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# Eco-trophic Relationships and Frequency of Occurrence of Mycobiota of Some Trees grown on the Roadside: the Case of Baku Highways

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**Abstract**

The current study is devoted to eco-trophic relationships and frequency of occurrence of fungal species involved in the formation of tree mycobiota along the highway. As a result, it was possible to determine the possibility of using determined indicators of tree mycobiota in assessing the environmental status of the area near the highway. With this purpose rhizosphere and mycobiota of the leaves of such trees as *Platanus orientalis, Populus nigra* and *Pinus eldarica* growing at various distances (10-100 m) from Baku highways with different traffic intensity have been studied. The samples were taken for the period from 2016 to 2018. The sensitivity of fungi species to pollution from the influence of motor transport has been evaluated. It has been established that the structure of micromycete complex spread both in leaves and rhizosphere of trees growing on the roadside is disturbed by the effects of highway. Near the highway the decrease of species diversity of fungi and increase of the number of dark-colored mycelium fungi species have been observed. This, in turn, leads to the disturbance of the ecological functions of roadside ecosystems. The possibility of using such species as *A.fumigatus, Botryotrichum piluliferum, P.cuslopium, P.funiclozum, St.chartarum* as indicators has been determined.

**Keywords:** highway, trees, mycobiota, number and species of composition, eco-trophic relationships, frequency of occurrence

## Introduction

Quality control of the environment using biological objects is currently considered as one of the ecologically oriented scientific approaches (Wang, 2017). Environmental monitoring is the organized monitoring of the environment which with the help of functional integrity and the state of natural ecosystems of ecological conditions is appreciated for both humans and other biological species (plants, animals, microorganisms and etc.) (Holt and Miller, 2010). Therefore, many different disciplines are involved in ecological evaluation, including biologists, conservationists, foresters, restoration ecologists, ecological engineers, economists, hydrologist, and geologists. Since ecological evaluation forms the basis for so many different types of environmental management, it seems reasonable to integrate management options to achieve economies of time, energy, and costs (Burge, 2008).

In the biodiversity of various ecosystems practice of using mycelium organisms that included in the microbial units of this ecosystem mainly is considered the most important (Haleem Khan and Mohan Karuppayil, 2012, Saikkonen et al., 2015). Fungi emerged as a ‘Third Kingdom’, embracing organisms that were outside the classical dichotomy of animals versus vegetals (Naranjo-

Ortiz and Gabaldon, 2019). For example, the mycelium structure causes the surface area to be quite large giving them to be in higher degree contact with environment. Fungi has a high growth and development rate, which makes it possible for them to completely cover the substrate in a short time and form a large number of structural elements in a state of homeostasis (Richards et al., 2017). Hyphal structure, intimate associations with prokaryotes and eukaryotes have made fungi very flexible at the genetic, physiological, and ecological levels. It is manifested with the fungal ability to perfectly exploit existing nutrient sources and plastically fit into a changing environment (Wrzosek et al., 2017). In addition, they have an ability to react quickly to the unfavorable effects of environmental factors by means of passing to anabiosis.

Recently, in the ecological-trophic characterization of fungi, attention is also paid to the presence of conventional pathogens, allergens and toxigens. Thus, these are important indicators in the ecological assessment of this or that cenosis (Bakshaliyeva, 2017, Rakhimova et al., 2017). More precisely, the species and number composition of fungi, as well as the ecological-trophic relationships are important indicators in the process of biological indication of the ecosystem.

Therefore, the purpose of the presented work was dedicated to the characterization of some plants growing on the motorway areas in the Azerbaijan conditions from the point of view of ecolo-trophy relations and prevalence of random mycobiota.

**Materials and Methods**

The researches were carried out on the areas near the roads of Baku city. Samples for the researches were taken from the leaves and rizosphere of trees, such as Platanus orientalis, Populus nigra and Pinus eldarica, according to the known methods.

Taking of sampling, initial passportization, and preparation for laboratory analysis were carried out according to known methods as well as those used in previous studies (Handbook of Mycological., 2006). In the course of the work for the cultivation and preservation of fungal strains were used nutrient medium such as rice agar (DA), potato agar (KA), wort agar, agarized Czapek.

Isolation of fungi in the pure cultures, species composition, ecological-trophic relationships, and random frequency were carried out according to the known methods (Handbook of Mycological, 2006) and based on the approaches applied in our previous work (Farzaliyeva et al., 2017).

Determination of fungus as well as their causing diseases was carried out according to the known determinants (Kirk et al., 2008, Satton et al., 2001). Naming and systematization of fungi were carried out in accordance with the currently accepted principles (Hawksworth, 2014), as well as the data on the official website of the International Association of Mycology (http://www.mycobank.org)

All experiments were performed in 4-6 replicates and the obtained results were statistically processed (Static methods, 2019). In all cases, the results corresponding to the formula of $m / M = P \leq 0.05$ were considered reliable and included into the dissertation. Here, M - is the average number of repetitions, m- average quadratic index of deviation, and P - confidence level.

**Results and Discussion**

From the samples taken during the 2016-2018 years 75 species of fungi were determined, among them 6 belong to Zygomycota, 6 to the telemorphs of Ascomycota, 7 to Bazidiomycota and 56 to the anamorphs of the Ascomycota. Evaluating these fungi from the point of view of ecological-trophic relations, it was found that the overwhelming majority of them belongs to polytrophs (polysaprotroph+polybiotroph), and saprotrophs are not found among them (tab. 1). As can be seen, in terms of the characteristics of fungi by ecolo-trophic relations certain differences are observed. It became clear that, both plants and fungi play a certain role in the formation of this specificity.

**Table 1. Characteristic of mycobiota of plants and its rhizosphere grown on the motorway areas by the ecological-trophic relationships**

| | | Saprotrophs | | Biotrophs | | Polytrophs | | Symbiotrophs | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-10 m | 100 m | 1-10 m | 100 m | 1-10 m | 100 m | 1-10 m | 100 m |
| P. orientalis | L | 5 | 8 | 5 | 4 | 27 | 28 | 0 | 0 |
| | R | 8 | 11 | 0 | 0 | 36 | 47 | 0 | 0 |
| P.nigra | L | 6 | 10 | 7 | 5 | 28 | 26 | 0 | 0 |
| | R | 9 | 14 | 0 | 0 | 51 | 51 | 0 | 0 |
| P.eldarica | L | 2 | 3 | 3 | 2 | 14 | 15 | 0 | 0 |
| | R | 4 | 6 | 0 | 0 | 33 | 33 | 0 | 0 |

Note: L - leaf; R – rizosphere

For example, on the samples taken from the leaves of P.orientalis planted closest to the motorway were found 5 species of saprotrophs, but 100 m from the road there were 8 of them. Only 3 types of them are found in both cases. Similar examples were observed in other cases.

The results of characterization of fungi by ecologically-trophic relations do not allow to give the full assessment of the conditions of the road section. It was considered appropriate for characterization as the manifestation form of ecological-trophic specialization (Bakshaliyeva, 2017) to give the assessment in the direction of toxicity and allergenic reaction. From the obtained results it became clear that as you move from the motorway the quantitative indicator of both indicators is reduced and this decrease is observed in all three plants and on the samples taken on the leaf and rhizosphere (tab. 2). So, the effect of motor transport not only causes impoverished numbers and species of fungi but at the same time affects the ecological-trophic specialization of mushrooms, more precisely is characterized as a factor that changes the ecologo-trophic structure of the micro-complex. It should be noted with regret that this changes are characterized by negative aspects for both indicators.

**Table 2. Characteristics of registered fungi species by allergenicity and toxicity (according to their share in total with-%)**

| | | Allergens | | Toxigens | |
|---|---|---|---|---|---|
| | | 1-10m | 100m | 1-10 m | 100 m |
| P. orientalis | L | 38,6 | 30,9 | 57,4 | 43,2 |
| | R | 40,5 | 31,2 | 60,6 | 45,3 |
| P.nigra | L | 36,6 | 28,9 | 59,5 | 44,2 |
| | R | 41,0 | 32,1 | 59,6 | 44,6 |
| P.eldarica | L | 32,8 | 26,3 | 52,2 | 40,3 |
| | R | 37,4 | 30,1 | 56,6 | 41,5 |

Note: L - leaf; R – rizosphere

Thus, the specific weight of both allergens and toxigens rises in the structure of the mycobiota inherent to all three studied plants when approaching the motorway.

The increase in the specific weight of these fungi, namely toxigenic and allergenic species in these areas, can be assessed negatively in both sanitary-hygienic and phytosanitary aspects (Rick et al., 2016). So that, second metabolites formed by toxigenic fungi (Keller, 2019) which commonly called mycotoxins is a source of danger for human health (Omotayo et al., 2019). It should be noted that, some mycotoxins according to the degree of dangerous are compared to weapons of mass destruction (http://www.e-osnova.ru/PDF/osnova_1_0_3.pdf) and for some of them it is not possible to determine the allowed dose (i.e., any such amount is hazard).

As for the allergens, although today it is impossible to say unambiguously about fungi matching to this characteristic, many of them directly or indirectly cause allergic diseases and this information is known on the scientific community and does not give rise to doubts. In general, it should be noted that, recently, the problem of mycogen sensitization has started to be special relevance, the reason for this is that micromycetes are one of the wide spread allergy sources in the environment (Rick et al., 2016). From the researches carried out up to date became clear that, among known 300 micromycetes that have this feature and according to various authors, the frequency of mycogenic allergies can vary from 6 to 24% of total population (Simon-Nobbe et al., 2008).

It should be mentioned that the most of the fungi registered in researches have both allergy and toxicity characteristics. Such fungi characterized as universals consist of 55% of toxigenes and 71% of allergens, so that the incline to allergy compared to toxicity is higher.

Fungi species taken in research such as *Aspergillus nidulans, Fuzarium oxysporium, F. Semitechum, Penicillium decumbens* have only toxicity, but *Moniliia sitophila, Penicillium granulatum* and *Trichoderma viride* have only allergenicity. Species auch as *Alternaria alternata, Aspergillus fumigatus, A.niger, Cladosporium herbarium, Penicillium expansum, P.chryzogenum, P.cyclopium* and others carry universal features, namely toxicity, allergenic, and even opportunistic. For example, *A.fumigatus, A.niger, M.himalis* and other species have a broader spectrum of features.

The information mentioned above reveals some interesting moments from the point of view of ecological characteristics of areas of motorway, but this is not enough for bioindication and in the best case can be characterized as useful information for general characterization. Therefore, for the next stage of research it was considered appropriate to characterize fungi by prevalence rate, involved in the formation of mycobiota of trees plant grown in the areas of motorway. For this purpose the individual analysis of each sample was carried out and prevalence rate at the each point was identified. As a result, were determined dominant (frequency of occurrence above 40%), commonly encountered (10-40%) and random species (less than 10%) for the each point and generally for the motorway areas.

7 species of fungi are characterized by the prevalence rate inherent to dominants and the distance factor affects their combinations. So that, although the number of dominants at both distances is 5, in nearby areas such species as *Aspergillus niger, Cladasporium herbarum, Penicillium chryzogenium, P.cuslopium* and *Stachybotrys chartarum* are dominated, but in the remote areas the following species dominate: *Aspergillus niger, Chaetomium cellulolyticum, Mucor globosus, Gliocladium virens* and *Tricnoderma hamatum*. Interestingly, only 1 specie (*A.niger*) mentioned above dominated in all research areas. At the same time it has been identified that this fungi dominates all three trees which were selected as objects of the research. This case manifests both in the phyllosphere, as well as in the rhizome.

Prevalence rate of species which dominate in the areas of 10 m to the edge of the motorway varies between 43,5-56,5%. Prevalence rate for species dominated on the rizosphere and phyllosphere of trees at the distance of 100 m to the edge of the motorway varies between 45,6-

54,3% (tab. 3). As seen, the prevalence rate of species which dominated on the samples taken from 100 m distance as control, at the samples taken from other areas in the best case is characterized as often encountered species. A similar case occurs when compared to the opposite, that is, those are dominants only at 10 m for other distance they are characterized as commonly encountered or random species. This fact allows to determine character change of fungi in the species composition on the motorway areas and the use of some species as indicator species during bioindicator. One of the main points about dominant species is their characterization of toxicity and allergenicity. As mentioned above, impact of motor transport as other technogenic influence is ability to change structure of mycobiota inherent to this or another biotope as well as the manifestation of their ecolo-trophic specialization. Motor transport influence changed the cheracteristic of dominant spices too. So that, fungi involved in the formation of the dominant nucleus of mycobiota in the area on 10 m has 80% toxicity and 80% allergenicity. The analog indicator is 40% and 20%, respectively, for the distance of 100 m. The fact of influence can also be attributed to the confirmation of that the indicator species are among of dominant species.

**Table 3. Prevalence rate of fungi species dominated on the samples taken from trees at the different distance from motorway**

| **Dominant species** | **Prevalence rate (%)** | |
|---|---|---|
| | **10 m** | **100 m** |
| Aspergillus niger | 56,5 | 46,6 |
| Chaetomium cellulolyticum | 7,7 | 52,4 |
| Cladasporium herbarum | 51,5 | 21,2 |
| Gliocladium virens | 6,5 | 48,6 |
| Mucor globosus | 12,3 | 51,2 |
| Penicillium chryzogenium | 54,7 | 10,4 |
| P.cuslopium | 53,4 | 7,8 |
| Stachybotrys chartarum | 51,2 | 1,1 |
| Trichoderma hamatum | 1,6 | 54,3 |

It should be noted that, the regional factor plays a significant role in the spread of fungi and it shows itself in the colors of fungi mycelium, or, more precisely in the formation of pigment (Zachynyaeva, 2006). In areas with sunny days, where the radiation background is relatively high, fungi with black colored mycelium are widespread. The exposure of motor transport to this indicator of mycobiota, also has a specific scientific and practical interest.

The fungi registered in the research can be seperated into three groups according to the color of the mycelium, so that, they have either white, gray or black-colored mycelium. The quantitative analysis allowed to determine that the fungi with black-colored mycelium are more frequent than others, so that, among of generally registered fungi 50,7% have black, 22,7% gray, and 25,6% white mycelium (tab. 4). Despite this, the color change of fungi mycelium varies according to the distance from the motorway, i.e, in the result of emitted the large amount of exhausted gas into the environment black mycelium fungi increases approaching to the motor road. For example, at the characterization of fungi isolatied from the rhizosphere of *P.orientalis* by this aspect, became clear that the colors of fungi mycelium respectively contain 24, 32 and 44% white, gray and black, which samples were taken from the rizosphere of the trees at a distance of 100 m from the roadside.

**Table 4. Characteristics by the color of mycelium of fungi recorded in the research (by the number of species)**

| White | Gray | Black |
|---|---|---|
| 17 | 20 | 38 |

Similar case is observed in the samples taken from leaves as well as in the instance (rhizoceros and leaves) of the other two trees (Eldar pine and Black popular) with small quantitative differences. Interestingly, the distance factor has impact on the prevalence rate of fungi. So that, black colour mycelium of fungi at 10 m, contains 60% of dominant core, but at 100 m it contains 40%. Characterized these indicators by the other prevalence rate became clear that, at the 10 m 50-55% of the commonly encountered species are having black, 15-25% white and 25-35% gray colour mycelium. The analog indicator contains 40-45, 25-30 and 30-35% respectively at the distance of 100 m.

As mentioned above, the black color of the mycelium is an adaptive feature that fungi obtain to protect from the lethal rays of sunlight and this case is encountered in areas where sunny days are more high. The Absheron Peninsula is also one of those places and preferences the domination black colouring mycelium of fungi is related with climatic features. In short, it is possible to note the impact of motor transport is a factor that destroys the regional symptoms of fungi spread.

It should be noted that, the seasons factor was also taken into consideration in our research during taken samples and as mentioned above, as two of the trees selected as objects of the research were viewed only in autumn period we could operate only with rhizosphere micromycetes and from the obtained results it became clear that, the mycobiota of rhizosphere of all three trees during the year have dynamic character (tab.5). From the research carried out at Baku-Airport highway, where the intensity of movement is higher became clear that, depending on the factor of season the number and species composition of the fungi is a changing indicator and according to both indicators the autumn season is more favorable. End of spring and early summer in particular, the month of June is considered to be conducive to fungi, but more clearly it is manifested in the form of number composition. The species composition in autumn, namely in September according to both indicators gets to maximum. It should be noted that, this case is manifested same effect both in the distance (10 and 100 m) and at the samples of every 3 tree.

**Table 5. Change of the number (CFU/q) and species composition (pieces) of fungi depending on the season factor (On the example of Baku-Airport highway)**

| | Platanus orientalis | | Pinus eldarica | | Populus nigra | |
|---|---|---|---|---|---|---|
| | number | species | number | species | number | species |
| Winter | $1,1 \cdot 10^4$ | 14 | $0,5 \cdot 10^3$ | 11 | $1,3 \cdot 10^4$ | 17 |
| Spring | $2,6 \cdot 10^4$ | 19 | $1,5 \cdot 10^3$ | 16 | $2,7 \cdot 10^4$ | 22 |
| Summer | $3,1 \cdot 10^4$ | 22 | $2,1 \cdot 10^3$ | 17 | $3,2 \cdot 10^4$ | 24 |
| Autumn | $3,5 \cdot 10^4$ | 23 | $2,3 \cdot 10^3$ | 18 | $3,6 \cdot 10^4$ | 26 |

Samples were taken from rhizospher and leaves of trees such as P.eldarica, P.orientalis and P.niqra growning at different distances of Baku city highways with different intensity, then quantity and species composition of mycobiota were determined by seasons (3 years). As a result of the research, it was assessed the sensitivity of registered fungi to the load caused by the auto transport. In order to assess technogenic impact including auto transport influence to the formation of mycocomplexes in the concrete areas different approaches are used and especially todays' the mostly wide-spread method taken from the work of A.V. Zachinyayev (2008). According to this approach, species involved in the formation of the micocomplex are grouped as follows and generally they are referred to 4 groups which, for each group, the following is typical:

1. Extreme sensitive species. These species are characterized as either dominant or frequently encountered species of micocomplexes of soils which used as a control and as a rule, they are not observed in polluted soils.
2. Sustainable species. These fungi belonge to dominant or commonly encountered species of microcomplexs, which were formed under the influence of or not under the influence of anthropogenic and technogenic influences.
3. The species belonging to this group are intensively developed at the expense of pollution in the biotope and in our opinion would be expediented to call this group technogenic.
4. Indicatorspecies. These fungi mainly belong to the dominant and commonly encountered species of microcomplex of biotopes exposed to the technogenic influence, that it is almost impossible to distinguish them from the background soils, more clearly from control soils.

According to that during characterization of typical micromicets under the impact of motor transport they are divided into 3 groups (Koretskaya, 2006) with the following features. First group, those are sensitive to anthropogenic impacts usually dominate at the distance of 100 m from the roadside and they are not encountered approaching the edge of the road. The second group of species is sustainable and their prevalence rate does not change significantly depending on the distance. Third group of species are indicator species that can identify the pollution and their prevalence rate is rising approaching the highway.

Although both approaches contain many logical elements, the recorded fungi were divided into 4 groups according to the effect of motor transport. From the obtained results it became clear that only 9 species from 34 are characterized as sustainable species which encountered all areas (tab. 6). As seen, only 5 species can be characterized as indicators and they belong to real fungi more precisely, to the anamorphs of sac fungi.

Most of the registered species in the all of research areas are sensitive species, which contain 1/3 of registered fungi. Interestingly, phytopathogens are also found among sensitive species (http://www.agroatlas.ru/diseases). For example, *Botrytis cinerea* causes gray rot, but *Trichothecium roseum* provokes pink mold on the plants.

Comparing obtained results with the similar research carried out in other areas, it becomes clear that, according to the noted division some of characterized species can change the reactivity depending on the environmental conditions (Svistova and Koretskaya, 2014). Fungi belonging to the genus Trichoderma can be this example. So that, some species of this genus according to reaction ability are characterized as resistant species, however, this was not found in our studies. In the other studies conducted in Azerbaijan it was determined that in general, this genus was unsustainable to the technogenic contamination and even on the oil-contaminated soils, they were not recorded.

**Table 6. Characterization of fungi recorded in rhizosphere of studied plants to the reaction of influence to the motor transport.**

| Groups | Suitable species |
|---|---|
| Sensitive species | *Botrytis cinerea, Cephalosporium griseum, Ch.cellulolyticum, Gliocladium virens, Mucor plumbeus,M. rasemous, Rhizopus nicricans, Rh.stolonifer, Sordariafimicola, Sporotrichum pruinosum, Trichothecium roseum* |

| Groups | Suitable species |
|---|---|
| Technogenic | *Aspergillus terreus, Candida lipolytica,Mortierella alpina, Penicillium brevi-compactum, P.expansum, Torula lipolytica, T.rubescens* |
| Sustainable species | *Alternaria tenuis, A.niger, A.ochrseus, A.ustus, Cladosporium cladospories, C.herbarum, Humicola grisea, Mucor globosus, M.himelis, Penicillium chryzogenium, P.tardum* |
| Indicator species | *A.fumigatus, Botryotrichum piluliferum, P.cuslopium, P.funiclozum, St.chartarum* |

As mentioned, only 5 types of fungi registered in the course of research carry features as such as pollution indicators at the around of motorways areas (Svistova et al., 2006). Interestingly, in other researchs these or other types always have the features of the indicator, that the pollution from the impact of motor transport everywhere creates a specific mycobiota.

It is necessary to turn to the issue related to the reduction of species diversity of fungi. According to some scientists, the reduction of biodiversity inherent in any ecosystem is also aimed at disrupting the integrity of the ecosystem itself. On the other side, fungi play an important role in the implementation of the circulation of nutrients. Therefore, it should be noted that the changes occurring in the structure of the mycocomplex inevitably lead to disruption of the ecological functions of roadside ecosystems.

## Conclusion

Thus, from the carried out researches it became clear that under the eddect of motor transport, violations of structure of the micromycetes complex spread both at the fillosphere and in the rhizosphere of trees grown on the roadside. It is resulted in the reduction of diversity of fungi species as well as in the increasing of the number of species of black-colored mycelium.

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