ABS ACTA BIOLOGICA SIBIRICA
A journal on the biodiversity of Siberia and the adjacent lands

Acta Biologica Sibirica 11: 1–12 (2025)
doi: 10.5281/zenodo.14637256
http://journal.asu.ru

RESEARCH ARTICLE

Technogenic effects on scots pine generative organs: a cross-country study

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Academic editor: R. Yakovlev | Received 14 September 2024 | Accepted 7 November 2024 | Published 15 January 2025

http://zoobank.org/2D836361-3BF1-4F3F-AC85-D7E20B03ECEE

Citation: Assylbekova G, Zhumabekova B, Klimenko M, Khan SM, Aubakirova K (2025) Technogenic effects on scots pine generative organs: a cross-country study. Acta Biologica Sibirica 11: 1–12. https://doi.org/10.5281/zenodo.14637256

Abstract

This article presents the results of a study assessing the state of morphological changes in the male generative organs of Scots pine (*Pinus sylvestris* L.) in Kazakhstan and Poland. The purpose of this study is to assess the transformation of pine pollen under different environmental conditions. Research and pollen sampling were carried out in 2023 in different environmental conditions and in different geographical areas: Astana (Kazakhstan), Bayanaul State National Natural Park (BSNNP) (Kazakhstan) and Poznan (Poland). For cytological analysis, microsporophylls were selected from the middle part of the microstrobilus, the material was stained with 1% Lugol's pharmaceutical solution, the preparations were studied on a Nikon eclipse E 200 microscope with a magnification of 10x15x40. The results of the analysis showed that the maximum number of disturbances in pine pollen was detected in samples from Poznan - 63%. In the Bayanaul Natural Park and in Astana, pine has fewer anomalies in pollen - 57% and 55%. The most common types of anomalies found in Scots pine pollen in the study areas are: deformed (26.3%), pollen with impaired shell formation (19%) and degraded (7.4%). Under conditions of technogenic pollution in coniferous trees (*Pinus sylvestris* L.), a higher level of abnormality of pollen grains was revealed than in relatively clean conditions, and a wide spectrum (sterile, deformed and small pollen grains with disturbances (without air sacs, with one and three air bags).

Keywords
Bioindication, cytological analysis, ecology, monitoring, phytoindication, pollen sterility, pollen transformation

Introduction

Phytoindication is the fastest method for assessing the ecosystem and response to anthropogenic impact. One of the striking examples of phytoindication is pollen analysis – an accessible, reliable, not requiring specialized technical equipment, relatively simple and at the same time effective way to assess the quality of the environment.

Woody plants of the most common species can be considered one of the phytoindicators, since they are perennial and can be studied over several years (Hlebova, Bychkova 2016; Kalashnik 2011; Valetova 2009). Pollen from herbaceous plants can also serve as a component of low-cost monitoring of soil and plant conditions: for example, pollen grains from dandelion and oxytrope react strongly to conditions of pollution from transport exhausts (Reshetova 2019).

Scots pine pollen (*Pinus sylvestris* L.) was used as bioindicators to assess the environment, since the cytological method is very sensitive in assessing the influence of unfavorable factors. Analysis of the results of transformation of *Pinus sylvestris* pollen reflects the technogenic influence on the morphological state of grains.

There is a great interest among scientists in studying the processes of microsporogenesis in coniferous plants subject to technogenic influence (Assylbekova 2012; Chropeňová et al. 2016; Noskova, Tret'jakova 2006). Research shows that under conditions of industrial pollution in coniferous woody plants, the number of pathologies in the process of microsporogenesis increases, leading to changes in the male generative system. All these indicators make it possible to use conifers as bioindicators for assessing the ecosystem.

The purpose of this study is to assess the transformation of Scots pine (*Pinus sylvestris* L.) pollen under different environmental conditions.

Materials and methods

The object of the study is Scots pine (*Pinus sylvestris* L.). Coniferous plants are widely used as test objects, since anthropogenic pollution has a pronounced effect on their condition. The object we have chosen is a forest-forming component, and currently there is a problem of preserving and restoring coniferous tracts near urbanized areas.

Research and sampling were carried out in 2023 in different environmental conditions and in different geographical areas: Astana (Kazakhstan), Bayanaul State National Natural Park (BSNNP) (Kazakhstan) and Poznan (Poland) (Fig. 1).

Brief characteristics of the study areas

The city of Astana is the capital of the Republic of Kazakhstan, covers an area 797 km^2. Operating enterprises are manufacturing industries: mechanical engineering (15.0%), production of building materials (33.8%), metallurgy (production of gold and silver) (23.5%), food industry (11.5%) and other industries (16.2%). Basically, air pollution is typical for the cold period of the year, accompanied by the influence of emissions from thermal power enterprises and private sector heating. The level of atmospheric air pollution in Astana over the past 5 years remains very high (Environmental protection ... 2023).

Bayanaul State National Natural Park (BSNNP), selected as background area, located in a mountainous area (altitude from 400 to 1027 m above sea level). The park area is 68,452.8 hectares, belongs to the Pavlodar region and is located 140 km from the city of Ekibastuz, which has a developed coal industry.

City of Poznan – ancient city with an area of 262 km^2, an important industrial center in Poland. Nearby enterprises: the largest machine-building plant named after Tsegelsky, enterprises of the chemical, food industries and electrical engineering. In the city, a large number of old high-rise buildings have stove heating.

To conduct the study on the territory of Poznan (Poland), samples were taken from three points: an urban area (Jan Henryk Dąbrowski St., 60), a wooded area (Bedrusko), and a botanical garden. In Astana and BSNNP, 2 points were selected from which samples were taken. The presented maps indicate the geolocation coordinates of all sampling points.

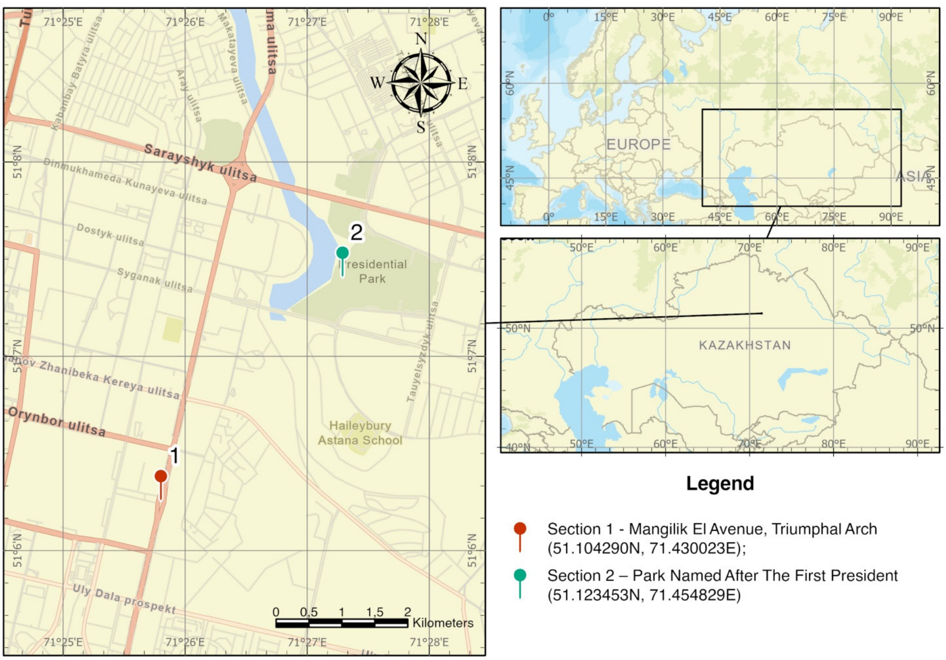


Figure 1. Maps of the study areas with sampling points. Continued on the next page.

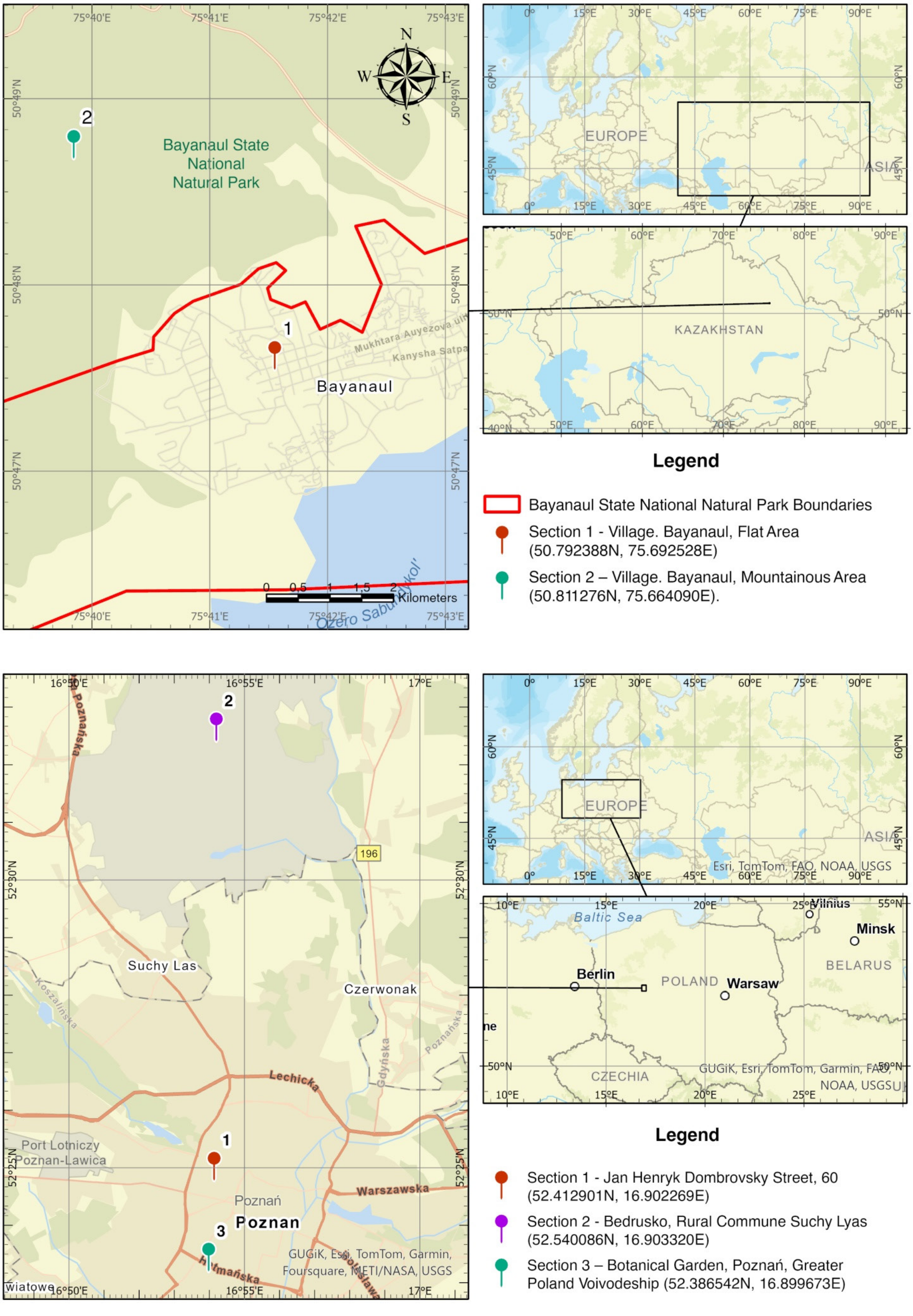


Figure 1. Maps of the study areas with sampling points. Continued from the previous page.

From each sample plot, 2000–3000 pollen grains from 5–10 trees were examined. Cytological analysis of pollen samples was carried out on the basis of the scientific center of biocenology and environmental research of Pavlodar Pedagogical University (Pavlodar, Kazakhstan).

For cytological analysis, microsporophylls were selected from the middle part of the microstrobila (*Pinus sylvestris* L.), the material was stained with 1% Lugol's pharmaceutical solution (Şeker et al. 2022), and the preparations were studied on a Nikon eclipse E 200 microscope with a magnification of 10x15x40. Temporary microslides were photographed using a Canon PowerShot A 95 digital camera.

The study was guided by generally accepted methods of statistical data processing using Microsoft Excel – descriptive statistics (mean, mode, median) and analysis of variance (statistical significant difference).

Results

The fertile pollen grain is a male gametophyte and has thin filmy scales with two large microsporangia – pollen sacs (Fig. 2). Pine pollen normally has an ellipsoid shape with two symmetrically located air sacs. The study and description of morphological changes in pollen of *Pinus sylvestris* have become the subject of a number of studies, during which various morphological types of pollen grains were determined. Natural polymorphism of pollen grains of this species is represented by a variety of morphotypes (Mollaeva, Tembotova 2022; Monoszon–Smolina 1949).

It is known that a large number of teratomorphic (with developmental pathologies) pollen grains – from 45 to 100% – are recorded in areas with high levels of gas pollution from transport exhausts, the presence of a large number of industrial enterprises and excess of the maximum permissible concentration of zinc, cadmium and lead in soils. While the percentage of natural polymorphism of pollen grains in plants in favorable environmental conditions usually does not exceed 5–10, less often 20% (Dzjuba 2006). Moreover, the worse the environmental situation, the higher the percentage of pathologically developed pollen and vice versa. As a result of meiosis, numerous tetrads of haploid microspores arise from cells of sporogenic tissue. Numerous anomalies of pollen grains are explained by disturbances during meiosis. Figure 3 shows different types of anomalies (Reshetova 2019).The quality (fertility) of *Pinus sylvestris* L. pollen is related to the environmental conditions for its growth.

The results of laboratory studies of pollen grains of coniferous trees collected in Astana (Kazakhstan), Poznan (Poland) and Bayanaul State National Natural Park (BSNNP) (Kazakhstan) showed that under pollution conditions the percentage of pollen grain anomalies increases and more a wide range of them. Table 1 shows the morphological changes in *Pinus sylvestris* L. pollen in numbers and percentages.

Comparative values of fertility indicators and pollen anomalies using the $\chi 2$ test indicate that the differences between study areas from contrasting environmental conditions are significant at high levels of significance.

The following types of anomalies were found: deformed, lens-shaped (26.3%) > impaired formation of membranes, fused (19%) > degrading (7.4%) > with 1 air sac (3.4%) > collar-shaped (1, 8%) > with 3 air sacs (0.6%) > 4 air sacs (0.3%) (Table 1, Fig. 3).

The maximum number of all types of anomalies – deformed and lens-shaped, 412 (39%) – were identified on the street. Jan Henryk in Poznan (Poland) (Fig. 4A–B). 24% of the total were pollen grains with disturbances in the formation of shells, collected on flat area BSNNP (Fig. 4C). Minimal numbers of deformed, lens-shaped pollen grains detected in the mountainous areas of BSNNP – 210 (18%), as well as with violations of the shells in pollen – on Mangilik El Avenue, Triumphal Arch in Astana – 106 (10.6%).

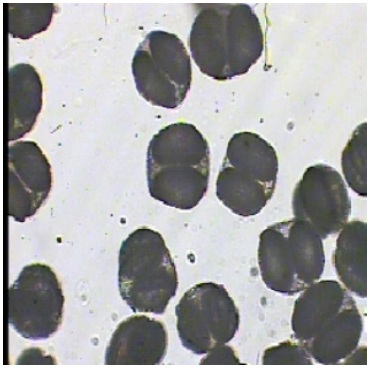


Figure 2. Fertile pollen grain of Scots pine (*Pinus sylvestris* L.).

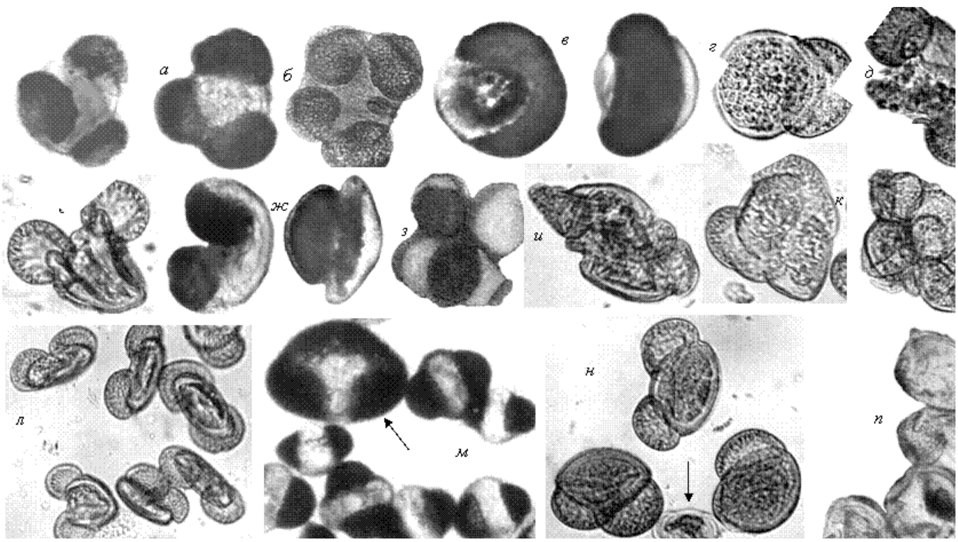


Figure 3. Anomalies of pollen grains (Reshetova 2019): **a** – pollen grain with three air sacs; **b** – diploid pollen grain with four air sacs; **c** – collared pollen grain; **d**, **e** – violation of the formation of membranes; **f**, **g**, **k** – pollen grains with body deformation; **h**–**k** – fused pollen grains; **n** – hypertrophied pollen grain; **p** – degraded pollen.

Table 1. Morphological state of *Pinus sylvestris* L. pollen in the study areas

№	Collection point	Total quantity pollen grains	Pollen grains (p.g.) with anomalies, number (%)							
			p.g. with 1 air bag	p.g. with 3 air bags	p.g. with 4 air bags	Collar p.g.	Deformed, lens-shaped p.g	Violation of the formation of membranes, fused, hypertrophied p.g.	Degrading p.g.	Total quantity abnormal p.g.
1	BSNNP, mountainous area (Kazakhstan)	1179	70 (6)	15 (1.3)	5 (0.4)	36 (35)	210 (18)	232 (19.7)	125 (10.6)	693 (58.8)
2	BSNNP, flat terrain (Kazakhstan)	1099	35 (3.2)	2 (0.2)	2 (0.2)	11 (10)	234 (21.3)	266 (24)	46 (4.2)	596 (54.2)
3	Astana, Mangilik El Avenue, Arc de Triomphe (Kazakhstan)	1000	26 (2.6)	8 (0.8)	4 (0.4)	21 (2)	223 (22.3)	106 (10.6)	38 (3.8)	426 (42.6)
4	Astana, Park named after First President (Kazakhstan)	1005	68 (6.8)	10 (1)	3 (0.3)	31 (2.8)	256 (25.5)	183 (18.6)	117 (11.6)	668 (66.5)
5	Poznan, st. Jan Henryk Dombrowski (Poland)	1045	18 (1.7)	1 (0.1)	1 (0.1)	9 (0.9)	412 (39)	204 (19.5)	127 (12)	772 (73.9)
6	Poznan Wooded area (Poland)	1007	16 (1.6)	5 (0.5)	2 (0.2)	10 (0.1)	344 (34)	201 (20)	40 (4)	618 (61.4)
7	Poznan, Botanical Garden (Poland)	1032	17 (1.6)	4 (0.4)	3 (0.3)	15 (1.4)	257 (25)	214 (20.7)	55 (5.3)	565 (54.7)
	Total number of p.g.	7367	250 (3.4)	45 (0.6)	20 (0.3)	133 (1.8)	1936 (26.3)	1406 (19)	548 (7.4)	4338 (58.9)

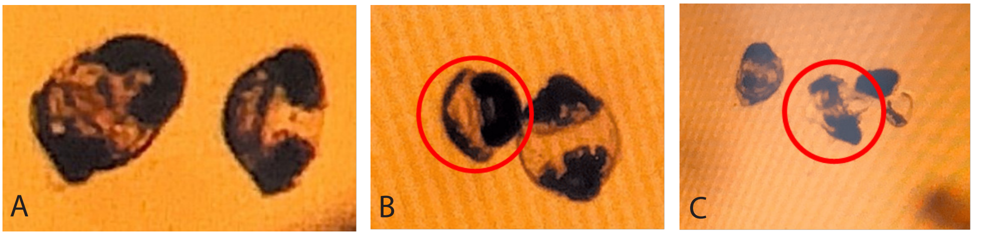


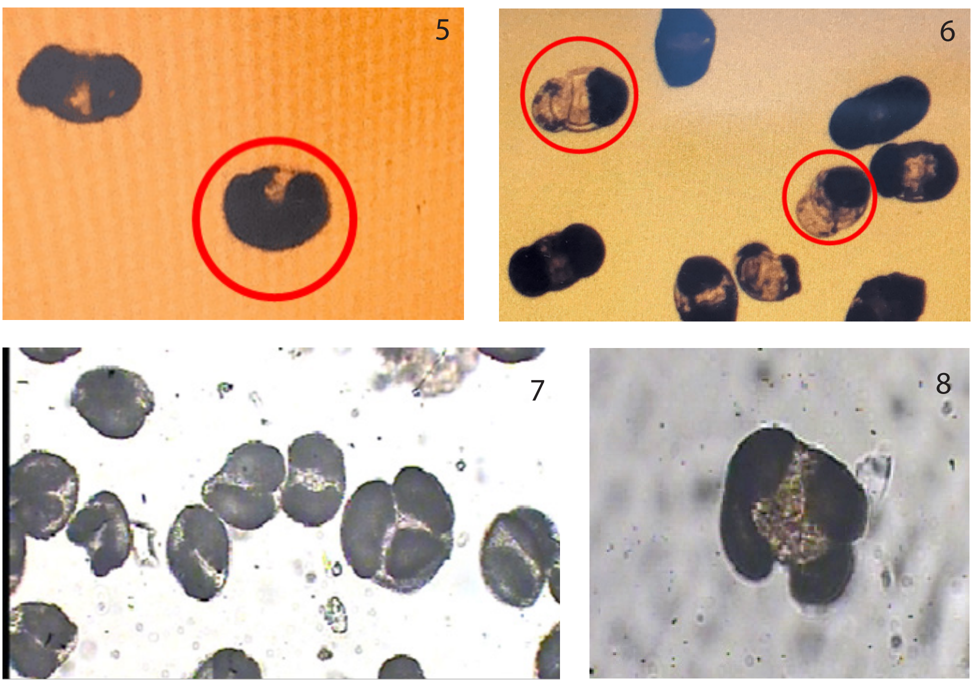
Figure 4. Pollen grain anomalies found during the study: **A** – deformed pollen (st. Jan Henryk Dombrowski, Poznan, Poland); **B** – lens-shaped pollen (Jan Henryk Dombrowski st., Poznan, Poland); **C** – pollen with damaged shells (flat terrain, BSNNP, Kazakhstan).

The most common degrading pollen is found in industrial areas of Poznan (Poland) on the Jan Henryk Dombrowski street – 127 (12%) and in the mountainous areas of BSNNP – 125 (10.6%). The smallest number of anomalous pollen was shown by pine growing on Mangilik El Avenue, Triumphal Arch in Astana – 38 (3.8%).

In the mountainous areas of the BSNNP, collared anomalous pollen grains predominate – 35% (Fig. 5), in the remaining study areas their percentage ranged from 10%–0.9%. In the First President park of Astana the largest number of pollen grains with 1 air sac was identified – 68, which amounted to 6.8% of the total number of items (Fig. 6). The lowest percentage of this anomaly was shown by areas in wooded areas and the botanical garden of Poznan (Poland) – 1.6% each (16 and 17 p.g., respectively).

Among the studied pine pollen grains, anomalous pollen with 3 air sacs was not common – 45 times (0.6%): from 1 (0.1%) per Jan Henryk Dombrowskiin st. Poznań up to 15 (1.3%) in the mountainous area of the BSNNP (Fig. 7).

The number of pollen grains with 4 air sacs is the smallest indicator of the total number of anomalous grains – 20 (0.3%), with a predominance in the mountainous areas of BSNNP – 5 (0.4%) and the minimum number on the Jan Henryk Dąbrowski street in Poznań – 1 (0.1%) (Fig. 8).



Figures 5–8. 5. Collared pollen grains (mountainous area, BSNNP, Kazakhstan); **6.** Pollen grains with 1 air sac (park named after First President Astana, Kazakhstan); **7.** Pollen with 3 air sacs (mountainous areas, BSNNP, Kazakhstan); 8. Pollen grains with 4 air sacs (st. Jan Henryk Dombrowski, Poznan, Poland).

For a general assessment of the transformation of pollen in Scots pine under the conditions of BSNNP, Astana and Poznan, a summary of data is presented in Table 2. In different environmental conditions of coniferous growth, 7 types of anomalies were identified in the pollen grains of Scots pine. The number of anomalous pollen grains (58.8) from the mountainous area of the BGNNP is higher than from the plain (lowland) (54.2) in the same area. This is confirmed by the fact that for conifers growing in unfavorable environmental conditions, such as atmospheric pollution, dryness, highlands, swamping of the territory, etc., an increase in pollen anomalies is observed (Benca et al. 2018). The results obtained from the territory of the Bayanaul State Nature Park showed that pollen collected from flat terrain has a higher number of fertile grains 45.8%, compared to other studied areas (Astana, Kazakhstan and Poznan, Poland). The studied samples from Astana have 55% of pollen anomalies, which is due to the high density of tree plantations, which act as a buffer for removing the technogenic load, being a phytoremediant. The maximum number of violations in pine pollen is contained in samples from Poznan – 63%. Perhaps the explanation for the high indicators is that Poznan is located near Krakow, which ranks 2nd in the world in terms of air pollution. In general, Poland has long had the status of a country with polluted air in Europe, especially high levels of smog during the heating season (Hofman, Wachowski 2010).

The processes of formation and development of pollen grains are extremely sensitive to the influence of chemical and physical factors. It has been revealed that with an increase in anthropogenic load, as well as with the intensity of car traffic, the amount of abortive and teratomorphic pollen increases (Hlebova, Ereshhenko 2012; Pausheva 1988; Tret'jakova, Noskova 2004; Tret'jakova et al. 1996; Shengnan et al. 2022).

Conclusion

Under conditions of technogenic pollution, coniferous trees (*Pinus sylvestris* L.) exhibit a higher level and a wider range of abnormalities of pollen grains (sterile, deformed and small pollen grains with disturbances (without air sacs, with one and three air sacs) than in relatively clean conditions (Table 2). In all studied points, a large amount of pollen with deformations and lens-shaped abnormalities, as well as with damage to the shells, was found less often in pine. The following types of anomalies were most often found in the pollen of Scots pine: deformed (26.3%), pollen with impaired shell formation (19%) and degraded (7.4%). The differences in the percentage of pollen anomalies between the studied samples are reliable and close to reliable (at $p = 0.05$).

Table 2. Transformation of pollen in Scots pine (*Pinus sylvestris* L.) in the conditions of BSNNP, Astana and Poznan

Collection point	Total pollen grains	Pollen grains with anomalies, number (%)							
		p.g. with 1 air bag	p.g. with 3 air bags	p.g. with 4 air bags	Collar p.g.	Deformed, lens-shaped p.g.	Violation of the formation of membranes, fused, hypertrophied p.g.	Degrading p.g.	Total number of anomalous p.g.
BSNNP, Kazakhstan	2278	105 (4.6)	17 (0.7)	7 (0.3)	47 (2.1)	444 (19.5)	498 (21.9)	171 (7.5)	1289 (56.6%)
Astana, Kazakhstan	2005	94 (4.7)	18 (0.9)	7 (0.3)	52 (2.6)	479 (23.9)	289 (14.4)	155 (7.7)	1094 (54.6%)
Poznan, Poland	3084	51 (1.7)	10 (0.3)	6 (0.2)	34 (1.1)	1013 (32.8)	619 (20.1)	222 (7.2)	1955 (63.4%)

Acknowledgement

The authors express their gratitude to the students of the Higher School of Natural Sciences of Pavlodar Pedagogical University named after Alkey Margulan – D.Zh. Bazarbaeva and A.Sh. Gubaidullina for the desk work carried out and E.R. Soldatova for sampling from Poznan (Poland).

Funding information. The article was prepared within the framework of a grant project funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, IRN AP19677807 "Assessment of the current state and dynamics of plant communities of Bayanaul National Park under the influence of climatic and anthropogenic factors".

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