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EXPERIMENTAL ARTICLES

Mycological Characterization of the Occupation Deposits in Excavated Medieval Russian Settlements

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Abstract—Microbiological analysis showed that the cultural layers of soils in excavated medieval Russian settlements differ from the surrounding soils in that the cultural layers have more fungal spores, their morphology is more diverse, the species diversity of microscopic fungi is higher, and the *Penicillium* species that are able to grow at an elevated temperature (37°C) are more frequent.

Key words: cultural layers, microscopic fungi, diversity, biomass.

Recent studies have shown that current anthropogenic impact can considerably alter soil microbiota [1–4]. At the same time, little is known about the effect of ancient anthropogenic factors on the microbiological, particularly mycological, characteristics of soils [5].

The aim of the present work was to study the mycological characteristics of the cultural layers of soils in excavated medieval Russian settlements and to evaluate the possibility of using such data for the bioindication of cultural layers in the soils subject to archeological excavation.

MATERIALS AND METHODS

Soils samples were collected during the years 1998–1999 in the territory of the Gnezdovo archeological complex (Novoselki and Gnezdovo settlements, IX–XI centuries) and in the surrounding areas. Soddy podzolic soil around Gnezdovo was sampled from horizons AO (forest litter from depths of 0–4 cm); A1A2 (organogenic horizon from depths of 4–18 cm); and B1f and B2f (illuvial ferruginous horizons from depths of 18–32 and 32–78 cm, respectively). Soddy podzolic soil around Novoselki was sampled from horizons A1A2 (organogenic horizon from depths of 0–20 cm); A2 (podzolic horizon from depths of 20–39 cm); and B (illuvial horizon from depths of 39–78 cm). Soil with cultural layers in the Gnezdovo settlement was sampled from horizons Ad (turf from depths of 0–4 cm); A_{til} (tilled horizon from depths of 4–32 cm); CL1, CL2, and CL3 (cultural layers from depths of 32–46, 46–77, and 77–80 cm, respectively); and B (illuvial horizon from a depth of 80–82 cm). Soil with cultural layers in the Novoselki settlement was sampled from horizons Ad (turf from depths of 0–7 cm) and CL1, CL2, and CL3 (cultural layers from depths of 7–20, 20–35, and 35–70 cm, respectively). The cultural layers were dated archeologically and by the carbon-14 dating technique.

The morphological, micromorphological, and chemical properties of soils with cultural layers considerably differed from those of the surrounding soils in that (1) the cultural layers 1.5 m in total thickness were rich in humus; (2) the content of organic matter in the cultural layers was comparable with that in the organogenic horizons of the surrounding soils (1–2.5%); and (3) the cultural layers contained greater amounts of bases and heavy metals (such as Cu, Zn, As, and Pb) than the surrounding soils. According to the observations of Zazovskaya *et al.* [6], these features of cultural layers are due to anthropogenic impact rather than to other factors.

The composition of fungal complexes was studied by plating soil suspension dilutions onto solid media or by the direct count technique. In preliminary experiments, soil samples were plated onto two media, Czapek agar and Mycosel agar. These experiments showed that the typical fungal species detected on these two media were the same; however, the fungal diversity evaluated with the use of Mycosel agar was poorer. For this reason, further studies were carried out using only Czapek agar. The species diversity of soil fungal complexes was evaluated in terms of the relative occurrence rate, the abundance of particular fungal species (both were expressed as a percentage of the total values), and the Shannon diversity index [7]. The level of similarity between the cultural layers and different horizons of the surrounding soils was estimated by the cluster analysis technique using STATISTICA software. Discriminatory analysis was carried out using the SPSS 9.0 software for Windows. The growth temperature ranges of fungi of the genus *Penicillium* were determined by growing them at 5, 25, and 37°C [8].

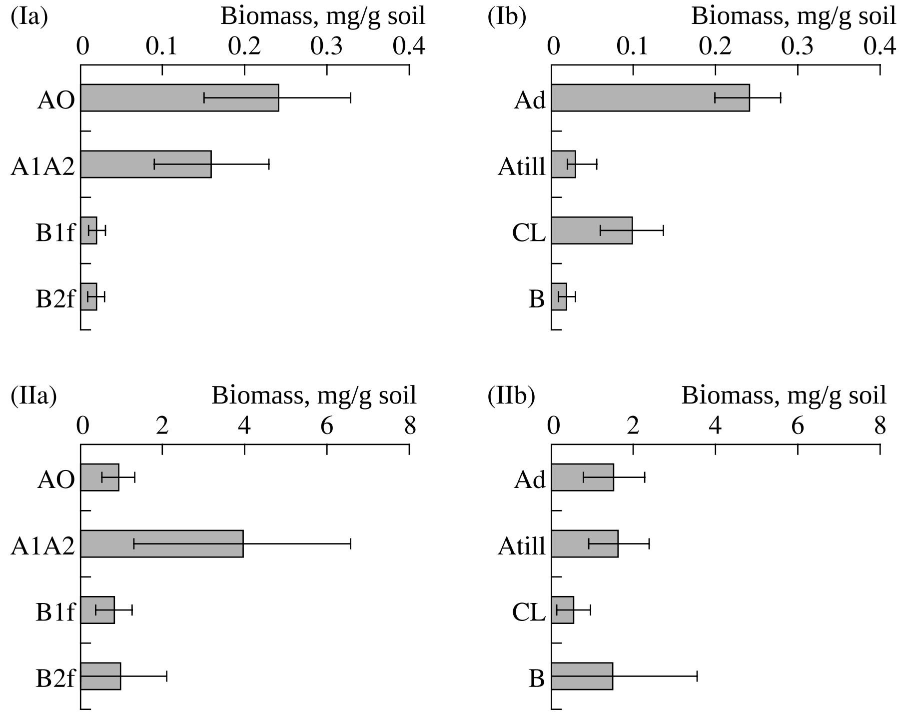

Fig. 1. The biomass of fungal (I) spores and (II) mycelium in (a) zonal soddy podzolic soil and (b) soil of the excavated medieval Russian settlement Gnezdovo.

The mass of mycelium and spores, as well as the morphological diversity of the latter, were estimated by luminescence microscopy with calcofluor [9].

RESULTS AND DISCUSSION

The content of fungal spores in the soils surrounding Gnezdovo declined in a direction from the upper (AO and A1A2) to the lower (B1f and B2f) soil horizons (Fig. 1, Ia). In the Gnezdovo soil, the content of fungal spores peaked in the uppermost Ad horizon and in the cultural layer (Fig. 1, Ib).

In the surrounding soil, the mycelium content was maximum in the subsurface A1A2 soil horizon (Fig. 1, IIa). In the Gnezdovo soil, the content of fungal mycelium showed a decline in the cultural layer (Fig. 1, IIb). Therefore, the cultural layers contained more fungal spores and less mycelium than the surrounding soil horizons from the same depth.

The morphological diversity of fungal spores in the surrounding soil was maximum in the upper AO and A1A2 horizons, which contained spores of five morphotypes, and minimum in the lower horizons, which were dominated by small oval spores. The morphological diversity of spores in the Gnezdovo soil exhibited two peaks, one in the upper Ad horizon and the other in the cultural layer. Spores in the cultural layer had specific features typical of fungi from the genera *Fusarium* and *Chrysosporium*.

In the surrounding soil, the species diversity of microfungal complexes, like the morphological diversity of fungal spores, was maximum in the upper horizons (Fig. 2, Ia and IIa), which is typical of zonal soddy podzolic soils [10]. In the Gnezdovo and Novoselki soils, the fungal diversity of the upper horizons was also high, but lower than that of the cultural layers (Fig. 2, Ib and IIb). The high fungal diversity of the cultural layers may be related to their neutral pH values, which are beneficial to fungi [11].

The generic composition of fungal complexes in the surrounding soil was typical of zonal soddy podzolic soils, which are dominated by the genera *Penicillium*, *Acremonium*, *Paecilomyces*, *Cladosporium*, and some others (these genera were also detected in the cultural soil layers of the medieval settlements). The fungal species *Mortierella ramanniana* (Moller) Lin., *Trichocladium asperum* Harz, *Aspergillus parvulus* Smith, and *A. versicolor* (Vuill.) Tiraboschi were detected in the surrounding soils but not in the settlement soils.

On the other hand, the settlement soils contained some fungal species—*Aspergillus fumigatis* Fres., *A. flavus* Link ex Gray, *A. aureolatus* Munt-Cvet & Bata, *Periconia minutissima* Corda, *Verticillium catenulatum* (Kamyschko ex Barron & Onions) W. Gams, *Tilachlidium brachiatum* (Batsch per Fr.) Petch, and some *Trichosporiella* species—that were not detected in the surrounding soils. The species *Penicillium janczewskii* Zaleski, *P. janthinellum* Biourge, and

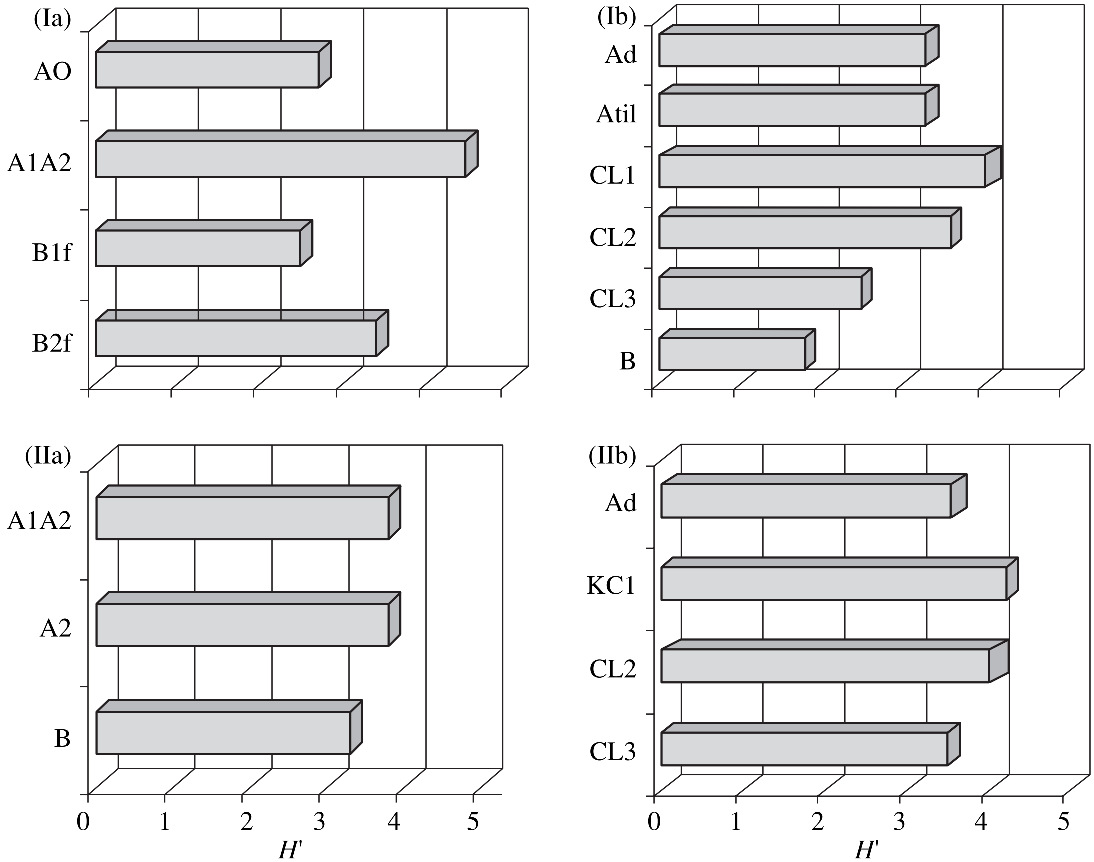

Fig. 2. The Shannon diversity index *H*' of microscopic fungi in various horizons of (a) zonal soddy podzolic soil and (b) soils of the excavated medieval settlements (I) Gnezdovo and (II) Novoselki.

Paecilomyces lilacinus (Thom) Samson exhibited higher occurrence rates and abundances in the settlement soils than in the surrounding soils.

It should be noted that the occupation deposit with the remains of wooden constructions was characterized by higher occurrence rates and abundances of the species *P. lilacinus, Penicillium aurantiogriseum* Dierckx, *P. decumbens* Thom, *P. funiculosum* Thom, and *P. corylophilum* Dierckx, whereas the cultural layer with the remains of household pits exhibited higher occurrence rates and abundances of the species *Aspergillus niger, Mucor hiemalis* Wehmer, *P. lilacinus, Penicillium aurantiogriseum, P. chrysogenum* Thom, *P. funiculosum*, and *P. purpurogenum* Stoll.

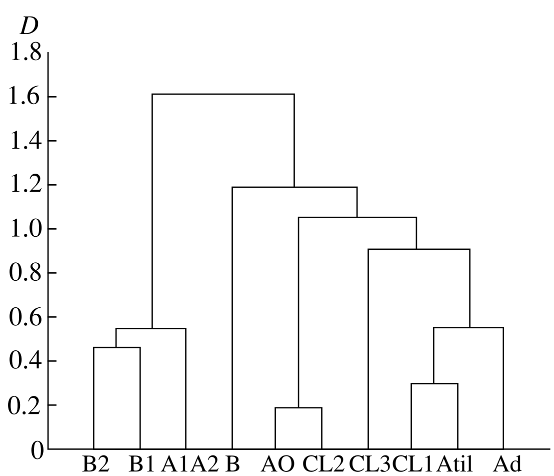

Fig. 3. Dendrogram illustrating the similarity of microfungal complexes in zonal soils (AO, A1A2, B1f, and B2f horizons) and the soil (Ad, Atil, CL1, CL2, CL3, and B horizons) of the medieval settlement Gnezdovo.

In general, the fungal diversity of the cultural layers was higher than that of the surrounding soils. Some fungal species, such as *A. fumigatis, A. niger, A. flavus, P. purpurogenum*, and *V. catenulatum*, that were revealed in these layers are also typical of modern urbanozems [2]. Cluster analysis confirmed that the mycobiota of the organogenic and mineral horizons of the surrounding soils differs from that of the medieval settlement soils and that the fungal complexes differ in various cultural horizons (Fig. 3).

Fungi of the genus *Penicillium* in the settlement soils were dominated by the species that are able to grow at an elevated temperature of 37°C (Fig. 4). At the same time, the *Penicillium* fungi of the surrounding soils were dominated by the species that have an optimal growth temperature of 25°C. Earlier, the same ten-

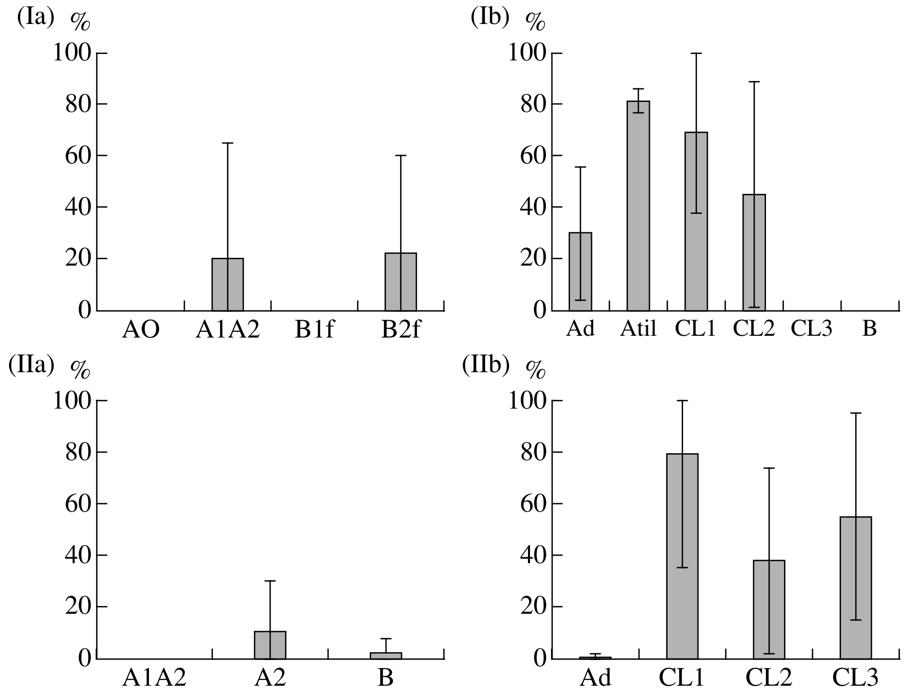

Fig. 4. Relative abundance (expressed as a percentage) of *Penicillium* fungi with an optimum growth temperature of 37°C in various horizons of (a) zonal soil and (b) soils of the medieval settlements (I) Gnezdovo and (II) Novoselki.

dency, i.e., the prevalence of the *Penicillium* species capable of growing at 37°C, was reported for urbanozems [2]. The prevalence of more thermotolerant fungi in the urbanozems may be related to the fact that this environment is characterized by higher annual temperatures than neighboring rural soils [12].

It is known that opportunistic fungal species are more frequent in urban than in rural environments [13]. Our studies, however, did not reveal any difference in the occurrence frequency of opportunistic fungal species in the medieval settlement and surrounding soils.

Thus, the cultural layers of soils in the medieval Russian settlements differ from the surrounding soils in that the occupation deposits have more fungal spores, their morphology is more diverse, the species diversity of microscopic fungi is higher, and the *Penicillium* species that are able to grow at an elevated temperature (37°C) are more frequent.

The mycological characteristics of the occupation deposits in the excavated medieval settlements were found to be similar to those of urbanozems [14]. The data presented show that mycological characteristics can be used for the bioindication of cultural layers in the soils subject to archeological excavation.

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