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STUDY OF FUNGAL DIVERSITY OF ZOOPORIC FUNGI IN POLLUTED YAMUNA RIVER FROM DIFFERENT CATCHMENT AREAS OF DELHI-NCR, INDIA

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ABSTRACT:- There is enormous pollution increasing day by day in the environment due to surplus increase of population and limitation of resources. All the important parts and habitats of animals have been destroyed and humans are exploiting the earth in his own disastrous manner. Water resources are also polluted hence forth. These water resources are not only useful for human's usage but is also the habitat of aquatic flora and fauna. The water pollution is adversely affecting the aquatic life and is no longer safe for humans. It is found that, with the increase in pollution in water bodies, some filamentous Zoosporic fungi gets accumulated and are thus are regarded as indicators of severe water pollution. The present study is hereby revealing the biodiversity of such Zoosporic fungi as per the geographical distributions and seasonal variations in different catchment areas of river, Yamuna.

Keywords: Catchment areas, Delhi-NCR, Filamentous fungi, geographical distributions, polluted water, seasonal variations, Yamuna river, Zoosporic fungi

1. INTRODUCTION

River, the natural stream of water comes from a Latin word- ripa "riverbank "plays an important role in human life by providing fresh water, fertile land and a major mode of transportation. Rivers provide excellent habitat and food for the aquatic creatures. It is unfortunate that, certain extensions of River Yamuna between Wazirabad barrage and Chambal river confluence are critically polluted and 22 km of Delhi range is the maximum polluted amongst all are much polluted. Various urban centers e.g., Delhi, Mathura, Agra etc., which are located on the banks of Yamuna river, draw fresh river water for various activities. The entire sewage generated by Delhi and NCR is discarded into the river. It leads to a major deterioration of Yamuna River water quality from urban agglomeration of Delhi up to Chambal River

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Confluence. Yamuna starts polluting by mixture of different pesticides and variety of fertilizers from Haryana thereafter, at Delhi. Nineteen known drains of Delhi drop into the Yamuna. On account of Delhi city poor sewage disposal, consequently sewage is discharged into these drains, and subsequently into the river Yamuna. Water-borne diseases constitute one of the major public health hazards in developing countries. Worldwide, in 1995, contaminated water and food caused more than 3 million deaths, of which more than 80% were among children under age five. Besides the conventional pathogens which are transmitted by water, several emerging water-borne pathogens have become increasingly important during the last decade or so The diseases due to fungi are Aspergillosis caused by different species of *Aspergillus,* Blastomycosis caused by *Blastomyces dermatitidis,* Candidiasis by *Candida albicans,* Cryptococcosis *Cryptococcus neoformans* Dermatomycosis by *Geotrichum candidum* and *Scytalidium dimidiatum,* Histoplasmosis b**y** *Histoplasma capsulatum* var. *capsulatum* Mycetoma by *Acremonium falciorme,* and *Acremonium recifei,* Zygomycosis by *Absidia corymbifera, Rhizomucor pusillus, Rhizopus arrhizus* and *Rhizopus oryzae* etc. Otomycosis by *Aspergillus fumigatus, Aspergillus niger, Candida albicans* and *Candida tropicalis* etc. Apart of above, other diseases like liver necrosis, nephritis and irritation of the gastrointestinal mucosa can produce cutaneous and nasal mucous membrane ulcers, dermatitis and cancer induces allergic reactions and contact dermatitis, Fungi may also cause conjunctivitis, eosinophilic, pneumonitis, and a potential human carcinogen resulting into lungs and nasopharyngeal cancers. Many fungi cause serious plant diseases while amongst zoosporic fungi like *Allomyces*, *Aphanomyces*, *Achlya*, *Dictuchus Saprolegnia*, *Pythium, Phytophthora* and are common and widespread fungal killers of fishes in addition to different genera of *Chytrids* groups on water amphibians. The study has been undertaken in understanding of the (filamentous and zoosporic) fungal diversity, their seasonal variation for the first time from Yamuna water of Delhi catchment area [1-3]. The study was thus subjected to determine the biodiversity of filamentous Zoosporic fungi under different environmental conditions passing through Yamuna catchment areas of Delhi region. The study was determined to study the relationship between seasonal variation on zoosporic fungi.

2. MATERIALS AND METHODS

2.1 Collection of samples from Delhi catchment area

During the present study, water and soil **s**amples were collected every month of winter, spring, summer, monsoon and autumn seasons from December 2015 to November 2016) from five different location of

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Yamuna river viz. Khyber Pass, ISBT, Maharani Bagh, Kalkaji and Sarita Vihar of Yamuna river bank. The water and soil samples collected from each location were stored and kept in icebox during the transportation to the laboratory.

2.2 Isolation and screening of fungus

2.2.1 Direct Incubation Method

The substrate (plant decaying parts and leaves, soft fresh woody material, scales, gills, fins of fishes with soil sediments were collected from five different sites of heavily polluted Yamuna water. Such material was turned into small pieces and place on triple folded previously wetted blotters in petriplates. Thereafter, these plates were incubated for 8-12 days at 20-25°C. After the incubation period, the colonies of zoosporic fungi having moist wetted cottony consisting white mycelium were examined under stereio-binocular. The fungal growth picked up aseptically by needle or forceps and transferred on a sterilized petriplates containing maize meal media incubated at room temperature and examined for zoosporic fungi through compound research microscope [4, 5].

2.2.2 Baiting Method

For isolation of Zoosporic fungi, Hemp seeds were used as baits. The seeds were first boiled for 10-15 minutes thereafter their cotyledons removed then dried placing in between two layers of dried sterilized blotter paper. The pre half-filled Petri plates with Yamuna river water taken out, the lid removed and earlier dried hemp seed floated on the surface of water. Such plates were kept in incubated at 20 - 25^0 C for 4-6 days. Thereafter, colony growth developing on baits was examined directly under stereo binocular for the zoosporic fungi. The sporangia developed of zoosporic fungi were picked up trough inoculating needle aseptically, dried further in between blotter sheets. After drying of sporangia, along with mycelium if any transferred-on culture media preferably maize meal or oatmeal in petriplates or culture tubes. Such plates or tubes again were incubated as earlier. After incubation a piece of growing mycelium is picked up and floated and baited as before and examined on glass slide under compound microscope for final identification at species level using of various standard monographs of Zoosporic fungal organisms [6-8].

2.2.3 Slide cultures

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The zoosporic conidial apparatus were delicate and difficult to mount successfully. The method of slide culture permits fungi to be studied virtually in situ, with as little disturbance as possible. A small block (7 x 7 x 2 mm) was cut from agar (1 %) in a Petri dish and was placed on a sterilized slide. The zoosporic fungi was inoculated at the center of each side of the agar square. A sterile cover slip was placed on the top of the agar and slides were placed in a damp chamber (supported on a pair of glass rods in a petri dish lined with damp filter paper) in an incubator under optimum temperatures, normally at 28 °C. After about six days the hyphae and sporangia get appeared and starts adhered to the lower side of cover slip. The cover slip was removed and placed colony in a drop of lactophenol cotton blue on a clean slide. The slides were then sealed using parafilm.

2.2.4 Depression slide method

The zoosporangia sporangia picked up from baits placed into depression slide or watch glass with four or eight two drops or 1-2 ml of distilled water and observed constantly the release of zoospores from sporangia. On release the swimming zoospores were picked with clean sterilized pipette and zoospore suspension transferred by dropping zoospore suspension on the plate on agar plate at pre marked points. Such plate's lid wrapped with parafilm to avoid evaporation, plates kept inverted for incubation and examined for identification.

2.3 Isolation of filamentous fungi

2.3.1 The soil sprinkles method

The soil sediment samples were collected around the years. The moistened soil samples were sun dried than sieved to get powdered sail. Out of sieved soil approximately 2 mg soil from each sample was sprinkled over the surface of each agar plate. A total of ten plates were sprinkled of each sample and then incubated at 25°C. The numbers of colonies developed were counted after 4^{th} – 7^{th} day of incubation and the number of fungi per gm of dry soil was calculated. The colonies developed of each species on Petri dishes were counted under stereo binocular microscope and expressed as number of colonies forming units (CFU) per gm of dry soil.

2.3.2 To estimate percentage frequency of an individual species

It can be calculated as,

Percent frequency of species= [(Total No. CFU in a sample)/ (Total number of CFU of a species)] x 100

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2.3.3 Soil dilution plate method

Soil fungi were isolated by soil dilution techniques. Soil sediment samples were collected from catchment areas of Yamuna river were first dried to remove the moisture contents, sieved followed with collection of fine soil powder. Thereafter, four cultures tubes were taken, the first tube was filled with 10 ml sterile water and other three tubes with 9 ml each of sterile water. These all four tubes were arranged in culture tubes. The dilutions were prepared as per following methodology and formulations.

2.3.4 Method to prepare soil dilution

Once the required soil dilution is prepared, was then poured by pour plate method on previously prepared 10 petri plates for each dilution of per soil sample containing potato dextrose agar medium. These plates were incubated at 25^0 C temperature for 5-7 days and were constantly examined for colony forming unit (CFU). These CFU was calculated per gm of soil samples and to determine the effect of seasonal variation on fungi species.

2.3.5 Calculation of total and individual fungal occurrence

All the calculation was made in terms of percentage. The total fungal occurrence during the entire sampling period was calculated by the number of fungi that appeared in the samples as against the total number of samples incubated, which was calculated by using the following formula.

Total percent (%) of fungal occurrence = [(Number of samples in which fungi appeared)/ (Total number of samples plated for isolation)] x 100

2.3.6 The individual fungal occurrence of each species

It can be calculated as,

Individual fungal occurrence = [(Individual fungal species that appeared on samples)/Number of colonies of all fungi that grow on the samples)] x 100

2.4 Microscopic examination

2.4.1 Whole mount

Slides were prepared from the 4 days old cultures growing baits. A small bit of hyphae or sporangia were taken was picked up by an inoculating needle from sporulating portion and put on to a slide in a drop of lactophenol acid fuchsine staining solution. Slides were slightly heated over the Bunsen flame and covered by cover slip. Excess mounting fluid was drained off with clean blotting paper strips.

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2.4.2 Stains and mounting media

2.4.2.1 Lactophenol cotton blue staining

Temporary slides were prepared by using Lactophenol-cotton blue stain for microscopic examination of morphology of conidia and conidiophores.

2.4.2.2 Lactophenol-acid Fuchsin

For preparation of temporary slides, Lactophenol-acid Fuchsin stain was also used. For this, 100 ml, lactophenol cotton blue was mixed with 1 to 5 ml of 1 % aqueous solution of acid Fuchsin.

2.4.2.3 DPX Mounting media

For preparing permanent slides, DPX mounting media was used. It has the advantage of rapid setting quality and does not require sealing with the wax. DPX is a colorless oily liquid which can be used directly for materials stained in lactophenol-cotton blue. For mounting stained material, the DPX mount is placed over the material. Due to its viscous nature, excess cotton blue diffuses out from the material thus giving a colorless background.

2.5 Micrometry

It is the measurements of the dimensions of conidiophores and conidia were done with the aid of ocular and stage micrometer. Calibration of monocular microscope was done with the help of ocular micrometer (I mm = 100 divisions) and stage micrometer (1 division = 0.01 mm) using l0 X, 40 X and 100 X objective lenses and l0 X and 15 X eye pieces. Twenty-five measurements of different conidiophores, conidia and spores were taken.

2.6 Camera Lucida drawing

Camera Lucida drawings were made of Zoosporic and filamentous fungi showing all possible details of morphology and ontogeny of reproductive parts (sporangia, sexual structures and zoospores etc.) under different magnification of compound microscope.

3. RESULTS AND DISCUSSION

The results of the present investigation revealed, total twenty-nine zoosporic fungi from Khyber Pass, ISBT, Maharani Bagh, Kalkaji and Sarita vihar along the cultivated land area during 1-year time period viz. from December 2015 to November, 2016. The study for isolation and identification of zoosporic fungi was conducted from five different catchment areas of Delhi viz. Khyber Pass, ISBT, Maharani

Bagh, Kalkaji and Sarita Vihar. The results are shown in **Tables 1-4.** The observation on seasonal variations were found closely parallel to findings [9-12] who observed large number of zoosporic fungi in different season at their place of study and observation.

4. CONCLUSION

The results of the present study suggest that, there is surplus biodiversity of microbes in the different catchment areas of river Yamuna. This fungal biodiversity varies as per seasonal variations. The enormous pollution in water bodies and their sediments enhances the microbial load and thus the biodiversity of such fungal isolates as reported in the study. The results of the study can be utilized as the basis to determine the indicators of water pollution of rivers, lakes and pond.

5. CONFLICTS OF INTEREST

Authors don't have any conflicts of interest.

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S.No.	Name of Fungi isolated from different stations of Yamuna	Khyber Pass		ISBT		Maharani Bagh		Kalkaji		Sarita vihar	
		CFU	%	CFU	%	CFU	%	CFU	%	CFU	%
01	Achlya *bisexualis*	1	2.63	0.	0.00	3	6.00	2	4.25	3	5.08
02	*Achlya diffusa*	0	0.00	2	4.44	0	0.00	2	4.25	2	3.38
03	*Achlya flagellate*	2	5.26	1	2.22	2	4.00	3	6.38	0	0.00
04	*Achlya klebsiana*	0	0.00	3	6.66	0	0.00	1	2.12	2	3.38
05	*Achlya oblongata*	1	2.63	4	8.88	0	0.00	0	0.00	3	5.08
06	*Achlya proliferoides*	3	7.89	0	0.00	1	2.00	0	0.00	5	8.47
07	*Achlya prolifera*	2	5.26	2	4.44	4	8.00	2	4.25	4	6.77
08	*Allomyces anomalous*	2	5.26	0	0.00	2	4.00	4	8.51	0	0.00
09	*Allomyces arbuscula*	1	2.63	0	0.00	0	0.00	1	2.12	2	3.38
10	*Aphanomyces laevis*	2	5.26	1	2.22	0	0.00	0	0.00	1	1.69
11	*Dictuchus sterilis*	2	5.26	2	4.44	5	10.0	2	4.25	3	5.08

Table 1: Diversity Zoosporic fungi from five different catchment areas in Dec., 2015 to Nov., 2016

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S.No.	Name of Zoosporic Fungi	Total CFU	Percentage Diversity (%)
01	Achlya *bisexualis*	09	3.89
02	*Achlya diffusa*	06	2.59
03	*Achlya flagellate*	08	3.46
04	*Achlya klebsiana*	06	2.59
05	*Achlya oblongata*	08	3.46
06	*Achlya proliferoides*	09	3.89
07	*Achlya prolifera*	14	6.06
08	*Allomyces anomalous*	08	3.46
09	*Allomyces arbuscula*	04	1.73
10	*Aphanomyces laevis*	04	1.73
11	*Dictuchus sterilis*	14	6.06

Table 2: Total Diversity of Zoosporic fungal from five different collection sites of Yamuna catchment

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Table 3: Seasonal variation of Zoosporic fungi during five different Seasons of Yamuna catchment area, New Delhi in December 2015 to November, 2016

Fungi isolated from Yamuna during different seasons	Winter Dec., 2015 & Jan , 2016 (5^0-25^0C)		Spring Feb., 2016 to March 2016 (20^0-25^0C)		Summer April 2016 to June 2016 (25^0-45^0C)		Monsoon July 2016 to Sep., 2016 (30^0C -35^0C)		Autumn Oct., 2016 to Nov., 2016 (20^0C -30^0C	
	Mean CFU	%	Mean CFU	%	Mean CFU	%	Mean CFU	%	Mean CFU	%
Achlya *bisexualis*	0	0.00	2	3.22	1	1.88	3	6.00	2	4.08
Achlya diffusa	2	3.57	3	4.83	2	5.77	2	4.00	3	6.12
Achlya flagellate	1	1.78	2	3.22	1	1.88	2	4.00	0	0.00
Achlya klebsiana	3	5.35	2	3.22	1	1.88	4	8.00	1	2.04
Achlya oblongata	1	1.78	3	4.83	2	5.77	2	4.00	2	4.08
Achlya proliferoides	3	5.35	2	3.22	2	5.77	1	2.00	1	2.04
Achlya prolifera	2	3.57	3	4.83	2	5.77	3	6.00	0	0.00
Allomyces anomalous	2	3.57	2	3.22	2	5.77	2	4.00	3	6.12
Allomyces arbuscula	1	1.78	2	3.22	0	0.00	1	2.00	1	2.04
Aphanomyces laevis	3	5.35	4	6.45	2	5.77	3	6.00	2	4.08
Dictuchus sterilis	3	5.35	2	3.22	3	5.66	2	4.00	3	6.12

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Table 4: Average seasonal variation of each Zoosporic fungi from total five different seasons of Yamuna catchment areas in December 2015 to November, 2016

S.No.	**Name of fungi**	**Total CFU**	**Percentage of Each species**
01	Achlya *bisexualis*	08	2.96
02	*Achlya diffusa*	12	4.44
03	*Achlya flagellate*	06	2.22
04	*Achlya klebsiana*	11	4.07
05	*Achlya oblongata*	10	3.70
06	*Achlya proliferoides*	09	3.33
07	Achlya prolifera	10	3.70
08	*Allomyces anomalous*	11	4.07
09	*Allomyces arbuscula*	05	1.85
10	*Aphanomyces laevis*	14	5.18
11	*Dictuchus sterilis*	13	4.81