EXPERIMENTAL ARTICLES =

The Distribution of Microscopic Fungi along Moscow Roads

A. B. Kul'ko and O. E. Marfenina

Department of Soil Biology, Faculty of Soil Science, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia Received November 21, 2000

Abstract—The distribution of microscopic fungi in soils and surface air along some Moscow roads was studied from 1994 through 2000 at several distances (0, 5, 10, and 20 m) from the roads. Soil fungal complexes in roadside zones with different levels of pollution from automobile engines were found to differ in their composition, species structure, and biomorphology. Relatively far from the roads, the content of fungal mycelium diminished, while that of fungal spores increased. Close to the roads, fungal complexes in soil and surface air were dominated by dark melanin-containing fungi. The microscopic fungal complexes of soil and surface air along the roadsides were similar but differed in the relative abundance of some species.

Key words: microscopic fungi, soil, air, pollution from automobile engines.

Soils and surface air along urban roads are usually polluted by different particulate and gaseous toxicants, such as exhaust fumes (primarily, carbon monoxide, nitrogen and sulfur oxides, and unsaturated hydrocarbons), heavy metals, oils, dust, and soot [1–3]. The use of deicing agents considerably contaminates soils along roadsides with easily soluble salts (sodium and potassium chlorides), thereby increasing the pH and salinity and impairing the moisture conductivity and aeration of these soils [4, 5].

In Moscow metropolis, more than 70% of air pollution is produced by automobile engines. Urban areas along the roadsides suffer the most from automobileproduced pollution [6, 7]. There is some information in the literature on plant and animal communities in these areas [1, 6]; however, little is known about the microbial complexes of these specific biotopes.

The aim of the present work was to study the effect of pollution from automobile engines on microscopic fungal complexes in soils and surface air along several Moscow roads.

MATERIALS AND METHODS

The object for study was microscopic fungal complexes present in soils and surface air along three Moscow roads, Frunzenskaya naberezhnaya, Leninskii prospekt, and Gogolevskii bul'var. Soils along these roads represent urbanozems of different thicknesses [7]. Soil and air samples were taken at several distances from the roads (0, 5, 10, and 20 m). Control air and soil samples were taken at a distance of 200 m from a road located in an urban forest park on Vorob'evy gory. The soddy podzolic soil of this park was only slightly polluted. Soil samples were taken in 5–10 replicates from the upper soil horizon in spring, summer, and autumn from 1994 through 2000. To analyze airborne fungi, air samples were taken at the soil surface and at a height of 1.5 m above the soil. The samples were analyzed by plating them on Czapek or nutritionally deficient agar. Microscopic fungal complexes were characterized using ecological indices, such as the occurrence rate and relative species abundance [8, 9].

The specific mycelium length and the number of fungal spores in the urban soils were evaluated by luminescence microscopy as of September and October 1997. Samples for this analysis were taken at distances of 0 and 10 m from the roads and stained with calcofluor [10, 11].

The results were statistically processed using Excel 5.0, Statgraphics, and Statistica software.

RESULTS AND DISCUSSION

Investigations showed that the specific mycelium length in the forest park (248.1 \pm 31.8 m/g soil) noticeably exceeded that in the urbanozems along the road-sides (77.9 \pm 17.2 to 105.3 \pm 21.4 m/g soil at a distance of 10 m and 12.3 \pm 3.1 to 25.2 \pm 5.3 m/g soil at a distance of zero from the roadsides).

Conversely, the amount of fungal spores was greater near the roads, making up 4.5–4.9 million spores/g soil at a distance of zero and 2.7–3.6 million spores/g soil at greater distances from the roadsides. The relative content of mycelium in the soil fungal biomass was 92– 96% in the forest park and at a distance of 10 m from the urban roads, while only 49–76% at a distance of zero from the roads.

The observed changes in the fungal complexes may be related to the impact of traffic on the environment,



Fig. 1. The discriminatory analysis of microscopic fungal complexes in urban soils: (1) urbanozems at a distance of zero from the roadsides, (2) urbanozems at distances of 5-20 m from the roadsides, (3) the soddy podzolic soil of the urban forest park, and (4) centroids.

i.e., the absence of a grass cover near urban roads, high alkalinity and an elevated content of chloride ions in adjacent soils, etc. [7]. At the same time, the presence of fungal spores in soils along the roadsides may be due to their precipitation from the air together with the road dust.

The impact of traffic also caused qualitative changes in the soil mycobiota of roadside areas, which were analyzed in terms of discriminatory analysis using ecological indices (the occurrence rate and relative species abundance). The analyzed information represented data on the relative abundance (expressed as a percent) of fungal species isolated from 44 urbanozem samples and 7 forest park soil samples collected during different seasonal periods. Analysis was performed using the following discriminant functions:

F1 = -2.5 + 0.22Z1 + 0.28Z2 - 0.01Z3 + 0.01Z4

+ 1.22Z5 + 1.18Z6 + 0.06Z7 + 0.1Z8 + 1.67Z9;

and

F2 = +0.28 + 0.12Z1 + 0.18Z2 - 0.02Z3 - 0.31Z4

+4.53Z5 + 0.25Z6 - 0.02Z7 - 0.07Z8 + 0.04Z9;

where parameters Z are the abundance (as a percent) of the following fungal species: Z1 – Alternaria alternata, Z2 – Aureobasidium pullulans, Z3 – Cladosporium cladosporioides, Z4 – Fusarium oxysporum, Z5 – Mortierella ramanniana, Z6 – Mucor hiemalis, Z7 – Penicillium vulpinum, Z8 – P. glandicola, Z9 – Phoma glomerata.

In the two-dimensional coordinate space depicted in Fig. 1, the experimental data fell into three groups: *I*, those pertaining to the soil samples that were collected at a distance of zero from the roadsides; 2, those pertaining to the soil samples that were collected at dis-

tances of 5–20 m from the roadsides; and 3, those pertaining to the soil samples that were collected in the forest park.

The soils adjacent to the roadsides were distinguished by the prevalence of dark-pigmented and sterile (i.e., non-spore-forming) fungal species (Fig. 2) in all seasonal periods. For instance, the melanin-containing species *A. alternata* and *C. cladosporioides*, which are usually encountered in urban soils in autumn, were very frequent in the soils adjacent to the roadsides in all seasons. In addition, the latter soils were found to be populated by dark-pigmented pycnidial fungi from the genera *Phoma* and *Coniothyrium*, as well as by the fungal species *Au. pullulans* and *Botrytis cinerea*, which are rarely encountered far from the roads. At the same time, the abundance of the genera *Penicillium*, *Aspergillus*, and *Fusarium* in the soils adjacent to the roadsides was low.

These data agree well with the results of our previous work [12], which showed that the snow cover along Moscow roads contains more dark-pigmented fungi than the snow cover in the environs and forest parks of Moscow. The resistance of the dark-pigmented fungi to the pollution from the automobile traffic is most likely due to the presence of melanin pigments in their cell wall, since there is evidence that these pigments are responsible for the resistance of dark-pigmented fungi to radiation and various industrial pollutants [13–15]. On the other hand, the high occurrence rate of dark-pigmented fungi in the soils adjacent to the roadsides, where the soil moisture content if often insufficient [7], may be due to a high tolerance of melanin-containing fungi to drought [16].

Pollution from traffic changed the species composition of *Penicillium* fungi in the soils along the roadsides. At distances of 0 and 5 m from the roadsides, the relative abundance of *P. chrysogenum* (a typical fungal dominant in Moscow soils) decreased and that of *P. vulpinum* and *P. glandicola*, which have coremia (sheaf-like aggregations of conidiophores), increased. It should be noted that the increase in the relative abundance of fungi with specific structures (coremia and pycnidia) is typical of the soil microscopic fungal complexes that occur close to roadsides. Presumably, coremial and pycnidial fungi are more resistant to extreme environmental conditions than those lacking such specific structures.

Thus, the mycobiota of soils along the roadsides of urban roads with intense traffic considerably differs from that of unimpacted soils. One of the factors that may affect soil fungal complexes is the precipitation of dust-borne fungal germs from the air to the soil surface. On the other hand, fungi that are present in soil dust may influence the composition of microbial complexes in the surface air. It should be noted in this regard that the transfer of dust in the roadside zones is very intense.

Irrespective of the season of observation (summer or autumn) and the level of pollution from traffic, airborne



Fig. 2. The relative abundance (as a percent) of (1) dark-pigmented fungi and (2) sterile light-pigmented mycelium in the vicinity of the Frunzenskaya naberezhnaya in (a) June 1997 and (b) October 1997.

urban mycobiota is dominated by dark-pigmented fungi (*A. alternata, Au. pullulans, C. cladosporioides,* and *Urocladium botrytis*). The dominance of airborne mycobiota by dark-pigmented fungi (*Cladosporium* spp. and others), which are known to be resistant to visible and ultraviolet radiation [13, 17], is generally recognized [18, 19]. Therefore, the dominance of the surface air along the roadsides in Moscow by dark-pigmented fungi is not surprising. The relative content of such fungi in the air along the roadsides was more than 60%, while it did not exceed 51% in the forest park air.

Urban surface air contained a large amount of phytopathogenic fungi of the species *Fusarium moniliforme, F. oxysporum, F. sporotrichioides*, and *B. cinerea*. This can be explained by the fact that the plant cover in Moscow is suppressed, especially along the roadsides, and therefore cannot prevent the development of phytopathogens.

In natural biogeocenoses, the microbial complexes of soil, litter, plant phylloplane, and air are distinguished by specific changes in a vertical direction [20]. As was shown earlier, these changes are much more prominent in rural than in urban zones [15].

Unlike the species structure of fungal complexes, the species composition of these complexes in soils and in surface air along the urban roadsides was similar (as follows from the Sörensen similarity coefficient, which varied from 0.38 to 0.65). The occurrence of *Penicillium* fungi was higher in the forest park soil and air, whereas the occurrence of dark-pigmented fungal species and *Aspergillus* fungi was higher along the urban roadsides.

The air near the roadsides (both at the soil surface and at a height of 1.5 m from the soil), showed high occurrence rates of *Penicillium* species with coremia (*P. vulpinum* and *P. glandicola*) and of the species *Paecilomyces variotii*, *Ph. glomerata*, and *Acremonium furcatum*, which are pathogenic to humans [21]. This fact can be explained by a high abundance of these species in adjacent soils.

MICROBIOLOGY Vol. 70 No. 5 2001

The cluster analysis of the data showed that the microscopic fungi detected in the soils and the surface air fall into two clusters (Fig. 3). As can be seen from the dendrogram presented in this figure, the fungal complexes of soils along the roadsides significantly differ from the airborne fungal complexes of the urban forest park.

To conclude, the fungal complexes of soils and surface air along the urban roadsides considerably differ from those of rural zones. The microscopic fungal complexes of roadside soils and adjacent environments (surface air and snow) are distinguished by the presence of dark-pigmented melanin-containing fungi, which are likely resistant to the pollution from automobile engines.





Fig. 3. Similarity dendrogram of microscopic fungal complexes present in urban soils and surface air. The first letter of the designations denotes either (A) air or (S) soil. The second letter indicates that the data refer to (F) Frunzenskaya naberezhnaya, (L) Leninskii prospekt, (G) Gogolevskii bul'var, and (P) urban forest park. The figure (if present) in the third place of the designations indicates that the data refer to zones at distances of (1) 0, (2) 5, (3) 10, and (4) 20 m from the roadside.

ACKNOWLEDGMENT

This work was supported by grants nos. 99-04-48126a and 00-15-97886 from the Russian Foundation for Basic Research.

REFERENCES

- 1. Kavtaradze, D.N., Nikolaeva, L.F., Porshneva, E.B., and Florova, N.B., *Avtomobil'nye dorogi v ekologicheskikh sistemakh: Problemy vzaimodeistviya* (Automobile Roads in Ecological Systems: Interaction Problems), Moscow: CheRo, 1999.
- Wareham, D.G., McBean, E.A., and Byme, J.M., Linear Programming for Abatement of Nitrogen Oxides Acid Rain Deposition, *Water Air Soil Poll.*, 1988, vol. 40, pp. 157–176.
- 3. Angold, P.G., The Impact of a Road Upon Adjacent Heathland Vegetation: Effects on Plant Species Composition, *J. Appl. Ecol.*, 1997, vol. 34, pp. 409–417.
- 4. Nikolaeva, L.F., Otskheli, O.V., Porshneva, E.B., and Florova, N.B., *Protivogololednye reagenty i ikh vliyanie na prirodnuyu sredu* (Deicing Agents and Their Effect on the Environment), Moscow: Dialog-MGU, 1999.
- Brod, H.-G., Assessing the Risks of Using Deicing Salts, in *Risiko-Abschatzung fur den Einsaltz von Tausalzen* ver kehrstechnic, Helt: N.W., 1995, vol. 21, pp. 65–76.
- 6. O sostoyanii okruzhayushchei prirodnoi sredy g. Moskvy v 1995 g.: Gosudarstvennyi doklad (About the Condition of the Environment in Moscow in 1995: A State Report), Moscow: Moskompriroda, 1996.
- 7. Pochva, gorod, ekologiya (Soil, City, and Ecology), Dobrovol'skii, G.V., Ed., Moscow, 1997.
- 8. Christensen, M.A., View of Fungal Ecology, *Mycologia*, 1989, vol. 81, no. 1, pp. 1–19.
- Miller, S.L., Functional Diversity in Fungi, *Can. J. Bot.*, 1995, vol. 73, pp. 50–57.
- Kozhevin, P.A. *Mikrobnye populyatsii v prirode* (Microbial Populations in Nature), Moscow: Mosk. Gos. Univ., 1989.

- Metody pochvennoi mikrobiologii i biokhimii (Methods in Soil Microbiology and Biochemistry), Zvyagintsev, D.G., Ed., Moscow: Mosk. Gos. Univ., 1991.
- Kul'ko, A.B. and Marfenina, O.E., Characterization of the Species Composition of Microscopic Fungi in an Urban Snow Cover, *Mikrobiologiya*, 1998, vol. 67, no. 4, pp. 569–572.
- 13. Zhdanova, N.N. and Vasil'evskaya, A.I., *Ekstremal'naya ekologiya gribov v prirode i eksperimente* (The Extreme Ecology of Fungi in Nature and Experiment), Kiev: Nauk. Dumka, 1982.
- Zhdanova, N.N., Vasil'evskaya, A.I., Artyshkova, L.V., and Gavrilyuk, V.I., The Species Composition of Actinomycetes in Radionuclide-contaminated Soils, *Mikol. Fitopatol.*, 1990, vol. 24, no. 3, pp. 293–308.
- Marfenina, O.E., Karavaiko, N.M., and Ivanova, A.E., Characterization of Microscopic Fungal Complexes in Urban Areas, *Mikrobiologiya*, 1996, vol. 65, no. 1, pp. 119–124.
- 16. Domsh, K.H., Gams, W., and Andersen, T.H., *Compendium of Soil Fungi*, London: Academic, 1993, vol. 1.
- 17. Mirchink, T.G., *Pochvennaya mikologiya* (Soil Mycology), Moscow: Mosk. Gos. Univ., 1988.
- Linch, J.M. and Hobbie, J.E., *Microorganisms in Action:* Concepts and Application in Microbiol. Ecology, Oxford: Blackwell Scientific, 1989.
- Ebner, M.R., Hastelwandter, K., and Frank, A., Indoor and Outdoor Incidence of Airborne Fungal Allergens at Low- and High-Altitude Alpine Environments, *Mycol. Res.*, 1992, vol. 96, no. 2, pp. 117–124.
- Zvyagintsev, D.G., The Structure and Function of Soil Microbial Complexes, in *Strukturno-funktsional'naya rol' pochvy v biosfere* (The Structure and Function of Soils in the Biosphere), Moscow: GEOS, 1999, pp. 101– 112.
- 21. Atlas of Clinical Fungi. Centraalbureau voor Schimmelcultures, The Netherlands, de Hoog, G.S. and Guarro, J., Eds., 1996.