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# Occurrence of the fungal flora in Natural environmental sources

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## Abstract

The present investigation was undertaken to study about the fungi in soil, water, and air. The samples of normal soil, garden soil, Rhizosphere, Rhizoplane, well water, sewage water are collected and processed by plating techniques. Totally, 52 fungal species were enumerated from all the samples by plating techniques. 35 fungal species were isolated from soil samples, 16 from Rhizoplane, 13 from well water, 13 from sewage samples, 15 from air samples. The distribution of fungi in classwise, one species belong to Oomycetess, 7 with Zygomycetes. One with Pyrenomycetes, 44 belongs to Hyphomycetes and one with Coelomycetes. Among the samples, 16 species were isolated from Rhizosphere and Rhizoplane respectively and 15 species of fungi were also isolated and enumerated from garden soil and air. The frequencies of occurrence of fungi were also studied in terms of their distribution in all the samples. Accordingly, 16 species were frequently occurred (+++) followed by 24 fungi less frequently (++) occurred and 12 (-) species were rarely isolated. Among the fungal isolates, the species of *Aspergillus* (32 species) were seemed to be dominant members followed by *Rhizopus* (2) and *Mucor (*2). In the present study, pathogenic fungi were also isolated and enumerated from all the samples. In those parts of the screening of amylase and protease enzymes, 9 species were found to be positive for the amylase enzyme and 12 species for protease enzyme respectively.

**Keywords:** fungi, plating techniques, Hyphomycetes, enzymes.

## Introduction

Fungi are ubiquitous in nature. The species of fungi ranges from 1,00,000 to 2,50,000. the true fungi (Eumycota) are grouped into five subdivisions on the basis of their reproduction. Fungi belonging to Mastigomycotina produce flagellate asexual spores and exist in unicellular or mycelial forms. The cellwall is composed of cellulose and other glucans or chitin. The zygomycotina fungi produce non motile asexual spores formed in a sporocarp. The thallus is usually mycelial and typically aseptate. Ascomycotina is the largest subdivision containing about 15,000 species. The vegetative structure consists of single cell or septate filaments. Most segments consists of nuclei. The cell walls are composed of chitin and glucans. Sexual reproduction leads to the formation of spores in ascus. Members of subdivision Deuteromycotina have a vegetative structure that is either unicellular or a septate mycelium. These fungi are also known as fungi imperfecti because of the absence or unknown or lost of sexual state. Vegetative reproduction is carried out by asexual reproductive structures called conidia.

Factors that influence the growth of fungi are moisture, temperature, $p^H$ and oxygen. Fungi cannot grow when submerged in water due to low oxygen availability. The mycelia are susceptible to desiccation and require more or less continuous source of water to grow. The optimum temperature for fungi to grow ranges from 25˚C to about 40˚C. the optimum $p^H$ level ranges from 4 to 7. Most of the aerobic. But yeasts are capable of facultative anaerobic existence.

Soils have both aerobic and anoxic regions. Fungi constitute a high proportion of the microbial biomass in soil. They may be either indigenous or allochthonous organisms. Fungi are found in the top 10cm of the soil and rarely below 30cm. Soil fungi are most abundant in well aerated soils. The most frequently isolated fungi from soils are members of fungi imperfecti like *Aspergillus, Geotrichum, Penicillium* and *Trichoderma.* Numerous ascomycetes and basidiomycetes also occur in soil in high numbers. The abundance of basidiomycetes in some soils is visibly apparent because of production of macroscopic fruiting bodies, the mushrooms. Fungi carry out metabolism when adequate moisture, aeration and relatively high concentration of utilizable substrates are available. Fungi remain dormant in the form of basidiospores, chlamydospores and sclerotia.

Fungi exhibit adaptations to local environmental conditions. Individual fungi exhibit adaptations to extreme conditions found in some soils. Example: *Blastocladiella* and *Penicillium* grow vegetatively at low oxygen tensions. *Fusarium* grows at unusually high $CO_2$ levels. In forests where air borne dispersal may be less effective than in open communities, some basidiomycetes and ascomycetes produce subterranean spores that are adapted to dispersal by subterranean animals.

Some fungi found in soil are pathogenic to plants and animals. Species of *Alternaria, Fusarium, Helminthosporium* and *Pythium* are commonly found in soil near roots and can become pathogenic to the above ground vegetation. The human pathogens *Histoplasma capsulatum* and *Coccidiodes immutis* occur in soil. These two fungi exhibit extreme localization related to the particular suitability of the habitat conditions for activity and survival.

Rivers, by nature contain high proportions of allochthonous microorganisms. Among the fungi, almost all Phycomycota are aquatic. Some Phycomycota in fresh water ecosystems are saprophytes. Others are parasites of fresh water algae, higher aquatic plants and animals such as crustaceans

and fish. Some Phycomycota even parasitize, other Phycomycota species. In lakes members of chytridiales and saprolegniales (both Phycomycetes) generally predominate. The difference in the distribution of fungal genera between lakes reflects variation in the organic substrates available for fungal parasites. Many fungi in fresh water lakes, rivers and streams are associated with foreign organic matter and are considered as allachthonous member of such ecosystem. Many Ascomycetes and Fungi imperfecti are found on wood and dead plant materials in rivers.

Saprophytic fungi are the natural scavengers decomposing the dead bodies of plants and animals and convert them into organic matter and further decompose them to elemental form. Thus fungi serve as an important link in the food chain. In the detritions food chain, the energy flow begins from decomposition of organic matter by fungi and other micro organisms. Thus microbes like fungi play a vital role in the initiation of detritions food chain and maintain the balance in the ecosystem.

Microorganisms can be transported with moving air rapidly across great distances. It is a major pathway for the dispersal of microorganisms. Several bacterial, viral and fungal diseases are spread through atmosphere. Clouds possess concentrations of water, there by promoting the growth of microorganisms. Light intensities and $CO_2$ concentration in cloud layers are sufficient to support the growth of photoautotrophic microorganisms. Industrial areas contain sufficient concentrations of organic chemicals in atmosphere to promote growth of heterotrophs. Many microorganisms that grow in hydrosphere or lithosphere can become air borne. There are no known autochthonous atmospheric microorganisms. Allochthonous microorganisms pass through atmosphere and reach terrestrial aquatic ecosystems. Dispersal through atmosphere insures survival of many microorganisms. Most microorganisms in atmosphere occur as spores. Spores, whose primary function is dispersal, are known as xenospores. Spores are produced by fungi, algae, protozoa, bacteria, lichens,-----etc. viruses that are metabolically inactive outside the host cells act as spores in the atmosphere.

The region in the vicinity of roots can be differentiated into many micro habitats. The term “rhizosphere” was introduced by German scientist Hiltner to denote that region of soil, which is subjected to the influence of plant roots. Rhizosphere is characterized by greater microbiological activity than the soil away from plant roots. The intensity of such activity depends on the distance to which exudations from root system can migrate. The term “rhizosphere-effect” indicates the over all influence of plant roots on soil microorganisms. Most of the bacteria, fungi and actinomycetes are present in rhizosphere soil. A wide range of enzymes of plant and microbial origin present in the rhizosphere catalyze the breakdown of organic materials. The rhizosphere to soil ratio can be calculated by dividing the number of microorganisms in the rhizosphere soil by the number in the soil free from plant growth.

Soil adhering to roots is removed and roots subjected to washing by sterilized water until the clean root surface is exposed. The washed roots are plated. Fungi that inhabit the root surface in a mycelial state belong to the genera *Fusarium, Pythium, Aspergillus, Mucor, Penicillium,*and *Trichoderma.*

Decomposition of organic matter in river, marine and estuarine waters is brought about by diverse aquatic biota namely bacteria, fungi, nematodes and worms. Aquatic fungi have been reported to improve the palatability and nutrient content of plant and animal remains. The survival and success of fungi as decomposers are largely depend on their ability to adapt to their environment immediately surrounding the substratum and to provide viable reproductive units. Along with nature of substrate ever changing factors like light, temperature, oxygen and hydrogen ion concentration act either singly or in concert to influence substrate colonization, growth and reproduction of fungus.
In a natural environment, substrate degradation is achieved by association of succession of taxonomically unrelated fungi and other organisms adapted to or tolerant to the special environment conditions associated with the fluid medium. Fungi play an important role in nutrient regeneration cycles as decomposers of dead and decaying organic matter.

The present study was initiated to evaluate the occurrence of the fungal flora in our college campus i.e., in the campus soils, well water and in sewage water. Accordingly in this line, attempts were made

1. To isolate, identify and enumerate the fungal species.
2. To study the fungal frequency and density and finally to screen the amylase and protease enzymes producing fungi.

## Materials and Methods

### Sample collection

Seven different samples were collected around the college campus.

| | | |
|---|---|---|
| Normal soil | | - building construction site |
| Red soil | | - garden of college campus |
| Rhizosphere soil | | - tomato plant cultivated soil |
| Sewage | | - women’s hostel |
| Water | | - well water |
| Rhizoplane | sample | - tomato plant |
| Air sample | | - P.G block |

All the samples were collected and used for the isolation of fungi.

### Isolation and enumeration of fungi

### Isolation of fungi from soil samples by dilution-plating technique:

The soil samples of normal soil, garden soil, rhizosphere soil, and non-rhizosphere soil were collected and processed using serial dilution and plating technique.

### Serial dilutions:

1 g of soil samples were measured and added into test tube containing 10 ml of sterile distilled water ($10^{-1}$dilution)

1 ml of the 10-1 aliquots was pipetted and transferred to another test tube containing 9 ml sterile distilled water ($10^{-2}$ dilution)

The same procedure was followed and dilution till $10^{-5}$ were prepared.

### Plating method:

Sterile Corn Meal Agar, Sabouraud’s Dextrose Agar, Rose Bengal Agar, Oat Meal Agar plates prepared aseptically and all the plates were aseptically marked with dilution ,soil type, sampling site.

0.1ml aliquots were pipetted out and transferred to agar plates.
Spread plate technique was performed using sterile glass L-rod.
The plates were incubated at room temperature for 4-5 days.
The plates were observed regularly for the growth of fungal colonies.
The number of colonies developed and their morphology were noted.

## Rhizoplane samples

Roots pieces from the first dilution was taken in screw cap bottles with 100ml sterile water and it was shaked well.

Serial washings of the roots with sterilized water was taken and plated on Corn Meal Agar, Sabouraud's Dextrose Agar, Potato Dextrose Agar, Rose Bengal Agar plates.

The plates were incubated at 25°C for 7days in an inverted position.

## Isolation of fungi from water samples:

The water samples of well water, sewage were subjected to serial dilution and plating technique.
The water samples were serially diluted up to $10^{-5}$ dilutions.

Sterile Sabouraud's Dextrose Agar, Potato Dextrose Agar, Rose Bengal Agar, Oat Meal Agar , were poured into sterile petriplates.
From the dilutions, $10^{-2}$ to $10^{-5}$, 0.1ml of sample was transferred to agar containing petriplates.
Spread plate technique was performed by using sterile glass L-rod.
All the inoculated plates were incubated at room temperature for 4-5 days.
After incubation periods, the developed fungal colonies were observed and number of colonies are noted.

## Isolation of fungi from air by open plate method:

Sabouaud's Dextrose Agar , Corn Meal Agar media were prepared, sterilized and dispersed onto sterile petriplates.

The plates were allowed to solidify the prepared plates were labeled appropriately for the time of exposure to air (5,10 and 15 minutes) as well as the place of sampling.
The plates were exposed to air for the required period of time at the selected plates.
After the exposure time, lids were replaced and the plates were incubated at room temperature (28°C) for 4-5 days.
After the incubation periods, the plates were observed and the number of colonies counted.

## Isolation of fungi

The incubated plates were observed for the development of fungi from the third day onwards. The number of colonies in each plates were counted and compared with the control plates. In addition to this cultural characters and colonies (colour and structure) were identified and enumerated.

## Characterization of fungi

The characterization of fungi was processed using lactophenol cotton blue staining method (Aneeja, 2001).

## Principle

It stains the fungal cytoplasm and provides blue bock ground against which the walls of hyphae can readily be seen.
It contains the four constituents:
Phenol, which serves as a fungiside.
Lactic acid which acts as a clearing agent.
Cotton blue which stains the cytoplasm of the fungus and
Glycerine which gives a semipermanent preparation.

## Procedure

A drop of lactophenol cotton blue was placed on a clean glass slide.
A small tuft of fungus, usually with spores and spore bearing structure was placed onto the drop using flamed, cooled needles.
The material was gently teased using two mounting needles.
The mold structure was gently mixed with stain.
The coverslip was placed over preparation taking care to avoid trapping air bubbles in the stain.

## Sealing of lactophenol cotton blue mounts

All the air bubbles were removed from the preparation by pressure, gentle heating or the addition of more lactophenol cotton ble.
The excess mountant removed from around coverslip with 70% alcohol on a cotton swab or with blotting paper.
A thin layer of nail polish was applied around the edge of the cover slip. The preparation was allowed to dry over night.
A second coat of nail polish was applied over the first coat.

## Observations

The preparation was examined under low power and high power objectives.
The hyphae, conidiospores, conidigerous cells, conidia and their arrangement on the conidiophores were also observed.
The representation microscopic field under low power and high power magnification were drawn.
The mold was identified on the basis of characterization features produced.

## Presentation of data

The semipermanent slides for the fungi isolated were prepared using lactophenol cotton blue staining methods (Dring, 1976) and sealed with DPX mountant.
The fungal species were photographed using photomicrographic instrument.

## Identification of fungi

The identification of fungal taxa is based on Hyphomycetes (Subramanian (1971)). Dematiaceous hyphomycetes and more dematiaceous hyphomycetes (Ellis, 1971 and 1976). And A manual of soil fungi (Joseph C. Gilman, 1959,1998).

## Enumeration of fungi

The distribution of these taxa different system is tested out and the homenclature followed is based on"**The fungi: An Advanced Treatise" Vol:IV B (Ainsworth *et.al.*, 1973**).

Each taxon is briefly described by its binomial followed by morphology and frequency such as most frequency, frequency and rare forms.

## Quantitative analysis

A quantitative study of the distribution of fungi was presented in terms of frequency and density (D).

The frequency was calculated as follows.

Frequency (F) =

$$\frac{\text{number of sampled stations where the species occurred.}}{\text{Total number of sampling stations studied.}}$$

The frequency of distribution of fungi for all the samples was calculated.

## Density

Density represents the mean number of colonies expressed as number of colonies per ml.

Density (D) =

$$\frac{\text{number of colonies in all sampling stations put together}}{\text{Total number of sampling stations studied.}}$$

## Screening of enzymes from the isolated fungi:

Screening of enzymes i.e., amylase and protease from the selected fungi were also studied.

## Screening of amylase enzyme:

Amylase commercially produced from various Aspergilli are used in the initial steps in several food fermentation processes to convert starch to fermentable sugars.

## Procedure:

The starch agar medium was melted, cooled to 45°and poured into the sterile petri dishes.
The plates were allowed to solidify.
Each of the starch agar plates were labeled with the name of the organisms to be inoculated.
Using sterile technique, a single streak was made of each organism into the center of its appropriately labeled plate.
The fungal inoculated plates were incubated at room temperature for 72-96 hours.
The excess iodine solution was poured off.

### Observations

The plates for the starch hydrolysis around the growth of each fungal colony i.e., the colour change of the medium were examined.

### Screening of protease enzyme

Some fungal members have to degrade the protein by producing proteolytic enzymes which breaks the peptide bond Co-NH by introducing water into the molecules liberating smaller chains of amino acids called peptides, which are later break down into free amino acids by extra cellular or intra cellular peptidases which are transported through the cell membrane into the intra cellular amino acids pool for use in the synthesis of structural and functional cellular proteins.

### Procedure:

Preparation of skim milk agar whose constituents per litre of medium are

| | | | |
|---|---|---|---|
| Skim milk powder | | - | 100g |
| Peptone | - | 5g | |
| Agar | | - | 15g |
| $p^H$ | | - | 7.2 |

The ingredients were dissolved; $p^H$ is adjusted to 7.2 and sterilized. The sterilized medium was poured into sterile Petri plates and allowed to solidify.
A single line streak inoculum was made from each fungal cultures into its labeled Petri plate across the surface of the medium.
The plates were incubated at room temperature for 72-96 hours.

### Observations

All the inoculated plates for any cleaning around the line of growth were observed.

## Results and Discussion

### Isolation and enumeration of fungi

### Isolation of fungi

The samples of Normal Soil, Garden soil, Rhizosphere soil, Rhizoplane, water, sewage and air fungi were isolated by plating technique. Totally 52 fungal species were isolated, identified and enumerated. 12 fungi were isolated from normal soil, 15 in Garden soil, 16 in Rhizophere soil, 16 in Rhizoplane, 13 in water, 13 in sewage and 15 in air. Among the isolated fungi, *Aspergillus* was the dominant genus represented with 29 species.

### Enumeration of fungi

The various fungi isolated by plating technique in all the samples were enumerated and classified based on "**The fungi – An Advanced Treatise, Vol.IVB (Eds). Ainsworth *et al.*, (1973**) is followed for the arrangement of genera under their respective orders and families.

Each taxon is briefly described its binomial followed by Frequency such as frequent, less frequent and rare. Density was also studied in the present investigation.

### Species diversity of fungi

During the study period, a total of 52 fungal species were enumerated from all the samples by plating technique. Among these 12 fungi were isolated in Normal Soil, followed by 15 in Garden soil, 16 in Rhizosphere soil, 16 in Rhizoplane, 13 in Water, 13 in Sewage and 15 in Air respectively.

When the fungal species diversity was analyzed in relation to different classes, it has been observed that the maximum number species recorded belongs to Hyphomycetes (12 genus 42 species). This was followed by Zygomycetes with 4 genus; 6 species. Among the Hyphomycetes, *Aspergillus* was the dominant represented by 29 species followed by *Penicillium*, and *Cladosporium* (2 species).

### Isolation of fungi from soil samples

Form this totally 35 fungal species were isolated and enumerated from Normal soil, Garden soil and Rhizosphere soil.
In normal soil, 12 fungi were isolated. Among these, *Aspergillus* was the dominant genus represented by 8 species.
15 fungal species were isolated and enumerated from Garden soil. In this, 2 with Zygomycotina and remaining 13 species were belongs to Deuteromycotina. Among the isolated fungi in garden soil, *Aspergillus* was common genus with 10 species.

In Rhizosphere soil, totally 16 fungal members were isolated and enumerated. In this, one with Mastigomycotina, 4 with Zygomycotina and remaining 11 were belonged to Deuteromycotina. Out

of 16 fungi isolated in rhizosphere soil, *Aspergillus* was dominant genus represented by 8 species, followed by *Rhizopus* with 2 species. This was well accepted with previous reports given by Zak (1992), *Absidia, Aspergillus, Chatomium, Fusarium, Mucor* and *Penicillium* were commonly isolated from soil using soil-dilution technique. Stamts and Chilton(1983) reported that the 111 fungi were isolated from 64 different soil samples (Tables1,2,3).

**Table1 Isolation of fungi from normal soil**

| S. No | Name of the fungi |
|---|---|
| | **Deuteromycotina** |
| 1. | *Aspergillus flavus* |
| 2. | *Aspergillus oryzae* |
| 3. | *Aspergillus quercinus* |
| 4. | *Aspergillus* terreus |
| 5. | *Aspergillus paramensis* |
| 6. | *Aspergillus sulphureus* |
| 7. | *Aspergillus repens* |
| 8. | *Aspergillus sydowi* |
| 9. | *Penicillium funiculosum* |
| 10. | *Alternaria alternata* |
| 11. | *Periconia* sp. |
| 12. | *Ascochyta vulgaris* |

**Table 2 Isolation of fungi from garden soil**

| S. No | Name of the fungi |
|---|---|
| | **Zygomycotina** |
| 1. | *Aphanomyces* sp. |
| 2. | *Rhizopus nigricans* |
| | **Deuteromycotina** |
| 3. | *Aspergillus conicus* |
| 4. | *Aspergillus flavus* |
| 5. | *Aspergillus fumigatus* |
| 6. | *Aspergillus ochraceous* |
| 7. | *Aspergillus oryzae* |
| 8. | *Aspergillus paramensis* |
| 9. | *Aspergillus repens* |
| 10. | *Aspergillus* sulphureus |
| 11. | *Aspergillus terreus* |
| 12. | *Aspergillus versicolor* |
| 13. | *Penicillium janthinellum* |
| 14. | *Trichoderma* sp. |
| 15. | *Fusarium oxysporum* |

**Table 3 Isolation of fungi from Rhizosphere soil**

| S. No | Name of the fungi |
|---|---|
| 1. | **Mastigomycotina** |
| 2. | *Pythium* sp. |
| | **Zygomycotina** |
| 3. | *Blackslea* sp. |
| 4. | *Mucor* sp. |
| 5. | *Rhizopus nigricans* |
| 6. | *Rhizopus oryzae* |
| | **Deuteromycotina** |
| 7. | *Aspergillus flavus* |
| 8. | *Aspergillus granulosis* |
| 9. | *Aspergillus nidulans* |
| 10. | *Aspergillus niger* |
| 11. | *Aspergillus* ochraceous |
| 12. | *Aspergillus oryzae* |
| 13. | *Aspergillus terreus* |
| 14. | *Aspergillus terricola* |
| 15. | *Botrytis* sp. |
| 16. | *Verticillium* sp. |
| | *Fusidium viridae* |

## Isolation of fungal from Rhizoplane

From the Rhizoplane, totally 16 fungi were isolated and enumerated. In this, one with Ascomycotina and remaining 15 were belonged to Deuteromycotina. Among the isolated fungi from the rhizoplane *Aspergillus* was the common genus with 12 species (Table 4).

**Table 4 Isolation of fungi from Rhizoplane**

| S. No | Name of the fungi |
|---|---|
| 1. | **Ascomycotina** |
| 2. | *Neurospora crassa* |
| | **Deuteromycotina** |
| 3. | *Aspergillus erythrocephalis* |
| 4. | *Aspergillus flaviceps* |
| 5. | *Aspergillus fumigatus* |
| 6. | *Aspergillus janus* |
| 7. | *Aspergillus luchuensis* |
| 8. | *Aspergillus oryzae* |
| 9. | *Aspergillus quercinus* |
| 10. | *Aspergillus repens* |
| 11. | *Aspergillus sacchari* |
| 12. | Aspergillus sydowi |
| 13. | *Aspergillus terreus* |
| 14. | *Aspergillus wentii* |
| 15. | *Cladosporium tenuissimum* |
| 16 | *Helminthosporium sp.* |
| | *Fusarium oxysporum* |

## Isolation of fungal from water samples (well water)

Totally 13 fungal species were isolated and identified from the water sample (well water). Among these, 1 with Zygomycotina, 1 with Ascomycotina and 11 with Deuteromycotina (Table 5) . In this, *Aspergillus* was the dominant genus represented by 8 species. *Achlya, Saprolegnia, Pythium* and *Aphanomyces* were isolated from pond water samples and their of isolated fungi frequency was calculated by Hyun Chul Park *et al*., (1973).

**Table 5 Isolation of fungi from water sample :- (well water)**

| S. No | Name of the fungi |
|---|---|
| | **Zygomycotina** |
| 1. | *Aphanomyces sp.* |
| | **Ascomycotina** |
| 2. | *Neurospora crassa* |
| | **Deuteromycotina** |
| 3. | *Aspergillus flavus* |
| 4. | *Aspergillus fumigatus* |
| 5. | *Aspergillus glacus* |
| 6. | *Aspergillus ochraceous* |
| 7. | *Aspergillus* oryzae |
| 8. | *Aspergillus repens* |
| 9. | *Aspergillus sydowi* |
| 10. | *Aspergillus versicolor* |
| 11. | *Pencillium janthinellum* |
| 12. | *Cladosporium tenuissimum* |
| 13. | *Fusidium viridae* |

## Isolation of fungal form sewage sample

From the sewage sample, total 13 species of fungi were isolated and identified. Out of 13 fungal species, one with Mastigomycotina, 2 with Zygomycotina and remaining 10 were belongs to Deuteromycotina (Table 6). Among these, *Aspergillus* was the common genus represented by 7 species. *Alternaria, Aspergillus, Cladosporium, Fusarium* and *Penicillium* found commonly as terrestrial fungi but frequently isolated from sewage sample was reported by Park (1972). Harvey(1952); Suzuki(1960) and Cooke and Bartsch(1959 and 1960), studied the fungi in sewage and more or less polluted water samples. Suzuki (1960) found that species of *Saprolegnia* and *Achlya* occurred the sewage effluent, where as species of *Aphanomyces* and *Pythium* were restricted to the entrance of the effluent.

**Table 6 Isolation of fungi from sewage sample**

| S. No | Name of the fungi |
|---|---|
| | **Mastigimycotina** |
| 1. | *Achyla* sp. |
| | **Zygomycotina** |
| 2. | *Mucor* sp. |
| 3. | *Rhizopus nigricans* |
| | **Ascomycotina** |
| 4. | *Neurospora crassa* |
| | **Deuteromycotina** |
| 5. | *Aspergillus carbonarius* |
| 6. | *Aspergillus conicus* |
| 7. | *Aspergillus flavus* |
| 8. | *Aspergillus gramlosis* |
| 9. | *Aspergillus ochraceons* |
| 10. | *Aspergillus oryzae* |
| 11. | *Aspergillus versicolor* |
| 12. | *Cladosporium uredinicolo* |
| 13. | *Verticillium* sp. |

## Isolation of fungi from Air samples

Totally 15 fungal species were isolated and enumerated from the air sample. In this, 2 with Zygomycotina, one with Ascomycotina and of 15 species of Deutromycotina (Table 7). *Aspergillus* was dominant genus with represented by 10 species. *Cladosporium tenuissimum* was also isolated form the air sample. Levetin (1995) reported that the spores are discharged from fungi growing as saprophytes or parasites in all the environments including atmospheric air.

**Table 7 Isolation of fungi from Air sample**

| S. No | Name of the fungi |
|---|---|
| | **Zygomycotina** |
| 1. | *Mucor recemosus* |
| 2. | *Rhizopus nigricans* |
| | **Ascomycotina** |
| 3. | *Neurospora crassa* |
| | **Deuteromycotina** |
| 4. | *Aspergillus candidus* |
| 5. | *Aspergillus clavatus* |
| 6. | *Aspergillus flavus* |
| 7. | *Aspergillus fumigatus* |
| 8. | *Aspergillus ochraceous* |
| 9. | *Aspergillus oryzae* |
| 10. | *Aspergillus querainus* |
| 11. | Aspergillus sydowi |
| 12. | *Aspergillus terricola* |
| 13. | *Aspergillus wentii* |
| 14. | *Cladosporium tennissimum* sp. |
| 15. | *Curvularia* sp. |

## Density and frequency of fungi

### Density of fungi

In Rose Bengal agar, the fungal density was calculated and the density (D) were found to 16 species.The fungal density was calculated from all the sample in SDA and the density was found to 21 species.

### Frequency of occurrence of fungi

The frequency of occurrence in all the samples wee calculated (in percentage) and it was represented in the following frequency groupings per sample.

Frequency → 7.9
Less frequency → 4 – 6 %
Rare → 1 - 3 %

In Mastigomycotina, 2 fungal species were formed to less frequency occurred. In Zygomycotina, *Mucor* sp. and *Rhizopus nigricans* were frequently occurred. *Neurospora crassa* were frequently occurred in Ascomycotina.

In Deuteromycotina, *Aspergillus* and *Cladosporium* were occurred frequently. Among the *Aspergillus* sp. *A.clavatus, A.flavus A.fumigatus, A. luchuensis, A. nidulans. A. niger , A. ochraceous, A oryzae, A. sulphreus, A.sydowi, A. terreus* and *A. versicolor*.

The genus of *Cladosporium, C.tenuissimum* were frequently occurred.
Total 52 fungal species, 16 fungi, were found frequently, 24 fungi wee less frequently occurred and remaining 12 species were rarely occurred (Table 8).

Hyun Chul Park *et al*., (1973) studied that the *Achlya, Saprolegnia, Pythium,* and *Aphanomyces* were isolated from pond water samples and their of isolated fungi frequency was calculated. In frequency of fungi, *Achlya* and *Aphanomyces*, each of which were isolated from approximately 33% of the samples.

**Table 8 Total number of fungi isolated and their frequency**

| Name of the fungi | Frequency |
|---|---|
| **Mastigomycotina** | |
| *Achlya* sp. | ++ |
| *Pythium* sp. | ++ |
| **Zygomycotina** | |
| *Aphanomyces* sp. | + |
| *Blackslea* sp. | + |
| Mucor recemosus | + |
| *Mucor* sp. | +++ |
| *Rhizopus nigricans* | +++ |
| *R.oryzae* | ++ |
| **Ascomycotina** | |
| *Neurospora crassa* | +++ |
| **Deuteromycotina** | |
| *Aspergillus candidus* | ++ |
| *Aspergillus carbonarius* | ++ |
| *Aspergillus clavatus* | +++ |
| Aspergillus conicus | ++ |
| *Aspergillus erythrocephalis* | ++ |
| *Aspergillus flavus* | +++ |
| *Aspergillus flariceps* | + |
| Aspergillus fumigatus | +++ |
| *Aspergillus fumiculosis* | ++ |
| *Aspergillus granulosus* | ++ |
| *Aspergillus glaucus* | + |
| *Aspergillus janus* | + |
| *Aspergillus luchuemsis* | +++ |
| *Aspergillus nidulans* | +++ |
| Aspergillus niger | +++ |
| *Aspergillus ochraceous* | +++ |
| Aspergillus oryzae | +++ |
| *Aspergillus paramensis* | + |
| *Aspergillus quercinus* | ++ |
| *Aspergillus repens* | ++ |
| Aspergillus secchari | ++ |
| *Aspergillus sulphureus* | +++ |
| *Aspergillus sydowi* | +++ |
| *Aspergillus tamerii* | ++ |
| *Aspergillus terreus* | +++ |
| *Aspergillus terricola* | + |
| *Aspergillus ustus* | ++ |
| *Aspergillus versicolor* | +++ |
| *Aspergillus wentii* | ++ |
| *Botrytis* sp. | ++ |
| *Penicillium jonthinellum* | ++ |
| *Penicillium funiculosum* | ++ |
| *Trichoderma* sp. | ++ |
| *Verticillium* sp. | + |
| *Alternaria alternata* | ++ |
| *Cladosporium cladosporides* | ++ |
| *Cladosporium tenuissimum* | +++ |
| *Curvularia* sp. | ++ |
| *Fusidium viridae* | + |
| *Helminthosporium* sp. | ++ |
| *Periconia* sp. | ++ |
| *Fusarium oxysporum* | + |
| *Ascochyta vulgaris* | + |

## Screening of enzymes from the isolated fungi:

### Amylase enzyme

14 species of fungi were selected for the screening of analyzes and protease enzyme out of 14 fungal species. 9 were found to positive for the enzyme production. In this Aspergillus was found to mostly producers of amylase enzyme which include species of *Aspergillus, A.candidus, A.fumigatus, A.flavus, A.oryzae,* and *A.terricola*. Ellaiah *et al.,* (2003) reported that the 30 soil samples screened for isolation of alpha amylase producing fungi.200 fungi were isolated and 100 of them were found to posses amylolytic activity by steak method plate method. Among these, *Aspergillus* species was found to posses the highest activity.

### Protease enzyme

Out of 14 species of fungi 12 fungal species were found to positive for protease enzyme. *Aspergillus* was found to most producers of protease enzyme which include *A.candidus, A.conicus , A.fumigatus, A.flavus, A.orzyae, A.tamerii, A.terreus, A.terricola* and *A.versicolor*. In *Cladosporium* sps. *C. tenussimum* was found to positive for enzyme screening (Table 9) .This well agreed with previous reports by Henryk (1998), *Aspergillus* species are highly variable and widespread and majority of species are non-pathogenic and non-toxin forming .The most frequency used species for enzyme production are the *A.niger* and *A.oryzae* groups.Intracelluar enzyme with properties similar to cysteine proteinase have been reported in *Trichosporon* sp, *Oidiodendron kalrai* and *Nannizzia fulva*. Extracellular cysteine protease have been observed in *Microsporium* sp,*A.oryzae* and *Sporotrichum rulentum* (Kalisz,1988).

**Table 9 Screening of amylase enzyme protease enzyme**

| S.No | Name of the fungi | Amylase | Protease |
|---|---|---|---|
| | **Mastigomycotina** | | |
| 1. | *Achyla* sp. | + | - |
| | **Zygomycotina** | | |
| 2. | *Blakslea* sp. | + | + |
| | **Deuteromycotina** | | |
| 3. | *Aspergillus candidus* | + | + |
| 4. | *Aspergillus conicus* | - | + |
| 5. | *Aspergillus fumigatus* | + | + |
| 6. | Aspergillus flavus | + | + |
| 7. | *Aspergillus oryzae* | + | + |
| 8. | *Aspergillus tamerii* | - | + |
| 9. | *Aspergillus terreus* | - | + |
| 10. | *Aspergillus terricola* | + | + |
| 11. | *Aspergillus versicolor* | - | + |
| 12. | *Penicillium furiculolosum* | + | + |
| 13. | *Cladosporium cladosporides* | + | - |
| 14. | *Cladospoium tenuissimum* | - | + |

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