%).

Studies have shown that samples from the impact zone of the Apatity Plant and Tailing Dump contain a lot of Scots pine pollen teratomorphs of normal, dwarf and giant sizes with pathologies of the sacci. It is known that the size of pollen grains is a stable trait of species and is characterized by very low individual, intrapopulation and intraspecific geographic variability [23]. Pollen sizes vary slightly in natural populations, unlike the industrial impact zones. G. M. Levkovskaya [24] noted that dwarf pollen grains are the general tendency of teratomorphism under geobotanical stresses. V. P. Bessonova [25] have shown that dwarf pollen is formed due to meiosis disorders, losing part of the genetic material, in the reason of damage of the division spindle or chromosomal mutations. The high content of giant pine pollen in the city of Apatity, presumably diploid, with various pathologies of sacci, is a consequence of mutagenesis and disturbances of the meiosis process. Such pollen, with a huge body and dwarf sacs, and vice versa, was described in the study of natural geobotanical catastrophes [24]. Pollen of *P. sylvestris* with the pathologies of pollen sacci loses the ability to be transported for long distances by air and the ability to pollinate. From the point of view of adaptive evolution, bilaterally symmetrical sacci of conifer pollen play the role of balancers on the nucellus of the ovule [21]. Disruption of their morphological structure prevents them of performing this function and thus limits reproduction. All these anomalies are highly pernicious to reproductive success. Moreover, these abnormalities can even change the breeding behavior by imposing total male sterility and changing the pollination system [26].

The frequent occurrence of pollen with large transverse or longitudinal cracks in the center of the body, as well as dwarf pollen with loop-shaped growth of the exine are the characteristic feature of the pathomorphosis of *P. sylvestris* in the impact zone of the Apatit concern. Similar teratomorphes were found near the Leningrad Nuclear Power Plant and in the city of Sosnovy Bor [22], they are considered as indicators of the presence of radionuclides and a high level of environmental pollution. The level of teratomorphosis of pine pollen in the city of Apatity (80.4–90.4%) exceeds the same indicators in the industrial cities of the Russian North, where similar studies were conducted [14, 27]. In the city of Monchegorsk, the center of non-ferrous metallurgy of the Murmansk region, the proportion of teratomorphic pollen of *P. sylvestris* varied from 10 to 55%, in the city of Murmansk from 54.2 to 70.6% [14]. In the city of Kostomuksha (Republic of Karelia), in the impact zone of mining and processing plant “Karelsky Okatysh”, which produce iron ore raw materials, proportion of teratomorphic pine pollen averaged 43% [27].

## 4 Conclusion

The palynological studies of *P. sylvestris* were conducted in the city of Apatity for the first time. A high level of pollen teratogenesis (80.4–90.4%) was revealed in all test sites, while the content of abnormal pine pollen grains in populations of northern forest ecosystems does not exceed 3–7% [1]. Anomalies of the development of Scots pine pollen in the city of Apatity indicate that the anthers of microstrobiles are exposed to the toxic dust emissions of the ANOF-2 and the Tailing Dump, which leads to disturbances of microgametogenesis. The formation of a lot of abnormal forms of Scots pine pollen and the feature of its teratology indicates that substances in the emissions of the Apatity Plant have a mutagenic effect. The results confirm the data of environmental monitoring conducted by scientists of the Moscow State University after M. V. Lomonosov of high pollution of the atmospheric air of the city of Apatity by the toxic compounds [20]. Studies have shown that *P. sylvestris* pollen can be used for the detection of the phytotoxic and mutagenic effects of pollutants, which is consistent with the results obtained in some industrial centers of Russia [4, 12, 27]. For the identification of the causes of teratomorphism of *P. sylvestris* pollen under the influence of industrial emissions of the Apatit Plant and for clarifying of environmental causes of genetic impacts, long-term monitoring is necessary.

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