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Diversity of Heavy Metals Tolerant and Antifungal Sensitive Fungal Community of River Ganga.

Diversidad de la comunidad de hongos tolerantes y antimicóticos sensibles al hongo del río Ganges.

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ABSTRACT

Ganga is the largest river in India and has both religious and economical importance to our country. Ganga water has very important reverence in various religious ceremonies as holy water along it had been used for drinking and irrigation purpose. In developing cites such as Haridwar Ganga start facing water pollution problems but still its water quality was maintain, it was may be due to its microbial community which may have an adorable capability to clean the Ganga river, All the isolated strains had showed highest heavy metal tolerance against As, Cu, Fe (200- 1000 mg/L) followed by Cr, Ni, Cd (200-800 mg/L) and the lowest tolerance to Hg (200-400 mg/L), along with this these strains are mostly sensitive to different antifungal such as Nystatin, Amphotherecin, Fluconazole and Ketomycin.

Keywords: Ganga, Haridwar, Fungal Community, Heavy Metals, Antifungal

RESUMEN

Ganges es el río más grande de la India y tiene importancia religiosa y económica para nuestro país. El agua de Ganges tiene una reverencia muy importante en varias ceremonias religiosas, ya que el agua bendita a lo largo de ella se había utilizado para beber y para riego. En ciudades en desarrollo, como Haridwar Ganga, comienzan a enfrentar problemas de contaminación del agua, pero aún así se mantuvo la calidad del agua, puede deberse a su comunidad microbiana, que puede tener una capacidad adorable para limpiar el río Ganges. Todas las cepas aisladas mostraron el metal pesado más alto. tolerancia contra As, Cu, Fe (200-1000 mg / L) seguido de Cr, Ni, Cd (200-800 mg / L) y la tolerancia más baja a Hg (200-400 mg / L), junto con estas cepas son principalmente sensibles a diferentes antimicóticos como la nistatina, la anfotericina, el fluconazol y la cetomicina.

Palabras clave: Ganga, Haridwar, Comunidad fúngica, Metales pesados, Antifúngico

INTRODUCTION

Ganga River has the largest basin in India, Draining as much as 10, 60,000 km^2 of country area (Bilgrami, 1991). After the run of about 280 km it's reaches Haridwar, Haridwar is a developing city and it has it's religious value too as it is located on the banks of river Ganga, it provide water to the city to meet their various requirement and in return the city disposed off the waste into the river along with this the festival bathing and consignment of half burnt bodies also add to the level of pollution of river Ganga (Shukla and Asthana, 1995)There are many sources of water pollution, but two main general categories exist: direct and indirect contaminant sources. Direct sources include effluent outfalls from industries, refineries contaminants that enter to water supply from soils/ground water systems and from the atmosphere via rain water. Some organic water pollutants include industrial solvents, volatile organic compounds, insecticides, pesticides and food processing wastes.Inorganic water pollutants that come from industrial discharge include heavy metals (Abdi and Kazemi, 2015) and (Sharma and Singh, 2018). The ability of microbial biomass to remove heavy metal ions from polluted aquatic systems has been reported and has also attracted much interest in recent years. Micro-organisms in fresh water environments can be divided on the bases of their feeding habits into two major groups, one the autotrophs which synthesize their complex carbon compound from environmental CO_2 this group involve microalgae and photosynthetic bacteria while the second are heterotrophs it include saprotrophy i.e. obtain their food from non- living material either by direct uptake of soluble compounds or indirect uptake by secretion of external enzymes followed by absorption of the hydrolytic product. Biological analysis of the environment is helpful in determining the health of the ecosystem, there is a correlation between chemical constituents of organic and inorganic nature coupled with physical attributes of the water body and microbial profile. The international Convention on Biological Diversity (CBD) defines biodiversity as "the variability among living organisms from all sources including, inter alia, terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part" (UNEP, 1992).For those concerned with quantitative assessments of biological diversity, the key issue in the CBD definition is how to measure variability quantitative indexes of biological diversity that are sensitive to environmental change have relied on three major concepts, namely scale, component, and viewpoint (Van Kooten, 1998). The scale aspect focuses on the criteria of species richness and the geographical distributions of individuals among the species (evenness). Species richness within a local ecosystem is referred to as alpha diversity. The variation in alpha diversity among ecosystems within the same landscape is referred to as beta diversity, and, when measurable, gamma diversity represents species richness at the regional and global scales. The simplest measures of species diversity rely only on the number of species (s) and the total number of individual Community representing all the species (N). For example, the Margalef index (Dm) computes the species diversity according to the following equation (Margalef, 1958 and 1963)

$$Dm = (s-1) + \sqrt{logN}$$

The Margalef index and similar indexes do not support the differentiation of communities that have identical s and N values because the evenness of the distribution of individuals within the communities is not considered. To correct this shortcoming, some investigators have introduced the concept of species dominance into measures of diversity. For example, Simpson (1949) demonstrated that if two individuals are selected randomly from a community, the probability (Pd) that the two individuals belong to the same species is a measure of dominance (ds), and it is given by the following equation:

$$df = 1 - Pd = 1 - \sum \quad ni\ (ni - 1) \div N\ (N - 1)$$

Where ni is the abundance of individual members belonging to species i. Simpson's measure of dominance has been modified to compute species diversity as follows:

$$Ds = 1 \div Pd = N(N - 1) \div \sum \quad ni\ (ni - 1)$$

Simpson's diversity index has been referred to as the probability of an interspecific encounter, which expresses the number of times required to select two independent individuals at random from the community before both are found to belong to the same species (Hurlburt, 1971; Brower et al., 1998). Estimates of Ds are based on the assumption that the data on the number of species and abundance of individual members are derived from randomly collected environmental samples. However, in cases where it is possible to conduct an exhaustive sampling of a community (e.g. microcosm experiments), where direct molecular methods that can capture the complete spectrum of diversity are used (see Figs. 11.2 and 11.3), or where other non-random methods are applied, modified Simpson's indexes of dominance (l) and diversity (Ds) are represented as follows:

$$\Delta s = 1 \div \lambda = N^2 \div \sum \quad ni^2$$

These probability-based measures of species diversity have not found as much use as measures based on the concept of uncertainty as defined by information-theoretic indexes. In a community with relatively low species diversity, there is a relatively high level of certainty that the identity of a species selected at random can be predicted. Conversely, in a relatively diverse community, the level of certainty in predicting the species identity of a randomly selected individual is low. The Shannon diversity index (H¢) is perhaps the best known of diversity measures rooted in information theory (Perkins, 1982; Shannon, 1963):

$$H' = -\sum \quad Pi\ log\ logPi'$$

Where pi is the fraction of the total number of individuals in the community that belong to species i. The equation can be rewritten to facilitate the calculation of H¢ without the need to convert abundances (ni) to proportions (pi) as follows:

$$H' = (N\ log\ logN - \sum \quad (ni\ log\ logni)) \div N$$

The microbial diversity has a great importance in the removal of heavy metal pollution in aquatic system. Bacteria (Rajkumar et al., 2009 and Rehman et al, 2008), Yeast (Chergui et al, 2007 and Ashwini et al, 2009), Fungi (Coreno- Alonso et al 2009 and Khambhaty et al, 2009) and Algae (Gupta et al, 2010) act as biosorbent. Out of these Fungi are ubiquitous in natural environment their cell wall and their components have a major role in biosorption process. Fungal cell walls and their components have a major role in biosorption and also take up suspended metal particulates and colloids. Fungi are ubiquitous in natural environments and important industrial processes. Their most important roles are as decomposers of organic material, with concomitant nutrient cycling as pathogens and symbiotic with animals and plants, and as spoilage organisms of natural and synthetic materials (Abdi and kazemi, 2015). White rot fungi are highly specialized groups of organisms. They are Basidomycetes, which include all the higher fungi that are characterized by their sexual fruiting bodies. Lentinussajor-cajuis a well known white rot, fungus whereas a little attention has been paid to the ability of its potential for the removal of mixed pollutants from environment (Yakup et al, 2004). So in present study we had focused on the isolation of river Ganga Fungal community during different season at four different sites, Haridwar, India. The isolated strains were morphological identified as *Aspergillus, Talaromyces, Fusarium, Curvularia*, etc.

MATERIAL AND METHODS

Site description: Four sampling sites selected for the study were as follows.

Site 1: (Bhimgodha) - (N 29o60’ 25.9’’/ E 078o 14’28.1’’). It is at 500m from Har kiPauri, it is a barrage on the Ganges River, it is a site of high tourist interest as this site has many mythological sites, and here the Ganga meets the waste of most of the hotels, dharamshala and residential sewage.

Site 2: (Kankhal) - (N 29o63’92.6’’/E 078o14’6.3’’). Kankhal is a small colony in Haridwar, Kankhal is one of Panchtirth within Haridwar. It is a site where according to Hindu religion ash of dead bodies made to dump in Ganga and various sewage in river.sewage opening dump direct

Site 3: (Prem- Nagar) - (N 29o55’48.8’’/E 078o 08’10.3’’). It is the site where one of the oldest ashram is situated along with this include the basic residential area of Haridwar and hence contribute to anthropogenic and mythological wastes.

Site 4: (Jwalapur) - (N 29o71’92.7’’/E 078o10’8.0’’). It is the site of Haridwar where a large number of sewage opening dump the human sewage directly in the river at Jatwara Bridge various human activities such as bathing, washing of clothes and disposal of industrial waste pollute Ganga River.

Sample collection: The sample was collected from all the 4 sites during 3 different seasons i.e. monsoon, winter’s and summer in sterile sampling bottles around 10 am at 30 cm depth.

Isolation and Enumeration of Fungi: Fungi were isolated from samples by the serial dilution method (Waksman and Fred, 1922). The 1ml of water sample was suspended in 9ml of sterile distilled water blanks and diluted up to (10 $^{-}$3). 0.1ml suspension of 10^{-1}, 10^{-2} and 10^{-3} were poured on Rose Bengal plates with 1ml of heavy metals stock solution and incubate them, further the enumeration of fungi community was done by determining Colony Forming Unit (CFU).

*Determination of Diversity Index:*Margalef index, Simpson's indexes,Shannon Wiener Diversity Index was determined (Shannon & Weaver, 1963).

Identification of Isolates: The isolates were identified as morph taxonomically at Agharkar Research Institute, Pune India. NFCCI. Pune.

Heavy metals tolerance ability: The Heavy metals tolerance ability of all Fungal isolates were calculated by well diffusion method (Hemambika et al., 2011). The PDA media was poured in Petri plates, allow to solidified and spread with 0.1ml of fungus culture and allow settling for 1 minute. Wells were prepared and poured with 0.1 ml of different concentration of different heavy metals and incubated at 28 ±1^{o}C and the metal tolerance ability was determined by determining the zone of inhibition as follows

$$Zone\ of\ inhibition = Diameter\ of\ Zone - Diameter\ of\ cork\ borer$$

Antifungal Sensitivity Test: The antifungal sensitivity of all the fungal isolates was carried out against four antifungal i.e. Nystatin, Amphotericin, Fluconazole and Ketomycin by disc diffusion method and determined by determining the zone of inhibition around the antifungal disc (Baurer et al., 1966).

RESULTS:

Enumeration of Fungi during different seasons are specified in table 1.

Table 1: Enumeration of Fungi community during different seasons.

Sites	Co-ordinate Elevation	Seasons	CFU ml^{-1} 10^{-1}	CFU ml^{-1} 10^{-2}	CFU ml^{-1} 10^{-3}
Bhimgodha		Winter	8.77 ± 0.33 ×10^{1}	7.15 ± 1.2 ×10^{2}	3.6 ± 0.33 × 10^{3}
	N29^{0}71'22.7''/ E078^{0}10'28.0''	Summer	10.78 ± 1.75 ×10^{1}	8.93 ± 0.33 ×10^{2}	5.63 ± 0.88 × 10^{3}
		Monsoon	15.23 ±1.2× 10^{1}	13.21± 1.75 × 10^{2}	10.33 ± 0.66 × 10^{3}
Kankhal		Winter	6.9 ± 1.2 × 10^{1}	4.4 ±0.33 × 10^{2}	3.12 ± 0.25 × 10^{3}
	N29^{0}55'48.8''/ E078^{0}08^{0}10.3''	Summer	10.72 ± 0.66 × 10^{1}	8.75 ± 1.2 × 10^{2}	6.23 ± 0.33 × 10^{3}
		Monsoon	12.83 ± 1.2 × 10^{1}	11.46 ± 0.88 ×10^{2}	5.16 ± 1.75 × 10^{3}
Prem-Nagar		Winter	5.8 ± 0.33× 10^{1}	2.7 ± 0.08 × 10^{2}	1.1 ± 0.03 × 10^{3}
	N29^{0}63'92.6''/ E078^{0}08^{0}14.63''	Summer	7.1 ± 1.66 × 10^{1}	5.2 ± 0.88 × 10^{2}	3.3 ± 0.23 ×10^{3}
		Monsoon	8.37 ± 0.23 × 10^{1}	7.12 ± 0.66 × 10^{2}	5.6 ± 1.2 ×10^{3}
Jwalapur		Winter	7.6 ± 0.33× 10^{1}	5.4 ± 0.66 × 10^{2}	3.3 ± 0.33 × 10^{3}
	N29^{0}60'25.9''/ E078^{0}14'28.1''	Summer	9.9 ± 0.85 × 10^{1}	7.2 ± 0.33 × 10^{2}	5.4 ± 0.11 × 10^{3}
		Monsoon	12.3 ± 0.85 × 10^{1}	10.88 ± 0.75 × 10^{2}	8.1 ± 0.66 × 10^{3}

The Fungi of different sites was identified on morphological and staining bases and they are found in table 2.

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Table 2: Identification of Fungi at different sites during different seasons

Isolates	Morphology	Number of colonies during different season (average of triplicates)											
		Winter				Summer				Monsoon			
		J	PN	K	B	J	PN	K	B	J	PN	K	B
Aspergillus terreus (HGF1) NFCCI 4468	Brownish in colour and become darker as it ages on culture media reverse is colourless. .	2.0±0.0	3.0± 0.0	1.8± 0.3	-	1.5 ± 0.7	-	0.8 ± 0.0	-	-	1.5 ± 0.2	1.0 ±0.0	-
Talaromyces(HGF2) NFCCI 4469	Greyish green in colour with moderate growth, reverse veiw is colourless	-	-	1.9± 0.0	1.7 ± 0.0	1.4 ± 0.0	2 ±0	1.8 ± 0.8	3.3 ± 0.6	3.9± 0.0	2.9± 0.7	1.9 ± 0.0	2.6 ± 0.3
Aspergillus fumigatus (HGF3) NFCCI 4470	Cottony, velvety or powdery, white first than darken to green, green gray or green brown with white margin, reverse veiw is white to tan.	2.0± 0.0	1.7 ±0.8	1.5 ±0.3	1.0 ± 0.0	-	-	-	-	2.0 ± 0.7	-	1.5 ± 0.0	-
Nodulisporiumgregarium(HGF4) NFCCI 4471	Colonies grow rapidly and resembles cottony candy, colonies darken with age, become gray, reverse veiw is bluish gray..	1.3±0.3	-	1.0±0.0	1.4 ± 0.0	1.0 ± 0.0	-	1.8 ± 0.0	2.0± 0.8	0.9 ± 0.1	-	1.3 ± 0.3	4.6 ± 0.5
Aspergillus fumigatus (HGF5) NFCCI 4472	Cottony, velvety or powdery, white first than darken to green, green gray or green brown with	-	-	-	-	1.6 ± 0.1	-	1.3 ± 0.7	-	-	-	-	2.0 ± 0.0

	white margin, reverse veiw is white to tan.												
Fusarium sp. (HGF6) NFCCI 4473	White cottony colonies with dense aerial mycelium, reverse veiwis tan to brown overtime.	4.0 ± 0.0	2.3 ±1.4	-	3.0 ± 0.6	1.4 ± 0.1	-	5.0 ± 0.3	2.2 ±0.6	1.0 ±0.3	3.2 ± 0.8	2.0 ±0.0	-
Aspergillus candidus (HGF7) NFCCI 4474	Cream to yellowish colour colonies, reverse veiw is uncoloured to pale brown.	-	-	-	-	-	-	1.2 ± 0.5	1.5 ± 0.3	-	2.0 ±0.0	-	4.5 ± 0.3
Aspergillus flavus (HGF8)	Granular, velvety or wooly, yellow or yellow to green, reverse veiw is golden to red brown.	-	3.8 ± 0.0	2.5 ± 0.3	1.6 ± 0.8	4.3± 0.5	-	1.1± 0.0	2.4 ± 0.3	9.3 ±0.4	2.5 ± 0.3	3.3 ± 0.3	2.0 ± 0.2
Acremonium sclerotigenum (HGF9)	White colonies flat with smooth, wet, velvety or floccose texture	-	-	-	-	-	-	1.5 ± 0.3	-	2.0 ± 0.0	-	3.5 ±0.1	1.7 ± 0.2
Non sporulating hyaline form (HGF10)	White cottony colonies, reverse veiw is gray to balck.	-	-	1.7± 0.2	3.2± 0.3	-	-	2.6 ± 0.6	-	4.8 ± 0.2	-	3.5 ± 0.8	2.4 ± 0.7
Aspergillus niger (HGF11) NFCCI 4476	White to pale yellow but quickly form jet black conidia, reverse veiw is gray to black.	-	-	3.6 ± 0.3	3.0 ±0.0	7.5± 0.2	-	2.5 ± 0.6	1.7 ± 0.3	5.0 ± 0.0	4.3 ± 1.7	5.3 ± 0.6	-
Curvularialunata (HGF12) NFCCI 4475	Flattened greyish colonies, reverse view is black.	2.0 ± 0.0	3.5± 0.3	-	-	-	6.0 ± 0.3	2.4± 0.2	3.3 ± 0.3	3.0 ±0.0	-	-	5.0 ±0.0
Sordariafimicola	Dark brown to	3.0 ± 0.0	-	3.5±0.7	-	-	5.3± 0.6	6.6 ±0.6	1.0 ± 0.0	2.2 ± 0.3	3.8± 0.08	4.8± 0.8	2.0 ±0.0

(HGF13) NFCCI 4477	gray colonies												
Tricodermalongibrachiatum (HGF14) NFCCI 4478	White colonies and yellow pigment secreted into agar	-	-	-	-	-	3.7 ± 0.7	-	2.0 ±0.0	5.2 ± 0.2	-	4.3 ± 0.3	1.0 ± 0.0

J= Jwalapur, PN= Prem nagar, K= Kankhal and B= Bhimgodha.

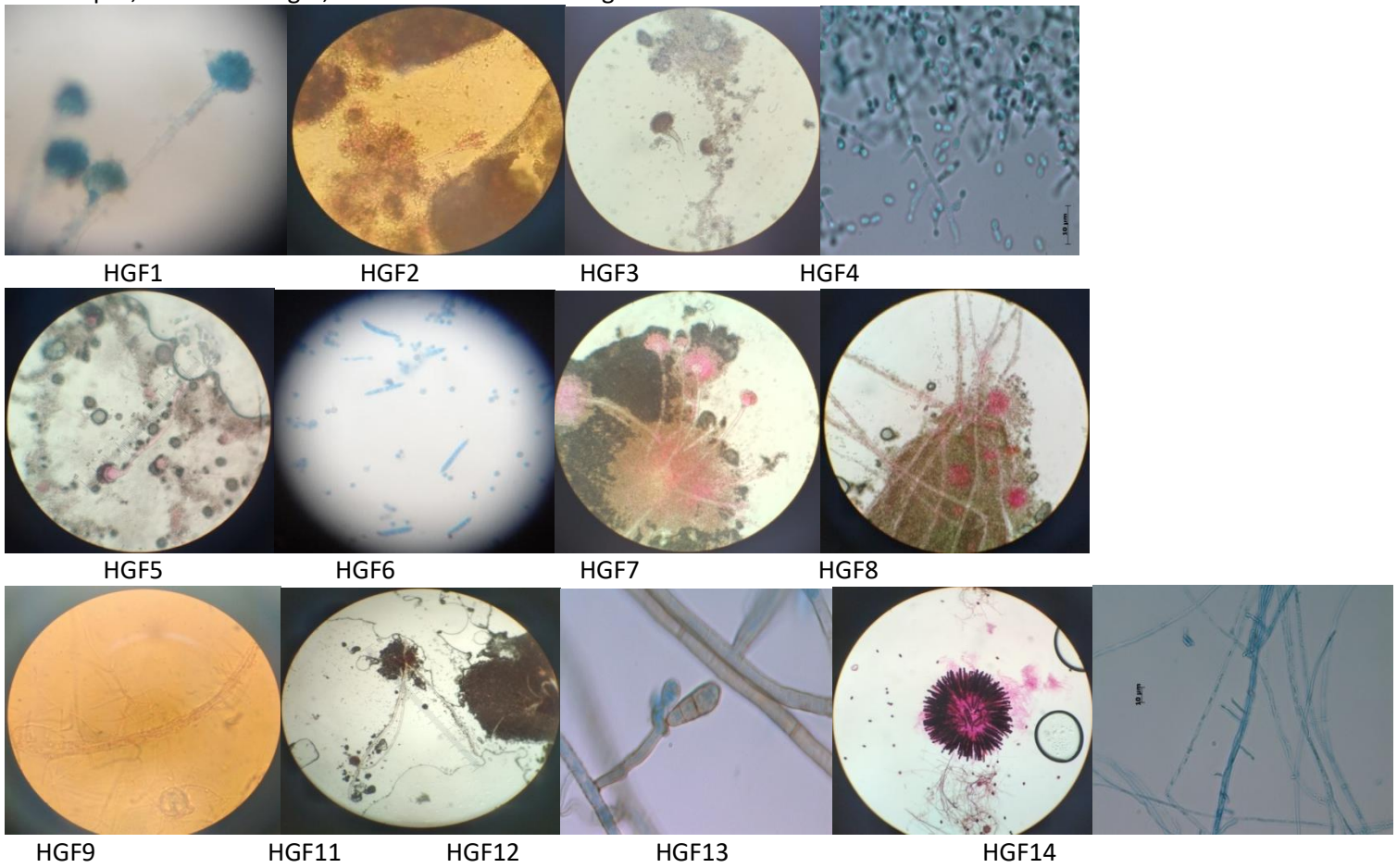

Fig 1: Microscopic view of different Fungi isolates

Quantitative Analysis: The Quantitative analysis such as Relative Density and Abundance of different fungi species in Ganga rive at Haridwar during different season were as follows (Table 3)

Table 3: Quantitative analysis

Isolates	Relative Density (%)			Abundance		
	W	S	M	W	S	M
HGF1	1.95	2,96	12.55	2	2.5	15.33
HGF2	10.38	11.58	9.66	3.5	9.25	8.5
HGF3	12.48	-	5.16	5.5	-	4.66
HGF4	8.24	5.48	5.4	4.33	3.66	6.8
HGF5	-	9.74	1.65	-	4.5	5
HGF6	10.38	6.69	2.58	5.66	5.33	2.33
HGF7	-	1.64	1.20	-	1.5	1.5
HGF8	10.38	4.04	11.38	5.66	8.33	10.75
HGF9	-	1.32	2.2	-	1	2.33
HGF10	3.14	-	4.11	1.5	-	2.5
HGF11	5.5	6.37	4.1	3.5	4.33	3.33
HGF12	4.33	8.33	2.03	2.5	6.66	4.5
HGF13	1.77	3.84	2.58	1	2	1.25
HGF14	-	2.52	2.33	-	2	2

M= Monsoon, W= winter, S= Summer

Species richness, Diversity and Dominance indices:

The Species richness, diversity and dominance were evaluated by Margalef's index of richness (D_{mg}), Shannon- Weaver diversity index and Simpson's index of dominance as follows (Table 4).

Table 4: Species richness, Diversity and Dominance indices

Index	Seasons		
	Winter	Summer	Monsoon
Richness			
Margalef Index (D_{mg})	1.78	2.11	2.20
Menhinick's Index (D_{mn})	0.79	0.89	0.73
Shannon Index (H')	2.86	3.06	3.44
Evenness & Dominance			
Shannon-Evenness index (E')	1.98	2.12	2.38
Simpson Index (D')	0.15	0.14	0.11
Berger-Parker Index (d)	0.24	0.27	0.20

Tolerance of Heavy Metals: the tolerance values obtained from different isolates are detailed in table 5.

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Table 5: Assessment of heavy metals tolerance ability of different isolates was as follows (average of triplicates).

Heavy metals	Isolates and the zone of inhibition (average of triplicates)													
	HGF 1	HGF 2	HGF 3	HGF 4	HGF 5	HGF 6	HGF 7	HGF 8	HGF 9	HGF 10	HGF 11	HGF 12	HGF 13	HGF 14
As														
200 mg/l	-	-	-	-	-	13.6 ± 0.6	-	-	-	-	-	8.6±0.6	-	-
400 mg/l	-	-	-	-	-	17.5 ± 0.1	-	-	-	-	-	12.3± 0.3	-	-
600 mg/l	-	-	-	-	-	17.3 ± 0.0	-	-	-	2.6± 0.6	-	18.3 ± 0.3	-	-
800 mg/l	-	-	11.3 ± 1.3	-	-	17.6 ± 0.5	-	-	-	4.1 ± 0.2	-	18.6 ± 0.6	-	-
1000 mg/l	-	-	9.3 ± 0.6	-	-	19.3 ± 0.6	4.8 ± 0.2	-	-	6.6 ± 0.6	-	22.6 ± 0.6	-	-
Hg														
200 mg/l	-	13.6 ± 0.6	7.5 ± 0.1	8.3± 0.3	5.1 ± 0.1	5.3± 0.2	5.6 ± 0.3	1.8 ± 0.1	-	10.6 ± 0.6	5.6 ± 0.6	14.0 ±1.0	-	-
400 mg/l	-	15.3 ± 0.3	7.4 ± 0.2	12.3 ± 0.6	5.2± 0.24	7.6 ±0.3	7.3± 0.3	1.3 ± 0.3	3.3 ± 0.6	12.6± 0.6	6.2 ± 0.7	14.0 ± 1.0	-	-
600 mg/l	7.3 ± 0.6	17.3 ± 0.6	7.5 ± 0.7	12.3 ± 0.3	7.42 ± 0.3	10.6 ± 0.3	8 .6 ± 0.3	4.6 ± 0.6	5.6 ± 0.6	14.0 ± 0.0	7.6 ± 0.6	14.6± 0.3	-	22.3 ± 0.3
800 mg/l	9.3 ± 0.6	17.0± 0.0	8.3 ± 0.8	13.5± 0.3	10.0 ± 0.0	15.6 ± 1.3	10.3 ± 0.6	4.2 ± 0.1	6.6 ± 0.6	16.6 ± 0.6	9.6 ± 0.6	14.0 ± 0.3	-	24.6 ± 0.8
1000 mg/l	10.0 ± 0.0	18.0 ± 1.4	12.0 ± 0.0	19.3± 0.5	10.6 ± 0.6	7.6 ± 0.8	12.6 ± 0.6	9.6 ± 0.3	11 ± 0.3	18.0 ± 0.1	10.6 ± 0.6	18 ± 0.1	-	27.3 ± 0.6
Cd														
200 mg/l	-	-	-	-	6.3 ± 0.3	-	19.8 ± 0.5	-	-	7.8 ± 0.6	-	11.4 ± 0.2	11.3 ± 0.6	16.8 ± 0.3
400 mg/l	-	-	-	11.6 ± 1.4	6.3 ± 0.6	-	28.0 ± 0.3	-	-	8.3 ± 0.7	-	19.3 ± 0.3	13.3 ± 1.6	17 ± 0.1
600 mg/l	10.1 ± 0.1	-	13.3 ± 0.3	15.6 ± 0.6	15.1 ± 0.1	-	29.5 ± 0.7	-	-	11.0 ± 0.3	6.0 ± 0.1	23.6 ± 1.8	21.0 ± 0.1	22.6 ± 0.3
800 mg/l	16 ± 0.88	-	15.3 ± 0.3	17.6 ± 1.2	15.3 ± 0.6	-	32.3 ± 0.3	9.3 ± 0.3	-	14.3 ± 0.6	7.6 ± 0.3	23.6 ± 0.6	21.6 ± 0.3	18.0 ± 0.6
1000 mg/l	21.3 ± 0.6	-	16.6 ± 0.3	22 ± 0.1	17.6 ± 0.3	-	34.6 ± 0.6	11 ± 0.6	-	18.6 ± 0.6	14 ± 0.3	33.3 ± 0.3	27.6 ± 1.2	23.3 ± 0.8
Cr														
200 mg/l	-	8 ±0.1	6.3 ± 0.33	-	5.2 ± 0.2	-	-	-	-	-	4.6 ± 0.6	11.0 ± 0.2	-	-
400 mg/l	-	11.1± 2.5	5 ± 0.1	-	5.6 ± 0.6	-	-	-	-	-	5.6 ± 0.6	20.6 ± 1.6	6.3 ± 0.3	-
600 mg/l	-	13 ± 1	6.3 ± 0.33	-	5.3 ± 0.3	-	-	-	6.2 ± 0.5	23.0 ± 0.3	6.3 ± 0.3	23.0 ± 0.3	6.7 ± 0.1	-
800 mg/l	7.3 ± 0.66	13.4 ± 0.8	7.5 ± 0.25	4.3 ± 0.3	5.6 ± 0.6	-	6.3± 0.3	-	-	2.2 ±0.1	8.3 ± 0.3	23.6 ± 0.2	7.0 ± 1.8	-
1000 mg/l	8.4 ± 0.31	18 ± 0.2	7 ± 0.6	7.0 ± 0.6	7.6 ± 0.6	-	6.0 ± 0.1	-	-	3.3 ± 0.3	9.2 ± 0.7	33.0 ± 0.3	6.0 ± 1.2	-
Cu														
200 mg/l	-	-	3.3 ± 1.5	-	-	4.6 ± 0.3	-	-	-	-	-	11.1 ± 0.1	7.3 ± 0.6	-
400 mg/l	-	-	8.3 ± 0.3	-	-	6.3 ± 1.1	-	-	-	-	-	10.6 ± 1.2	7.0 ± 0.3	8.0 ± 0.0
600 mg/l	-	-	10.5 ± 0.2	4.3 ± 0.33	-	7.3 ± 0.66	-	-	-	-	-	17.6 ± 0.3	7.5 ±0.7	17.3 ± 0.6
800 mg/l	-	-	16.8 ± 0.1	5.3 ± 0.02	-	10 ± 0.66	-	-	-	-	-	18.3 ±0.1	7.2 ± 0.0	18.1 ± 0.1
1000 mg/l	-	-	18.8 ± 0.0	8.4 ± 0.4	-	12.6 ± 0.2	-	-	-	12.6 ± 0.2	-	20.6 ± 1.7	7.5 ± 0.5	23.3 ±1.7
Ni														
200 mg/l	-	7.3 ± 0.3	3.3 ± 0.6	-	-	6.6 ± 0.6	-	-	-	-	-	12.3 ± 0.3	-	-
400 mg/l	-	12.6 ± 0.3	6.6 ± 0.3	-	-	9.0 ± 0.6	6.6 ± 0.1	-	-	2.3 ± 0.1	-	13.3 ± 0.3	-	-
600 mg/l	-	22.2 ± 0.0	15.8 ± 0.3	-	-	10.8 ± 0.1	7.3 ± 0.3	-	-	4.3 ± 0.33	-	12.6 ± 1.2	-	5.6 ± 0.33
800 mg/l	12.0 ± 0.0	25.0 ± 0.6	17.6 ± 0.3	-	-	12.2 ± 0.0	10.0 ± 0.0	-	6.6 ± 0.3	8.4 ± 0.2	-	15.6 ± 0.6	-	7.6 ± 0.33
1000 mg/l	11.6 ± 0.3	26.6 ± 0.3	21.1 ± 0.1	-	-	15.5 ± 0.7	12.0 ± 0.1	-	8.5 ± 0.7	9.9 ± 0.3	-	18.6 ± 0.6	-	11.3 ± 0.6
Fe														
200 mg/l	-	-	-	-	4.3 ± 0.3	5.2 ± 0.4	-	-	-	-	-	16.6 ± 0.3	-	-
400 mg/l	-	-	-	9.3 ± 0.6	8.3 ± 0.6	6.3 ± 0.6	-	-	-	-	-	17.3 ± 0.3	6.0 ± 0.0	-
600 mg/l	-	-	6.6 ± 0.6	11.8 ± 0.0	9.4 ± 0.2	14.3 ± 0.3	4.0 ± 0.0	-	-	-	-	13.0 ± 0.0	6.0 ± 1.0	-
800 mg/l	-	-	10.6 ± 0.6	11.1 ± 0.0	10.3 ± 0.6	12.2 ± 0.4	7.3 ± 0.6	-	-	-	-	23.3 ± 0.3	7.0 ± 0.3	-
1000 mg/l	-	-	13.0± 0.5	18.6 ± 0.3	11.0 ± 0.0	15.0 ± 0.0	5.3 ± 0.6	-	-	-	-	24 ± 0	7.0 ± 0.6	-

Antifungal Sensitivity: - The antifungal sensitivity of different fungal isolates was determined against four antifungal i.e. Nystatin, Amphotericin, Fluconazole and Ketonazole is specified in table 6.

Table 6. Antifungal sensitivity obtained from different fungal isolates.

Isolates	Antifungal			
	Nystatin	Amphotericin	Fluconazole	Ketonazole
HGF1	19mm	10mm	13mm	15mm
HGF2	16mm	14mm	10mm	11mm
HGF3	33mm	12mm	19mm	31mm
HGF4	12mm	16mm	14mm	13mm
HGF5	-	-	11mm	-
HGF6	32mm	15mm	18mm	13mm
HGF7	15mm	13mm	14mm	37mm
HGF8	14mm	-	26mm	-
HGF9	10mm	13mm	10mm	16mm
HGF10	17mm	21mm	19mm	15mm
HGF11	24mm	11mm	-	12mm
HGF12	12mm	-	-	-
HGF13	21mm	17mm	15mm	10mm
HGF14	28mm	18mm	-	-

(a) (b)

Fig 2: (a) Heavy metals tolerance ability and (b) antifungal sensitivity

DISCUSSION

In present study four sampling sites i.e. Jwalapur, Prem-nagar, Kankhal and Bhimgodha were selected on a bases of industrial and anthropogenic activities loads during three season i.e. Monsoon, Winters and Summers for determining the fungal diversity and there capability to tolerate heavy metals. From all the four sites total 7 genera and 13 species were isolated which are *Aspergillus terreus, Talaromyces*sp., *Aspergillus fumigatus, Nodulisporiumgregarium, Aspergillus fumigatus, Fusarium* sp., *Aspergillus candidus, Aspergillus flavus, Acremonium sclerotigenum, Aspergillus niger, Curvularialunata, Sordariafimicola and Tricodermalongibrachiatum*. It had been found that maximum fungi population was during monsoon followed by the summers and least in winters (Table 2), this might be due to the presence of high load of organic matter during monsoon, the same

was observed by Moddodi et al., 2009 while studying diversity of aquatic hypomycetes of the western ghat rivers. *Aspergillus* and *Talaromyces*genera were found to be the most abundant respectively, followed by other genera at lower frequencies (Table 3). Similar results were reported by Sengupta and Chaudhuri (1995), when they isolated fungi from the sediment and from estuary of the Ganga river in India, Gomes et l., (2008) and Doi et al., (2018) studied the most diverse genus in the dry season Casa Caiada beach in Orlinda, Pernambuco state, Brazil and araca Bay in Sao Sebastiao, Sao Paulo, Brazil respectively and they also found *Aspergillus* and *Penicillium* as dominant genera. According to Piedras et al.,(2006), Shannon's diversity index may provide information on conservation and impacted environment, they also suggest that values between 1 and 3 reflects moderately polluted water indicating good diversity relative to environment (Piedras et al., 2006). In present studies the value of Shannon's index were 3.443 for monsoon, 3.06 for summer and 2.869 for winters, which were somewhat around the limits provided by them. In the presence of fungal spore and hyphae in aquatic bodies indicate that they are capable of utilizing nutrients from the polluted water. Filamentous fungi have been found to be very effective in removing heavy metals from aqueous solutions due to their greater resistance to these elements (Kurek et al., 1982, Collins and Stotzky, 1992). The capacity of isolated fungi was examined by plate diffusion method by determining zone formation which indicates the ability of the isolates as heavy metal resistant or sensitive against different concentration of various heavy metals same method was used to determine MIC of different fungal isolates against different heavy metals by Hemambika et al., 2011. In present study it had been found that all the isolates had heavy metal tolerance ability to some extant but out of all isolates *Aspergillus* sp. especially

Aspergillus flavus had maximum tolerance ability. Similarly Khan 2001, and Iram et al., 2009 shows the Aspergillus sp. have maximum ability as compared to other filamentous fungi. Along with this heavy metal tolerance ability it was found that mostly all fungi are sensitive to antibiotics such as Nystatin, Amphotericin, Fluconazole and Ketonazole.

As conclusion, the isolation of fungi from Ganga River helps us to determine their biological diversity and their ability of interaction with their ecosystems. This provides knowledge about their role in conservation, preservation and remediation process. Ganga pollution is one of the most concern matters but by determining the ability of heavy metals tolerance of Ganga isolate shows that the Micro-organisms are one of the important for the conservation of Ganga existence.

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