Analysis of the Diversity of Microscopic Fungi in the Soils of Adjara, Georgia

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Abstract. The publication deals with the studies on the diversity of species composition of fungi in various layers of soils (red, sod-podzolic, marsh, urban) of the Adjara. The aim of the study was to identify and determine the composition of fungi; Establishment of the scale of development and spread of fungi in adverse and favorable conditions; Ecological status evaluation of soils in Batumi City. For the isolation of fungi in various soil samples, a sterilization method, serial dilution, and a scattering method were used. The presence of fungi was detected in various soil samples based on morphological characteristics, percentage frequency, growth rate, and colony forming units. The study has found 59 species taxa of soil fungi, belonging to the divisions of Ascomyota (39 species), Zygomyta (15 species), Basidiomycota (2 species), Deuteromycota (3 species). 7 species of fungi involved in forming of the consortium have been specified as well. The most widely distributed fungi in soil samples were of the genera Aspergillus (A. flavus, A. niger, A. versicolor), Paecilomyces (P. variotii), Trichoderma (T. koningii, T. hamatum, T. polysporum, T. viride), Mucor (M. circinelloides, M. hiemalis, M. racemosus, Mucor sp.), Rhizopus (R. oryzae) the genus of phytopathogens Fusarium (F. oxysporum, F. solani), Verticilium (V. lateritium, V. sp.), Phytophthora (Ph. sp.), Rhizoctonia (R. sp.), Pythium (P. sp) and of genus Sporothrix (S. schenckii). Mong them Aspergillus niger, A. flavus, Chaetomium sp. And Acremonium sp. were dominant fungi in all soil samples. Frequency percentage showed that marsh soil is rich in fungal population as compared to red and sod-podzolic soils, yet the greatest biomass is inorganic soil. A lower level of biological activity in the urban soils was found. Morphometric trait differences in test objects activated on the soil samples have been observed. The study was found specialized species of fungi from each ecotype of soil. The soil samples collected from polluted sites were more affected by waste water which affected the population densities of fungi. Experiments have shown that Aspergillus niger, A. flavus, Fusarium oxysporum, Trichoderma koningii, T. Hamatum and T. Viride grew well on contaminated soil containing heavy metals. On this basis, we will study them further in order to determine their stability to heavy metals.
1. Introduction
Soil is the habitat for many organisms (bacteria, fungi, algae, viruses and protozoa). Among them, fungi are crucial for the environment as well as for the production of food. These recycle soil nutrients and generally have a symbiotic relationship with most plants. Fungi provide diverse physical, chemical and biological habitats, are found in large numbers in soil usually between one and ten million are present per gram of soil [1, 2]. Azaz [3] reported that 1 g of fertile land soil micro fungi are around 400,000.

   Fungi (saprophytes) commonly active around woody plant residue. Fungal hyphae have advantages over bacteria in some soil environments. Under dry conditions, fungi can bridge gaps between pockets of moisture and continue to survive and grow, even when soil moisture is too low for most bacteria to be active. Fungi are able to use nitrogen up from the soil, allowing them to decompose surface residue which is often low in nitrogen. Soil which becomes anaerobic for significant periods generally loses its fungal component. Anaerobic conditions often occur in waterlogged soil and in compacted soils.

   Fungi are especially common in forest lands. An increase in forest productivity was observed as the fungal biomass increased. In Georgia, up to 500 species of microscopic fungi were identified in such soils [4]. In this region, Mycorrhizal Fungi is well studied [5-7]. As for the other soils (red, sod-podzolic, marsh, urban) of Georgia, the present time has not been studied.

   Outcomes of this, the purpose of the study was in these soils to identify and determine the composition of microscopic fungi; Establishment of the scale of development and distribution of fungi in adverse and favorable conditions; Assessment of the ecological state of the soil of the city of Batumi.

2. Materials and methods
The object of the study was various soils and fungi in the agrocenoses of the Adjara. The samples were collected from the 27 points: Adjaria, Georgia, in the vicinity of Batumi Bense, Garadoki 18 July 2009 and 13 September 2012, Shainidze and Lamparadze; Kobuleti (Cixisdziri, Chakvi, Cexlauri, Alambari) 5 November 2012, 20 August 2012, 05 October 2013, 27 August 2014 and 7 October 2015, Beridze; Chelvachauri (Acharisckali, Akhalsheni, Chutuneti, Gonio, Kvariati, Mirveti, Sarfi), 17 August 2002, Chkubadze; Keda (Dandalo, Tshmorisi, Pirvelimaisi, Kvash, Kokotauri) 11-17 July 2016, Diasamidze; Xulo (Agara, Didachara, Khikhadziri, Sxalta, Oktomeri, Dioknisi, Danisparauli) and Shuakhevi (Samoleti, Zamleti), 22-25 August 2016, Lominadze and Shainidze.

   For isolation of fungi sterilization technique, serial dilution technique were used.

   Collections of the species have been examined by standard light microscopy (Pereval, Carl Zeiss, Jena and Olympus, BX50, Hamburg, Germany). The SEM micrographs have been prepared by means of a JSM-35 (Japan) SEM microscope. The specimens examined are deposited at HAL, KW and TGM (23). Species identification was based on the morphological characteristics of single-spored isolates as described by [8, 9].

2.1. Isotypus
LAB M F BSU (Laboratory Mikologi and Fitopatologi, Batumi Shota Rustaveli State University, Adjara, Georgia).
2.2. Sterilization technique
Petri plates, media bottles, distilled water, syringes were sterilized in the autoclave. For sterilization purpose, all apparatus was autoclaved for 30 minutes at 121°C. After autoclaving, all sterilized material was dried in an oven at 90°C.

2.3. Cooking serial dilution
The purpose of serial dilution was to determine the occurrence and frequency of fungi. One ml of wastewater was taken from each sample. Serial dilution was set up by carefully taking the 10 ml of distilled water in McCartney bottles. Then these bottles were autoclaved for 30 minutes at 121°C. From the sample of wastewater 1 ml was dissolved in 10 ml of sterile distilled water in McCartney bottle to give (1:10) and shaken well. The McCartney Bottle 2 was inoculated with 1 ml from bottle 1 to give 1:100 dilutions. McCartney Bottle 2 was also shaken well. McCartney Bottle 3 was inoculated with 1 ml from bottle 2 to give 1:1000 dilutions. McCartney Bottle 4 was inoculated with 1 ml from bottle 3 to give 1:10000 dilutions.

The method was applied in the case of soil samples making dilutions up to 1:1000. To complete the serial dilution a micropipette was used with sterilized tips. Estimation of the fungal population was done by standard spread plate dilution method described [10].

2.4. Isolation of fungi
Spread plate technique was used for enumeration of fungi from given samples. From each McCartney bottle, 0.5 ml of sample was taken separately with the help of micropipette along with sterilized blue tips. Then these diluted samples were inoculated on sterile PDA plates with the help of micropipette and L shape rod was used to spread the diluted sample on the PDA plate. Repeat the same step with all other wastewater and soil samples. Then these plates were incubated at 30°C for 7 days and then the colonies were counted [11].

2.5. Identification of fungi
The identification of fungi was carried out by using the modern identification guides. The cultures were identified at genus level on the basis of macroscopic - colonial morphology, color, texture, shape and appearance of morphology and microscopic characteristics - septation in mycelium, presence of specific reproductive structures, shape and structure of conidia [12-15].

3. Results and discussions
The study has found 59 species taxa of soil fungi in Adjara (Table 1), belonging to the divisions of Ascomyota (39 species), Zygomycota (15 species), Basidiomycota (2 species), Deuteromicota (3 species). From the table, it is visible that the richest detachment was Hypocreales (25.4% of species), Mucorales (22.0% of species); from the genera - Aspergillus (5 species), Trichoderma (4 species) and Mucor (4 species).

7 species of fungi involved in forming of the consortium have been specified as well (Mucor circinelloides, Aspergillus flavus, Fusarium oxysporum, Trichoderma koningii, Rhizopus oryzae, Rhizoctonia sp., Verticillium lateritium). Formation of the consortium begins when air and soil temperatures are 24 to -30°C, and an optimum temperature is about 27°C. High humidity (90-95%) hastens the formation of the consortium.
**Table 1. Systematical structure of micobiota soil Adjara**

| Exciter     | Division      | Class          | Order              | Family        | Genus             | Species |
|-------------|---------------|----------------|--------------------|---------------|-------------------|---------|
| Chromista   | Oomycota      | Oomycetes      | Peronosporales     | Pythiaceae    | Pythium           | 1       |
|             |               |                |                    | Phytophthoraceae | Phytophthora     | 1       |
| Zygomycota  | Mucormycotina | Mucorales      | Mortierellaceae    | Mortierella   | 2                  |
|             |               |                | Choanephoraceae    | Blakeslea     | 1                  |
|             |               |                | Mucoraceae         | Actinomucor   | 1                  |
|             |               |                |                    | Mucor         | 4                  |
|             |               |                |                    | Rhizopus      | 3                  |
|             |               |                |                    | Filobolus     | 1                  |
|             |               |                |                    | Zygorrhynchus | 2                  |
| Fungi       | Ascomycota    | Sordariomycetes| Hypocreales        | Acremonium    | 2                  |
|             |               |                |                    | Gliocladium   | 1                  |
|             |               |                |                    | Paecilomyces  | 1                  |
|             |               |                |                    | Trichoderma   | 4                  |
|             |               |                |                    | Niessliaceae  | 1                  |
|             |               |                |                    | Monocilliun   | 1                  |
|             |               |                |                    | Bionectriaceae| 1                  |
|             |               |                |                    | Clonostachys  | 1                  |
|             |               |                |                    | Cordycipitaceae| 1                 |
|             |               |                |                    | Lecanicillium | 1                  |
|             |               |                |                    | Cordycipitaceae| 1                 |
|             |               |                |                    | Ophiocordycipitaceae| 1     |
|             |               |                |                    | Verticillium  | 2                  |
|             |               |                |                    | Neottieospora | 1                  |
|             |               |                | Sordariales        | Fusarium      | 3                  |
|             |               |                |                    | Chaetomiaceae | 1                  |
|             |               |                |                    | Pseudeurotiaceae| 1             |
|             |               |                |                    | Pseudeurotium | 1                  |
|             |               |                | Ophiostomatales    | Ophiostomataceae| 1            |
|             |               |                |                    | Sporothrix    | 1                  |
|             |               |                | Dothideales        | Aureobasidium | 1                  |
|             |               |                |                    | Didiodendron  | 1                  |
|             |               |                | Pleosporales       | Pleosporaceae | 1                  |
|             |               |                |                    | Helminthosporium| 1                |
|             |               |                |                    | Sempylium     | 2                  |
|             |               |                |                    | Alternaria    | 1                  |
|             |               |                |                    | Curvularia    | 1                  |
|             |               |                | Saccharomycetes    | Saccharomycetales| 1          |
|             |               |                |                    | Dipodascaceae | 1                  |
|             |               |                |                    | Geotrichum    | 1                  |
|             |               |                | Leotiomycetes      | Heliotiales   | 1                  |
|             |               |                |                    | Sclerotiniaceae| 1                 |
|             |               |                |                    | Monilinia     | 1                  |
|             |               |                | Eurotiomycetes     | Eurotiales    | 1                  |
|             |               |                |                    | Trichocomaceae| 1                  |
|             |               |                |                    | Aspergillus   | 4                  |
|             |               |                |                    | Penicillium   | 2                  |
| Deuteromicota| Coelomycetes  | Sphaeropsidales| Sphaeropsidaceae   | Phoma         | 1                  |
|             |               |                |                    | Sphaeropsis   | 1                  |
|             |               |                |                    | Darluca       | 1                  |
| Basidiomycota| Agaricomycetes| Cantharellales  | Ceratobasidaceae   | Rhiisoctonia  | 1                  |
|             |               |                |                    | Atheliales    | 1                  |

Frequency percentage fungi showed that from all of the soil, the maximum quantities of fungi in marsh soil that was 82% (Figure 1), in sod - podzolic soil 34%, in red soil 23%, the lowest frequency of occurrence of fungi was shown in urban soil 14%.
Figure 1. Percentage distribution of fungi in different types of soil of Adjara

From the collected soil samples, the occurrence of species in different samples soil is shown in Table 2 (presence"+" and absence"−").

From the table, it is visible that marsh soil is rich in fungal population as compared to red, sod-podzolic and urban soils.

Such a large composition of fungi on Marsh soils is due to the fact that it is not contaminated with heavy metals, wastewater and et al.

The following species dominates in marsh soil: *Acremonium alternatum, Acremonium sp.*, *Actinomucor elegans, Aureobasidium pullulans, Cladosporium herbarum, Clonostachys rosea, Curvularia sp.*, *Darluca sp.*, *Geotichum candidum, Gliocladium penicilloides, Leucanillium lecanii, Mortierella alpine, Mortierella sp.*, *Umbelopsisis abellina*, and et al.

In the present study a lower level of biological activity in the urban soils was found (total of 8 species fungi were isolated: *Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Curvularia sp.*, *Fusarium oxysporum, Trichoderma koningii, T. hamatnum and T. viride*), because urban soils are contaminated of heavy metals and sources are automobiles exhaust, sewage water, industrial ast, Batumi Oil Terminal and et al.

Similar results were received by almost in red and sod-podzolic soils that were receiving wastewater of Kintrishi and Chakvis ekali for several years.

Wastewater is often the only source of water for irrigation in these areas. The reality is that wastewater generated in Adjara receives no treatment at all. The use of wastewater for irrigation may affect the whole biological community, including species diversity and accumulation of toxic contaminates in the food chain.

Despite this, we identified a total of 20 species fungi (*Acremonium sp.*, *Aspergillus flavus, Aspergillus niger, Aspergillus versicolor, Chaetomium sp.*, *Fusarium oxysporum, Fusarium solani, Fusarium sp.*, *etc*.)
Mucor circinelloides, M. hiemalis, M. racemosus, Mucor sp., Neottiospora sp., Phytophthora sp., Pythium sp., Rhizoctonia solani, Rhizopus oryzae, Trichoderma harzianum, T. koningii, Verticillium sp.) in sod-podzolic soil and in the 14 species (Acremonium sp., Aspergillus flavus, Aspergillus niger, Aspergillus versicolor, Chaetomium sp., Fusarium oxysporum, Fusarium solani, Mucor hiemalis, M. racemosus, Pythium sp., Rhizoctonia solani, Rhizopus oryzae, Trichoderma harzianum) in the red soil (Table 2).

Experiments have shown that most widely distributed fungi in soil samples were of the genera Aspergillus (A. flavus, A. niger, A. versicolor), Paecilomyces (P. variotii), Trichoderma (T. koningii, T. hamatum, T. polysporum, T. viride), Mucor (M. circinelloides, M. hiemalis, M. racemosus, Mucor sp.), Rhizopus (R. oryzae) the genus of phytopathogens Fusarium (F. oxysporum, F. solani), Verticillium (V. lateritium, V. sp), Phytophthora (Ph. sp), Rhizoctonia (R. sp), Pythium (P. sp.) and of genus Sporothrix (S. schenckii).

Among them Aspergillus niger, A. flavus, Chaetomium sp. And Acremonium sp. were dominant fungi in all soil samples.

| Fungi                  | Soil type          |
|-----------------------|--------------------|
|                       | Marsh soil | Sod–podzolic soil | Red soil | Urban soil |
| Acremonium alternatum | +          | -                | -        | -          |
| Acremonium sp.        | +          | +                | +        | +          |
| Actinomucor elegans   | +          | -                | -        | -          |
| Aureobasidium pullulans | +        | -                | -        | -          |
| Alternaria fasiculata | +          | -                | -        | -          |
| Aspergillus flavus    | +          | +                | +        | +          |
| Aspergillus niger     | +          | +                | +        | +          |
| Aspergillus terreus   | +          | -                | -        | -          |
| Aspergillus versicolor| +          | +                | +        | -          |
| Athelia               | +          | -                | -        | -          |
| Blakesleatrispora     | +          | -                | -        | -          |
| Chaetomium sp.        | +          | +                | +        | +          |
| Cladosporium herbarum | +          | -                | -        | -          |
| Clonostachys rosea    | +          | -                | -        | -          |
| Curvularia sp.        | +          | -                | -        | -          |
| Darluca sp.           | +          | -                | -        | -          |
| Fusarium oxysporum    | -          | +                | +        | +          |
| Fusarium solani       | -          | +                | +        | +          |
| Fusarium sp.          | -          | +                | -        | -          |
| Geotrichum candidum   | +          | -                | -        | -          |
| Gliocladium penicilloides | +    | -                | -        | -          |
| Monilia sp.           | +          | -                | -        | -          |
| Monocillium sp.       | +          | -                | -        | -          |
| Helminthosporium sp.  | +          | -                | -        | -          |
| Lecanicillium lecanii | +          | -                | -        | -          |
| Mortierella alpina    | +          | -                | -        | -          |
| Mortierella sp.       | +          | -                | -        | -          |
| Mucor circinelloides  | -          | +                | +        | -          |

Table 2. The distribution of fungi in different soil types Adjara
Among identified fungus, one species of *Fusarium sp.* can be considered new to mycobioti. It differs significantly from other fusariosis species not only by the shape and size of the spore, but also by aggression [16].

In laboratory studies, *Fusarium sp.* is characterized by rapid growth in pure culture. On agar (of CLA), the fungi forms slimy colonies, and then it is cove characterized by rapid growth in pure cultured with a thick, aerial hyphae of white to light purple in color (Figure 2). The fungus produces three types of spores: Macroconidia (Figure 3A) Microconidia (Figure 3B), and chlamydospores (Figure 3C). Macroconidia are spindle-shaped, less ellipsoidal, 20-65×2.5-5 mm, with a pointed tip at both ends with five-septate (by determinant 3-5 septate less); where as microconidia are non-septate, elliptical, less cylindric, 20-21.3 × 1.5-3 mm (by determinant, microconidias are oval, colorless and one-two-celled). Microconidia are borne on simple phialides arising laterally, elliptical, less cylindrical, 20-21.3 × 1,5-3 mm (by determinant, microconidias are oval, colorless and 1-2 septate). Chlamydospores colorless, oval, elliptic, 5-15 mm, smooth and rough walls, abundant and form a terminally or on the basis of a leap. They are usually solitary but sometimes form in pairs or chains. No perfect stage it is not known.
Figure 2. Cultures of *Fusarium sp.* showing purple and pink pigmentation

Figure 3. Spores of *Fusarium sp.* A - Macroconidia B - Microconidia C - Chlamydospores

Figure 4 showing the number of cases of isolation and percentage frequency of fungal isolates from the collected soil samples.

From all of the soil samples, 276 colonies of fungi were isolated. How visible from Figures 4 the frequency of *Aspergillus niger* was high in all the locations and it was highest in red soil that was 100%. Frequency of *Aspergillus flavus*, *Aspergillus fumigatus*, *Acremonium alternatum*, *Paecilomyces variotii* and *Chaetomium globosum* was high also in all the location that are accordingly 98, 95, 89, 88, 86%.
Trichoderma koningii, T. hamatum and T. viride were found only in red soils that are accordingly 75,73,71%. Percentage of Mucor circinelloides, M. hiemalis, M. racemosus, Mucor sp. Was the highest in marsh soils that are accordingly 67,75,62,61%.

The species of phytopathogens Fusarium oxysporum, F. solani, Verticilium lateritium and Phytophthora sp. was found only in red soils that are accordingly 67,59,52, 48% (Figure 4).

Similar results were received by almost many researchers in different countries.

Figure 4. Percentage frequency of fungal isolates from the collected soil samples

4. Conclusions

The study has found 59 species taxa of soil fungi, belonging to the divisions of Ascomyota (39 species), Zygomycota (15 species), Basidiomycota (2 species), Deuteromycota (3 species). 7 species of fungi involved in forming of consortium have been specified as well.

Most widely distributed fungi in soil samples were of the genera Aspergillus (A. flavus, A. niger, A. versicolor), Paecilomyces (P. variotii), Trichoderma (T. koningii, T. hamatum, T. polysporum, T. viride), Mucor (M. circinelloides, M. hiemalis, M. racemosus, Mucor sp.), Rhizopus (R. oryzae) the genus of phytopathogens Fusarium (F. oxysporum, F. solani), Verticilium (V. lateritium, V. sp.), Phytophthora (Ph. Sp.), Rhizoctonia (R. sp.), Pythium (P. sp.) and of genus Sporothrix (S. schenckii). Among them Aspergillus niger, A. flavus, Chaetomium sp. And Acremonium sp. were dominant fungi in all soil samples. Frequency percentage showed that marsh soil is rich in fungal population as compared to the red and sod-podzolic soils, yet the greatest biomass is inorganic soil. A lower level of biological activity in the urban soils was found. Specialized species of fungi from each ecotype of the soil were also found.

Among identified fungus, one species of Fusarium sp. can be considered new to mycobioti.

It turned out the frequency of Aspergillus niger was high in all the locations and it was the highest in the red soil that was 100%. The frequency of Aspergillus niger was high in all the locations and it was the
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The soil samples collected from polluted sites were more affected by wastewater which affected the population densities of fungi.

Experiments have shown that Aspergillus niger, A. flavus, Fusarium oxysporum, Trichoderma koningii, T. hamatum and T. viride grew well on contaminated soil containing heavy metals. On this basis, we will study them further in order to determine their stability to heavy metals.

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