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Pollen from Surface Samples as an Environmental Indicator

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Abstract—Palynological analysis of surface samples was accomplished within the framework of integrated ecological investigation of northwestern Timan–Pechora oil-and-gas-bearing province (sampling depth, 0.015 m). Palynological results corroborated geochemical conclusions and allow the author to suggest that this area is gametopathogenically hazardous. This hypothesis is supported by the peculiarities of teratomorphic pollen grains found in the surface samples.

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INTRODUCTION

Studies of the palynological spectra of surface samples are conventionally used to refine and facilitate paleogeographical and paleoecological reconstructions made on a palynological basis. Each vegetation zone is correlated to a particular type of spectrum, in which the ratio of components ranges within certain limits, and each component is related to vegetation of a definite character.

This study was accomplished within the framework of an integrated investigation in the Nenets Autonomous Region in connection with the evaluation of the negative impact of aggressive formation fluid on downhole equipment and environment and the evaluation of resistance of the environment to possible gas intake from deep levels of gas fields of the area studied (Dzyuba et al., 2005).

Currently, the area under study belongs to the Nenetskii State Nature Reserve (Kumzhinskoe Field) and the Nenetskii State Zoological Refuge (Korovinskoe Field).

The climate in this area is rather severe, with excessive humidity, which is characteristic of polar areas and is controlled by low solar radiation during the summer, by the proximity to the Arctic Ocean, intense western transportation of air masses, and cyclones from the Atlantic Ocean. The area under study falls in the spodosol zone of northern and southern tundras. The landscapes are of the subarctic type, with the typical and southern tundras. The vegetation cover mostly comprises mosses, lichens, herbs, subshrubs, and shrubs. Perennial plants dominate, including widespread evergreen members of the family Ericaceae, which are abundant.

When determining the ecological state of any natural or natural–technogenic system, an integrate evaluation of their stability is necessary, including the evaluation of biological component. The formation of pollen grains is known to be very sensitive to various chemical and physical factors. In general, the response of the generative sphere of plants (in particular, pollen grains) and animals (man included) is adequate; the response of plants is much more rapid than that of animals. Unfortunately, our visit did not coincide with mass flowering of plants in the region, so we decided to study pollen and spores from surface soil samples.

MATERIAL AND METHODS

For palynological analysis, surface soil samples were collected in the region under study as well as surface soil samples and peat from a prospect hole (depth 0.20 m deep) from relatively distant areas. The samples were processed according to modified Post's method for peats and sapropels, with sodium pyrophosphate and hydrofluoric acid (Dzyuba, 1984).

Pollen grains were screened in temporary slides (in glycerol) to be able to move each specimen, study it in detail, and take photographs in different positions. The study and microphotography were accomplished with the aid of an LM Leica DMLS camera, using the *Video–Test–Structura–Master* image-analyzing system.

Pollen grains, where possible, were determined to the family, genus, or, ideally, species. Particular attention was paid to teratomorphic (with pathologies) pollen grains, since the presence or absence of such pollen indicates the environmental conditions at the time of the plants' reproduction. In each sample, we studied the upper layer of the sediment no more than 1.5 cm thick; this allowed us to obtain the characteristics of the environment during the last approximately 30–50 years, based on palynological analysis, taking into account the sedimentation rates in the region under study.

A reference collection of modern pollen was used for accurate identification of some taxa. Pollen grains in the spectra were counted by the group method, a conventional method for Quaternary palynological samples (*Pollen Analysis*, 1950; Karevskaya, 1999). In addition, the presence or absence of teratomorphs in each sample were registered, their numbers were counted, and their morphology analyzed.

Pollen grains were usually counted in six cover glasses; however, in some cases, pollen grains were counted in 10 to 12 slides instead of 1 or 2 (as is more usual in paleopalynology), because of the low concentration of pollen grains and spores in the samples.

In total, the palynological spectra of 24 samples were studied.

RESULTS AND DISCUSSION

All samples from the Korovinskoe Field, including samples from supposedly clean background areas, considerably exceed the maximum allowable concentrations (MAC) of ions of zinc, lead, copper, and arsenic. No obvious regularities of their distribution were found.

All samples from the Kumzhinskoe Field also considerably exceed the MAC of ions of zinc, lead, copper, and arsenic. The maximum concentrations of ions of heavy metals, arsenic (21 mg per kg), and sulfur were discovered in borehole 8. Samples from clean background areas are an exception.

THE CHARACTERISTICS OF STUDIED PALYNOLOGICAL SPECTRA IN RELATION TO THE QUALITY OF THE ENVIRONMENT

1. Korovinskoe Field

Sample B1 (Background 1 for the boreholes of the Korovinskoe Field, situated 5 km west of the field).

The palynological spectrum of the sample is dominated by herbs and subshrubs (51.1%), represented nearly exclusively by pollen grains of the Ericaceae. Normally (Pl. 3, figs. 1, 2), this family is characterized by tetrahedral tetrads of pollen grains rather than by monads, i.e., single pollen grains (Kupriyanova and Aleshinskaya, 1972). Within this taxon, we revealed 7.5% of pollen grains (tetrads) showing teratomorphic (atypical) morphology. The tetrads are asymmetrical, the pollen walls are thinned and folded, additional tetrad scars are distinctly visible on the tetrad surface. Until now, we only recorded numerous specimens with such an abnormality (tetrad scars on the surface of pollen grains) in gymnosperm plants, growing mostly in sites with high radiation (Chernobyl zone).

Trees and shrubs (38.4%) are represented by pollen grains of birch, spruce, and pine. It is most probable that the majority of these pollen grains are strangers, i.e., they come from outside the study area. Nonetheless, 99.9% of them were found to have morphological anomalies, i.e., virtually all were teratomorphic.

In gymnosperms (pine and spruce), the pathology is expressed in the cappa and in underdeveloped or distorted sacci. In pollen grains of birch, which we diagnose only as *Betula* sp. not subdividing into arboreal and shrub groups, the following pathologies are found: single-layered pollen wall, thinned layers of the pollen wall, distorted mesocolpia, thickened pollen walls, and the presence of tetrad scars on apocolpium. Many pollen grains retain their inner content (partially or completely) after sufficiently long treatment, which is characteristic of pollen grains produced by plants growing under conditions of high background radiation.

Background samples B2 (0–1.5 cm)–B2 (19.0–20.0 cm) for boreholes of the Korovinskoe Field (situated 10 km west of the field). Samples of peat were collected from a 0.20-m-deep prospect hole in a small peat bog. Samples were collected every 1.5 cm of sediments downward in the section. In total, seven samples were collected and studied.

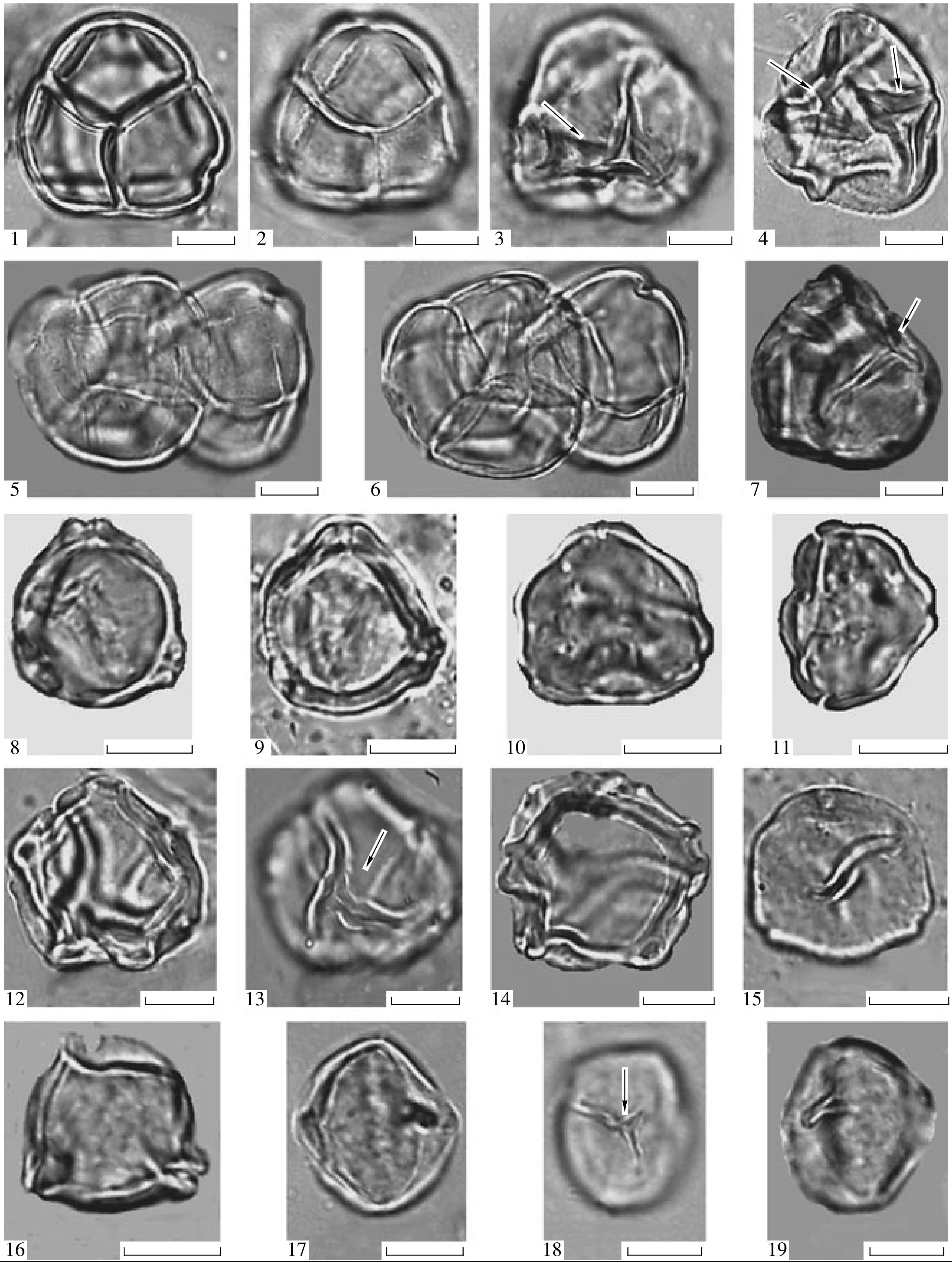
Sample B2 (0–1.5 cm). The palynological spectrum of this sample is dominated by pollen grains of trees and shrubs (57.6%), mostly by birch, pine, and spruce. The teratomorphosis in the pollen grains of arboreal plants is 3.1% (of the total of arboreal pollen grains) and is observed in pollen grains of *Betula* sp. The majority of these pollen grains are probably strangers.

The group of herbs and subshrubs (31.8% of the total) is mostly represented by the Ericaceae. Among these pollen grains/tetrad, 53.6% are teratomorphic. The tetrads are asymmetrical, the walls of their members are thinned and folded. The most typical new formations are tetrad marks in the shape of bordered triradiate scars on the surface of different members of the same tetrad. One to three such new formations may be present on the surface of different tetrads (Pl. 3, figs. 3, 4, 7). Such pathology (tetrad marks) may be caused by the exposition to high radiation of the plants that produced such pollen grains (tetrads). In addition, some tetrads of the Ericaceae were united into polyads of two tetrads (Pl. 3, figs. 5, 6).

It is pertinent to note that pollen of the Ericaceae usually does not spread far, but is accumulated locally, virtually in the same site where it was produced. Consequently, the quality of this pollen (its morphological features) reflects the state of the environment in the area from which it was sampled. On the other hand, pollen grains of arboreal plants are transported for much longer distances; therefore, the quality of this pollen extracted from surface samples usually characterizes the environmental state of a larger area (at the time of reproduction of this pollen); in our case, these areas are most probably rather distant from the region studied.

We also found teratomorphic pollen grains of other angiosperm taxa (Magnoliopsida fam., gen. indet.). The degree of teratomorphosis prevents the determination of these specimens due to the strong alterations of morphological characters (Pl. 3, figs. 16–19).

Plate 3



In total, the pathology of pollen grains of arboreal plants in this sample is 3.1%, that of herbs and shrubs is 51.4% (of the total for this group).

Samples B2 (1.5–3.0 cm), B2 (3.0–4.5 cm), B2 (4.5–6.0 cm), B2 (6.0–7.5 cm), B2 (7.5–9.0 cm), and B2 (19.0–20.0 cm) were collected at lower levels than the above-described sample. We did not find teratomorphic pollen grains in these spectra.

Sample 64 (mouth of borehole 64, Korovinskoe Field). The palynological spectrum is very poor: the screening of 12 cover glasses revealed only 75 pollen grains and spores, among which green mosses prevail (55 specimens), 14 pollen grains of birch, 1 of pine, and 5 of sedges. One reworked pollen grain of *Tsuga* was found. No teratomorphic pollen grains and spores were found.

Sample 66m (mouth of borehole 66). The spectrum of this sample is also very poor and includes mostly spores of green mosses. *Betula* dominates among arboreal plants. The pollen grains of this taxon vary in equatorial diameter, which is also a pathology constituting 62.5% in the group of arboreal plants. We found ten pollen grains of herbs and subshrubs, including three teratomorphic grains of *Artemisia* sp. and one more member of the Asteraceae, determined as cf. *Achillea* sp.

Sample 63 (50 m west of the mouth of borehole 63) is virtually empty: we found only 25 pollen grains and spores in 12 cover glasses. No teratomorphic pollen grains were observed, and one teratomorphic spore of *Lycopodium* sp. with a proximal scar of irregular morphology was revealed.

Sample 76 (mouth of borehole 76). A total of 80 pollen grains and spores were counted in 12 cover classes. Arboreal plants mostly include pollen of *Betula*, 100% of which show one or another example of pathology. Most often, the thickness of the exine, the number of its layers, and the equatorial diameter vary within a wide range. In general, 88.2% of arboreal pollen grains are pathological. Teratomorphic tricolporate pollen grains were registered in the group of herbs and subshrubs, which are impossible to identify more precisely. In general, there are 88.2% pathological specimens among the pollen grains of this group. In spores, we found three specimens of *Lycopodium* with morphological deviations: damaged or lacking tetrad scars.

Sample 46N (10 m north of the mouth of borehole 46) is characterized by a slight prevalence of herbaceous pollen, mostly Poaceae and Cyperaceae. About 50% of cereal pollen grains show pathologies in pollen wall morphology. In addition, one more teratomorphic specimen was determined as *Achillea* sp. Arboreal plants are represented by pollen grains of birch, which significantly vary in the thickness of the pollen wall and in the equatorial diameter. Among spore-bearing plants, green mosses prevail. Two spores of *Lycopodium* have atypical tetrad scars.

Sample 46S (10 m south of the mouth of borehole 46). Pollen grains of herbaceous plants, mostly the Poaceae and Cyperaceae, dominate in this spectrum. Two tetrads of the Ericaceae were found, both with pathologies (additional tetrad scars on the members of the tetrads) and three teratomorphic pollen grains of cf. *Achillea* sp. In total, 8.7% pathological pollen grains were found. In the group of arboreal plants, pollen grains of *Betula* prevail, 41.8% of which show pathologies. *Sphagnum* dominates among spore-bearing plants; one teratomorphic spore of *Lycopodium* sp. was found.

Sample 46/50 (50 m south of the mouth of borehole 46). The spectrum of this sample is dominated by pollen grains of herbaceous plants, mostly cereals, 55.2% of which show distinct pathology, in particular, three biaperturate pollen grains and three pollen grains with a tetrad scar. Three tetrads of the Ericaceae with a single-layered, thin, and strongly folded exine, and one pollen grain of the Rosaceae with a crooked mesocolpium were found. In total, 58% of pollen grains with pathologies were found in the herbaceous group. *Betula*

Explanation of Plate 3

Figs. 1 and 2. A normally developed tetrad of pollen grains of Ericaceae.

Figs. 3, 4, and 7. Teratomorphic tetrads of pollen grains of Ericaceae, arrows show distinct tetrad scars on the sporoderm.

Figs. 5 and 6. A polyad containing two tetrads of pollen grains of Ericaceae gen. indet. at different depths of field.

Fig. 8. A teratomorphic pollen grain of *Betula* sp. A tetrad mark is seen (arrow), resembling a coarsely bordered tetrad scar of spore-bearing plants.

Fig. 9. An asymmetrical pollen grain of *Betula* sp., with a strongly thickened sporoderm. Inner content retained after treatment is distinct within the pollen grain.

Figs. 10 and 11. An asymmetrical pollen grain of Betulaceae gen. indet., with wavy deformed mesocolpia, in different positions.

Figs. 12–14. A teratomorphic dyad of pollen grains of *Alnus* sp., at different depths of field. The poles of the pollen grains in the dyad are directed towards each other, the pollen grains are situated one after the other. A tetrad mark resembling a bordered tetrad scar is distinct in one member of the dyad.

Fig. 15. A folded pollen grain of Betulaceae gen. indet. with a very thin, single-layered pollen wall.

Fig. 16. A teratomorphic angiosperm pollen grain (Magnoliopsida fam., gen. indet.), polar view.

Figs. 17 and 19. The same pollen grain (Fig. 16) in different position, equatorial view. A papilla-like process is developed on the mesocolpium of this pollen grain.

Fig. 18. The same pollen grain (Figs. 16, 17, 19), equatorial view. A tetrad scar is clearly seen on the mesocolpium.

Scale bar, 10 μm. Figs. 1–7, 10, 11: sample B3, sampling depth 0–0.015 m. Figs. 8, 9, 12–15: sample 2m, sampling depth 0–0.015 m. Figs. 16–19: sample B2, sampling depth 0–0.015 m.

prevails among arboreal plants, while *Sphagnum* prevails among spore-bearing plants. No pathologies were discovered in the two groups.

2. Kumzhinskoe Field

Sample B3 (background sample for the boreholes) is virtually empty; we found only 12 pollen grains and spores. Nonetheless, four of the pollen grains show pathologies. In particular, all pollen grains of *Betula* have thin single-layered pollen walls and distorted mesocolpia (Pl. 3, figs. 10, 11); the only pollen grain of *Alnus* is strongly folded, has six irregularly formed apertures, and a single-layered pollen wall. No teratomorphic spores were found.

Sample 3 (50 m northwest of borehole 3) shows a very poor palynological spectrum, mostly represented by spores of green mosses. In the arboreal group, we found seven pollen grains of *Betula,* one of which is clearly pathological, has a single-layered exine. There are eight pollen grains and tetrads in the herbaceous group, three tetrads of the Ericaceae including. These tetrads are strongly folded due to thin single-layered pollen walls.

Sample 1 (mouth of borehole 1). The spectrum of this sample is dominated by spores of green mosses (83.5%), the proportions of pollen grains of arboreal and herbaceous plants are 11.4 and 6.1%, respectively. In the arboreal group, 80.4% of pollen grains have pathologies: a pine pollen grain with reduced sacci and virtually all pollen grains of birch that were found (their exine is atypically thin and single-layered). Pollen grains of cereals prevail in the group of herbs and subshrubs. All these pollen grains have thin exines that are strongly folded, but there are no grounds in this case to describe them as teratomorphic forms.

Sample 2m (mouth of borehole 2). The spectrum resembles the previous one and includes mostly spores of green mosses (80.9%). There are 9.7% pollen grains of arboreal plants and 10.4% of herbs. *Betula* sp. prevails among arboreal plants. All its pollen grains are very small and include both pollen grains with a strongly thickened exine (Pl. 3, fig. 9) and with a thin single-layered exine. Pollen grains of birch with wavy deformations of the mesocolpium (Pl. 1, figs. 10, 11) or tetrad scars (Pl. 1, fig. 8) as well as dyads of such pollen grains were found. Several pollen grains were found that have a thin single-layered exine and are determinable only as Betulaceae gen. indet. (Pl. 3, fig. 15). Pollen grains of *Alnus* were observed having one layer of the exine, six apertures, and many folds as well as teratomorphic dyads with tetrad scars on their members (Pl. 3, figs. 12–14). The pathology is 58.6% in this group. The group of herbs and subshrubs is dominated by pollen grains of cereals; no unequivocal cases of pathology were found.

Sample 8 (mouth of borehole 8). The spectrum of this sample predominantly includes spores of green mosses, which constitute 94%. 11 specimens are pollen grains of arboreal plants (4.4% of the total), mostly birch. The exine of these pollen grains varies widely in thickness even within the same mesocolpium; some pollen grains have a single-layered exine, and others have a bilayered exine that is two or three times as thick as the normal one. Four specimens belong to the group of herbs and subshrubs (1.6% of the total): the Cyperaceae, Polygonaceae, Rosaceae, and the Ericaceae. Two of them are pathological: the pollen grain of the Rosaceae gen. indet. has a thin single-layered exine, and the tetrad of Ericaceae gen. indet. is formed by pollen grains with single-layered, very thin, and strongly folded walls.

A reworked Neogene spore of Osmundaceae gen. indet. and a pollen grain of cf. *Tsuga* sp. were found. This sample contains numerous microparticles of burnt organic matter.

Sample 14 (mouth of borehole 14). The palynological spectrum includes 133 palynomorphs, 113 of which (85%) are spores of green mosses. Pollen grains of trees and shrubs constitute 5.2% of the total, and the proportion of herbs and subshrubs is 9.8%. The arboreal group includes exclusively teratomorphic pollen grains of birch (pathology reaches 100%). Pollen grains of herbs and subshrubs also include teratomorphs, a pollen grain of the Chenopodiaceae and a tetrad of the Ericaceae. Members of the tetrad have a thin single-layered exine and an additional tetrad scar on the surface of one of its members.

Sample 15 (mouth of borehole 15) is nearly empty; only 19 palynomorphs were recorded in 12 cover glasses. There were no normal specimens among the pollen grains, five of which belong to arboreal plants and seven to herbs. We did not find teratomorphs among the spores.

Sample 19 (mouth of borehole 19). The spectrum is dominated by pollen grains of herbaceous plants (42.6%), 20% of which are teratomorphic. These are mostly pollen grains of cf. *Achillea* sp. and *Artemisia* sp. We did not find clear pathologies in pollen grains produced by arboreal plants (24.4%), but dominating pollen grains of birch are polymorphic (varying parameters are mostly equatorial diameter and polar axes).

CONCLUSIONS

The samples analyzed in terms of palynoindication of the environmental quality and described above have been studied to provide detailed characteristics of the state of the environment in the region under study using available biological objects.

It was shown as early as the 1990s that palynomorphological studies could provide information about the presence of gametopathogens in the environment as well as about the degree of pollution by such compounds in different regions and particular zones within them (Dzyuba, 1993, 1999; Meyer-Melikian, 1993; Dzyuba et al., 2001), as pollen development is very sensitive to the influence of various environmental factors.

This was supported using modern material, i.e., pollen grains extracted from the anthers of living plants. However, surface samples contain pollen grains that are already to various extents fossilized. In the present case, the palynomorphs were accumulated over approximately the last three to five decades.

To leave no doubt of the validity of palynoindication of the environmental state by the study of surface samples, we collected peat samples from a 0.20-m-deep prospect hole in a small peat bog, 10 km west of the Korovinskoe Field. The samples were collected every 1.5 cm upwards in the section. We revealed that teratomorphic pollen grains are only present in the upper layer of the sediments (1.5 cm thick), i.e., in the surface sample. We failed to find pathological palynomorphs downwards in the section; this is evidence that environmental aggression against living organisms has increased during the last three to five decades and has probably resulted in gametopatogenic risk in the area under study and adjacent regions. The study of surface samples from other regions confirms this supposition. Moreover, it was revealed that most of the pollen grains of the samples studied partially or completely retain their inner content after a long treatment. Our previous experience shows that this is characteristic of pollen grains produced in highly polluted sites and, especially, with high level of radiation.

It is essential that pollen grains of herbs and subshrubs are not transported far, but accumulated in the sites where they are produced, so that they reflect the environmental conditions of the area where the surface sample was collected. Pollen grains of arboreal plants, especially pine and birch, are spread by air currents for a much longer distance. Therefore, the quality of pollen grains of *Pinus* and *Betula* extracted from surface samples characterizes the contemporary environmental state not only of the sampling area, but also that of much more distant territories. If surface samples are collected in woodless areas and contain pollen of arboreal plants, these pollen grains are, as a rule, strangers from other, sometimes very distant areas. Our results have shown that both the areas studied and adjacent territories are at serious ecological risk.

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