Review Paper:

Microbial diversity in wetlands of India

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Abstract

Wetlands are considered to be the surface of the earth which is kept in water for a whole year or part of the year. They are regarded as the most productive ecosystems that are essential to life support systems and harbor a wide range of microbial species. India has several natural wetlands including inland and coastal habitats of wetlands, covering an area of about 1.5 million ha or 18.4% of the country's geographical area. These wetlands serve as a source of water for human consumption in various ways including drinking, washing, bathing. Microbes are considered to be the first living organisms on the Earth. So, the microbial diversity is the oldest and the broadest unexplored pool among all the types of biodiversity on the planet "Earth". Microbes are present almost everywhere in the nature. Wetlands have high possibility of the presence of microbes as these microbes can survive in the diverse and extreme environments.

Microorganisms have immense importance in the function in of a wetland as many biogeochemical conversions are mediated by microbes. This leads to ultimate control of vegetation composition in wetlands. Understanding of these microbes in the wetland systems helps us in measuring the wetland health conditions. Microbial indicator populations in a wetland along with physical, chemical and biological parameters are essential for the overall assessment of the wetland health condition. Moreover, wetlands' microbes, plants and wildlife form a part of global cycles for biogeochemical cycles. Microbial indicators will be helpful for wetland restoration and management practices.

Keywords: Bacteria, Fungi, Algae, Actinomycetes, Wetlands, India.

Introduction

Biological diversity relates to the variety of species living in a particular area and results due to billions of years of evolution shaped by natural processes. Ramsar Convention perfectly defined wetlands as "areas of marsh, fen, peat land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six meters.[66]" About 6% of Earth's land surface is covered by wetlands. Globally wetlands extend from the tropical to the tundra. Ecological sustainability of a region depends on the wetlands of the region. Wetlands occur in all climatic areas and at all altitudes in the Himalayas up to about 6000 m above m.s.l.

Animals and plants which are adapted to the aquatic environment enable wetlands to exist wherever water accumulates for a sufficiently long period of time. They are an essential part of human civilization that meets many crucial needs for life on earth such as drinking water, recreation, production of protein, fodder, biodiversity, purification of water, energy, storage of flood, transportation, sinks and climate stabilizers. Almost all the major man-made wetlands or water bodies have been managed by us and we are controlling their aquatic (floral and faunal) diversity.

Many paddy fields and fish ponds are created from the natural wetlands. Increased human activity, unplanned growth, lack of management structure and lack of adequate legislation are the major causes contributing to wetland loss. Wetland plant roots create oxic–anoxic conditions which facilitate the growth and activity of aerobic and anaerobic microorganisms.

Wetlands are increasingly recognized in nature and in natural processes that extend far beyond their geographical locations and boundaries for their vital role. Compared to any other ecosystem group worldwide, they are the largest producers of biomass, far above the estuaries of the sea, tropical rain forests and even coral reefs.[67] Wetlands and other forms of deepwater are estimated to cover about 6% of earth surface.[50]

Types and functions of wetlands: Wetlands are vital for the survival of diverse flora and fauna. About 20% of the known species of life depend on wetlands for their survival, as they are their primary and important seasonal habitats. Paddy fields, acid sphagnum dominated peat lands, riparian wetlands, black mangroves and shallow marine sediments are also considered as wetlands that were also reported by researchers from different origin.[1,14,33,38,43,45,64,79] Wetlands are classified as "areas of marsh, fen, peatland or stream, natural or artificial, permanent or temporary, with water that is stagnant or flowing, new, brackish or salt, including marine water areas with a depth not exceeding 6 m at low tide" (Secretariat R, 2013). Wetlands also play a unique role in regulating global biogeochemical cycles. They serve as filters that help in the bioremediation process by removing toxic pollutants

including organic compounds and other hazardous wastes. The functions of the wetlands include primary production, providing habitats and also helping sustain human habitations, regulation of hydrological regimes such as recharge of groundwater and mitigating the flood problem.

About 3 billion people are entirely depending on wetlands for their regular activities such as drinking, food, livelihood security and other recreation activities. Direct economic benefits include agriculture, fisheries, recreation and tourism, water supply, wildlife, transportation supporting a wide range of flora, fauna and cultural heritage. Indirect benefits include enhanced water quality, collection of surface runoff, elimination or retention of nutrients, treatment of organic waste reduction of sediment before it reaches open water and cultural aspects.

All natural ecosystem functions include energy transfer from plants to food chains and biogeochemical cycling. Wetlands are one of the world's most important ecosystems.[50] The world's projected wetland area is about earths land surface of 5 to 8%. Wetlands have an immense diversity of microorganism which needs to be explored.[56] Particularly, soil microbial communities in wetland ecosystems contribute a lot to biogeochemical cycles of nitrogen, phosphorus, carbon, sulphur and iron cycle.

Wetlands of Indian subcontinent: India is the seventh largest country in terms of area that lies in the south of Asia. It is a member of Like Minded Megadiversity Countries (LMMC) group. India is rich in diversity both qualitatively and quantitatively. It is having unique biological diversity with a wide range of landform types. Wetland is an ecosystem that is either seasonally or permanently flooded by water and oxygen-free processes prevail.[42] The important factor that differentiates wetlands from other forms of land or water bodies is unique vegetation of aquatic plants.[9]

Wetlands are very crucial that play a number of important functions including purification of water, storage of flooded water, various biogeochemical cycles, stabilization of shorelines and support of plants, animals and microorganisms. Wetlands are also considered the most biologically faunal and microbial life. Wetlands can also be constructed by human beings. Wetlands in India on the basis of topographical variation can be divided into four major categories. These are Himalayan wetlands, wetlands in the Gangetic plains, wetlands in the desert and coastal wetlands.

In India, wetlands are distributed across the country in different geographical regions ranging from the Himalayas in the north to the Deccan plateau in the south[51] (Table 1; Figure 1). Wetlands can be classified into Glaciatic Wetlands, Oxbow Wetlands, Lagoons, Tectonic Wetlands, Crater Wetlands, seagrasses, estuaries, thermal springs, Saltwater Wetlands, Urban Wetlands, Ponds/Tanks, man-made Wetlands, Reservoirs, Mangroves, Coral reefs and Creeks.[51]

These wetland systems are associated directly or indirectly with the Ganges, Brahmaputra, Narmada, Tapti, Godavari, Krishna and Cauvery river systems. Among all types of wetlands, river or streams are the largest category covering an area of 5.26 mha (34.46%). In contrast with other biological systems, wetlands are spread over a small space. Many wetlands like mangroves, coral reefs, estuaries and ocean grass beds are rich in microbial biodiversity. Indian subcontinent contains a rich biodiversity due to its geographical location, fluctuated geography and atmosphere. India's contribution to the total worldwide biodiversity is around 8%.

There are wetlands, marshes, peat lands, wet grasslands, streams, lakes and rivers in the Hindu Kush-Himalayas which act as host sites for migratory birds. The wetlands and rivers store over 90% of the water on earth surface and are significant for human improvement. The most significant are Western ghats, North-eastern slope districts Andaman and Nicobar Islands, Mangrove timber lands of Sunderban region, Bastar locales occupied by tribals, Silent Valley of Kerala, Playas of Rajasthan, Chilka pool of Orissa, Sonar Lake of Maharashtra and the Himalayan area. Microbes present inside a wetland supplement nutrient cycling.[74,89]

Overall inland oceanic biological systems is spread about 18 million ha.[30] Gopal[29] has differentiated Indian wetlands into two different types, one in which water logging is consistent and the second type where evaporation of the wetland takes place and replenishes based on the season.

Biswas[7] has described about 1193 wetlands in India that covered an area of about 3.9 mha in 274 locations within India. They harbor around 1,800 algal species and 1250 higher plant species.[31] About 5% of the world's mangrove vegetation at the global level has been accounted for India. These biological systems are found in between tidal zones of protected shores, estuaries, backwaters and tidal ponds.

In mangroves, one hundred and sixteen plant species have been recorded.[3,4] Faunal diversity in mangrove wetlands of India consists of Crustaceans, Molluscs, Fishes, Reptiles, Birds, Mammals, Microbenthos, Shellfishes.[39]

The International Center for Integrated Mountain Development (ICIMOD) identified 1,439 ice sheets with an all out territory of 4,060sq km and 127 cold lakes in Uttarakhand[10] which form the wetlands of Uttarakhand. Vembanad Lake (Kerala) is the India's largest tropical wetland ecosystem on the southwest coast.

Apart from that, as a Ramsar site, Vembanad Lake has tremendous conservation significance. It covers an area of over 1512 km and is bordered by Alappuzha, Kottayam, Ernakulum and Thrissur districts. Thannermukkom saltwater barrier is a unique characteristic of the lake. Kumarakom region of this lake is the most unexplored ecosystem as far as the microbial diversity is considered.

Ashtamudi Lake is the second largest brackish water lake in Kerala and has been designated as a Ramsar site. The lake flows into the Arabian Sea and the water is exchanged by tides. The lake has optimum nutrient concentration levels. Bhitarkanika wetland presents a variety of habitats in which the faunal diversity is high in comparison to other mangrove forest areas of Odisha.[87]

Bhoj wetland is a man-made lake which satisfies the domestic needs of the residents of Bhopal, Madhya Pradesh. The upper lake is the source of water for 40% of the population of Bhopal with rich biodiversity.[81] Chandertal Wetland is a known Ramsar site situated near little below the Kunzam Pass. It is situated at an elevation of 4,830 m above m.s.l. in the Tibetan Plateau. It exhibits half-moon shaped rock basin Chandertal Lake that is a glacier-fed lake formed by glacial melt in land which drains down into Chandra River. In this biogeographic zone, characterized by extreme cold, low precipitation and alpine cold desert, 11 mammalian species belonging to 11 genera under 06 families and 03 orders have been observed.[75]

East Kolkata wetlands are complex natural and artificial wetlands lying east of Kolkata. It is the largest sewage-fed aquaculture of the world. These wetlands are shrinking because of various reasons such as rapid urbanization, excessive silting, human habitation etc.[70] Harika Lake is rich in aquatic biodiversity including flora and fauna and spreads into the four districts of Punjab, India. It forms a part of the Indo-Gangetic alluvial plain of Holocene age. This artificial lake not only recharges ground water but also provides water for irrigation to parts of Punjab and Rajasthan.[12]

Hokera wetland is located at an elevation of 1,584 m above m.s.l. and originates from Dudhganga watershed in Pir Panchal range of the Himalaya and Sukhnag stream from the west. It is an important source of food, spawning and various other activities including the feeding and breeding ground to lots of aquatic birds.[16]

Table 1
Ramsar sites in India

S.N.	Name	Site	State
1.	Ashtamudi	Wetland	Kerala
2.	Bhitarkanika	Mangroves	Orissa
3.	Bhoj	Wetland	Madhya
4.	Chandertal	Wetland	Himachal Pradesh
5.	Chilika	Lake	Orissa
6.	Deepor	Beel	Assam
7.	East Calcutta	Wetlands	West Bengal
8.	Harike	Lake	Punjab
9.	Hokera	Wetland	Jammu
10.	Kanjli		Punjab
11.	Keoladeo	National park	Rajasthan
12.	Kolleru	Lake	Andhra
13.	Loktak	Lake	Manipur
14.	Nalsarovar	Bird Sanctuary	Gujarat
15.	Calimere	Wildlife and bird sanctuary	Tamil Nadu
16.	Pong	Dam Lake	Himachal Pradesh
17.	Renuka	Wetland	Himachal Pradesh
18.	Ropar	Lake	Punjab
19.	Rudrasagar	Lake	Tripura
20.	Sambhar	Lake	Rajasthan
21.	Surinsar-Mansar	Lakes	Jammu
22.	Tsomoriri	Lake	Jammu
23.	Upper Ganga	River	Uttar Pradesh
24.	Vembanad-Kol	Wetland	Kerala
25.	Wular	Lake	Jammu
26.	Sasthamkotta	Lake	Kerala

Figure 1: Map showing State-wise number of wetlands in India. (Note: Figure in parenthesis represents total number of wetlands in respective State.); (Source: Bassi et al)[5]

Chilika Lake of Odisha state is a brackish water lagoon and identified as one of the biodiversity hotspots in India. India designated Chilika lagoon as a wetland of International importance under the Ramsar convention in 1981. It hosts over 230 species of birds including the migratory birds from the Caspian Sea, Russia, Central and South Asia, Ladakh and Himalaya.[54] Keoladeo wetland occupies a total area of the park as about 29 sq. km with a gentle slope towards the centre forming a depression. For the purpose of groundwater monitoring, the area is divided into three zones of which zone 1 is the wetland.[73]

Techniques for studying microbial diversity of wetlands: Microbial communities are the key members of many ecosystems on the earth.[46] The word microbial communities can be described in the terms of richness or evenness as well as composition i.e. an abundance of taxa and genes in the sample. They play a significant role in crucial biological functions ranging from nitrogen and carbon cycles in the environment to regulation of metabolic and immune responses from inhabited animal and human hosts.[36,46] The exploration of microbial communities through culture-dependent methods was used from ancient time, but on the standard laboratory media less than 1%, bacterial species of the environmental communities were culturable.

Metagenomics now offers a viable framework for tracking environmental cultures by high throughput methods to resolve traditional culture approaches. In aquatic and terrestrial environments, these techniques revealed taxonomic and functional diversity.

The term metagenomics is the sequencing of total DNA isolated directly from the environmental samples.[21] By applying this approach, functional and taxonomic microbial diversity can be described and then changes in communities can be monitored over time and space in response to anthropogenic or environmental impacts related to human health.[62]

Another method for estimating biomass is Substrate Induced Respiration (SIR). The SIR method tests the output of CO_2 from a sample exposed to a surface that is readily available. The activity of microorganisms refers to a measure of biological processes that occur in wetlands that are microbially induced. Enzymatic activity processes can be studied in the wetlands for understanding the various biological processes that take place in them. Standard enzymatic processes are often estimated and can provide information in the sample. Enzymatic activities help us to understand the mechanisms of degradation of pollutants.[41]

Isotope tracers with different isotope ratios can be introduced into the wetland and track various metabolic pathways to determine the activity of various microbial consortium. Electron transportation system activity with the use of tetrazolium salt can also be applied in a successful manner to a wide range of microbes to assess the dehydrogenase activities.

Microscopic observation of specimens under a conventional optical microscope was used to classify protozoan and metazoan populations.[63] Biofilm formation on gravel surfaces can be studied with electron microscopy.[47] Fluorescence *in situ* hybridization (FISH) probe helps in isolating and enumerating the specific microbial population.[22] With appropriate FISH probes, it is possible to differently stain two or more specific bacterial populations e.g. nitrifying and denitrifying bacteria within a biofilm.

Phospholipids fatty acid profile (PLFA) allows examination of microbial communities and population changes over time which is an indirect density analysis as well as an estimate of diversity. Garland and Mills[26] have applied this to the study of complex microbial communities while Osem et al[58] have reported the disparity in microbial community arrangement.

Microbial diversity of Indian Wetlands: Microbial group is necessary to numerous biogeochemical processes in wetlands. Microbial network has been developed from various environments present in different wetlands. Wetlands consist of fish, timber, reeds; therapeutic plants which help in maintaining a sustainable environment to the population living around these wetlands. They add to the amphibian nourishment networks and biogeochemical cycles. Increases in heterotrophic bacterial number can be utilized to screen natural contamination of the earth.[44]

Researchers have recognized about 1.7 million living species on our planet. Studies show the 5,000 distinguished types of prokaryotes of which 1 to 10% are bacterial species.[78] Macrophytes present in wetlands such as *Arundo donax* can produce up to 20 tons of biomass per hectare per year. Some algae such as *Chlorella, Spirulina, Scenedesmus* and *Dunaliella* can be used both for food and medicine. High organic production in wetlands causes a reduction in the atmospheric carbon dioxide which is sequestered in the plant or animal biomass.[85] In few wetlands, decomposition of organic matter can be reduced significantly by the anaerobic environment resulting in peat formation at extreme low temperature and acidic conditions that reduce microbial activity. Advances in wetland microbiology are reviewed by the research workers Kolb and Horn,[43] Lamers et al, Lovell and Davis and Pester et al.[59]

Methanotrophs and denitrifiers were studied by Kolb and Horn.[43] Pester et al[59] reported sulfate reduction in freshwater wetlands. Lovell and Davis highlighted role of nitrogen-fixing diazotrophs in the management of nutrient-limited salt marshes. Preston et al used several approaches to describe depth-independent microbial community structure and function

Bacterial diversity of potable spring water of Indian Himalayan Region showed that the phylum wise distribution showed the abundance of *Bacteroidetes, Proteobacteria, Verrucomicrobia, Planctomycetes, Armatimonadetes* and to a lesser extent, *Parcubacteria, Actinobacteria* with other minor classes such as *Aquificae, Firmicutes, Hydrogenedentes, Acidbacteria, Nitrospirae, Deinoococcus, Thermus* and *Chloroflexi*.[76] *Planctomycetes, Bacteroidetes, Proteobacteria, Armatimonadetes* and *Verrucomicrobia* were the major phylum from western spring water.[76]

Algal Biodiversity in India: India, with its varying geological and climatic systems consists of different kinds of wetland natural surroundings.[62] In Valthur Lake during the pre-monsoon period, Bellandur Lake inlets were dominated by the members of Cyanophyceae followed by Bacillariophyceae while Chlorophyceae such as *Chlorococcum* sp. and *Monoraphidium* sp. dominated outlets[49] which indicates increased photosynthesis by green algal members.[88]

During the post monsoon, the Cholorophycean members dominated such as *Scenedesmus sp., Anabaena sp*. and *Anacystis* sp. *Chlorococcum* sp. and *Monoraphidium* sp. were present during the monsoon season. Algal samples at inlets revealed predominance of *Gomphonema* sp. and *Nitzschia* sp. during the monsoon.[49]

Fungal Diversity in India: There are plenty of bacteria and fungi in the wetlands (Table 3 and Table 4). Although a large number of environmental studies are performed across the globe on different wetlands, the aspects of bacterial and fungal diversity are often overlooked. This is despite the fact that in the decomposition of dead organic matter and in biogeochemical cycles, these micro-organisms play a vital role.

The intermittent flooding of wetland soils and the proximity of wetland plant roots make dynamic oxic-anoxic interfaces that give living spaces to a wide variety of aerobic and anaerobic organisms.

Table 2
Algal Biodiversity in Wetlands in Lower Ganga plain[55]

Name of the Algal species
Azollapinnata
Eichhorniacrassipes
Lemna minor
Pistia stratiotes
Salvinia auriculata
Hydrilla verticillata
Otelliaalismoides
Vallisneria spiralis
Ceratophyllumdemersum
Ludwigiaadscendens
Ludwigiaperenis
Nelumbo nucifera
Nymphoidescrystata
Nymphaea nouchali
Nymphaea stellata
Victoria amazonica
Alternanthera philoxeroides
Alternanthera sessilis
Marsileaquadrifolia
*Cyperus*spp.
Polygonum spp.
*Scirpus*spp.

Behera et al[6] reported the presence of sulphur oxidizing microscopic organisms from mangrove soil of Mahanadi wetland delta of Odisha, India. From morphological and biochemical studies, the majority of the isolates were recognized as *Micrococcus spp., Bacillus spp., Pseudomonas spp.* and *Klebsiella spp.* Nath and Kalam[56] isolated various fungi from East Kolkata wetland in Czapex Dox Agar media and reported *Aspergillus* sp., *Penicillium* sp., *Sphaeropsis* sp., *Pericouic* sp., *Fusarium* sp., *Xylophpha* sp., *Umbelopsis* sp., *Cylindrocladium* sp. and *Llelicocephalum* sp. D'Souza and Rodrigues observed diversity of AM growths in Mangroves of Goa.[15]

Glomus was the dominant genus followed by Acaulospora, Rhizophagus, Funneliformis and Racocetr a. Choudhury et al[13] studied the diversity of Arbuscular mycorrhizal fungi (AMF) in Deepor Beel Ramsar site of Assam, India which revealed mycorrhizal colonization of various plant species. The *Vetiveria zizanioides* L. from the family Cyperaceae showed most elevated (86.47%) level of root colonization.

Despite the above mentioned microbial research in the wetlands of the country, more in-depth studies have to be done in various wetlands for micro-organisms. Persistent efforts to culture fungi along with interdisciplinary understanding are required to understand of microbial communities in wetlands. Studies of Romanowsky et al[68] revealed that wetlands located in the Eastern Kolkata have varieties of microbial population. Microbial diversity includes *Rhodococcus sp., Bacillus sp., Pseudomonas sp., Azotobactor sp., Aeromonas* sp. etc. These bacteria help in bioremediation process.[28]

Springs of East district were dominated by Proteobacteria while springs of West district were dominated by Planctomycetes. Planctomycetes was least dominated in the East as compared to the West dist. Proteobacteria dominated the springs of the eastern district, while Planctomycetes dominated the springs of the western district.[24]

Specific bacterial phyum Armatimonadetes has been identif ied from western district samples which are not present in other district springs. *Verrucomicrobia* genus was found in freshwater and soil of these samples.[32,35]

Table 3
Fungal Biodiversity in Wetlands in East Kokata[56]

S.N.	Region of East Kolkata Wetland	Identified genus
1.	Gadakhalifari ghat	*Aspergillus sp.*
2.	Gadakhalifari ghat	*Penicillium sp.*
3.	Gadakhali	*Penicillium sp.*
4.	Gadakhali Sundari tree	*Sphaeropsis sp.*
5.	Malta bridge	*Pericouic sp.*
6.	Malta bridge	*Llelicocephalum sp.*
7.	Motghora pond	*Fusarium sp.*
8.	Motghora	*Xylohypha sp.*
9.	Motghora	*Cylindrocladium sp.*
10.	Gadakhali	*Umbelopsis sp.*

Table 4
Bacterial biodiversity in India

S.N.	Name of the organism	Taxonomical Group
1.	*Bacillus anthracis*	Firmicutes
2.	*Rhodococcus rubber*	Actinobacteria
3.	*Bacillus megaterium*	Firmicutes
4.	*Rhodococcus sp.*	Actinobacteria
5.	*Rhodococcus rubber*	Actinobacteria
6.	*Rhodococcus sp.*	Actinobacteria
7.	*Bacillus cereus*	Firmicutes
8.	*Sphingobacterium sp.*	Bacteroidetes
9.	*Bacillus sp.*	Firmicutes
10.	*Pseudomonas sp.*	Proteobacteria
11.	*Bacillus sp.*	Firmicutes
12.	*Bacillus sp.*	Firmicutes
13.	*Pseudomonas sp.*	Proteobacteria
14.	*Bacillus sp.*	Firmicutes
15.	*Bacterium* Dcu	Birchwood
16.	*Aeromonas sp.*	Proteobacteria
17.	*Aeromonas sp.*	Proteobacteria
18.	*Azotobacter sp.*	Proteobacteria

The spring water of North was most diverse out of the three districts having major dominance of *Arcicella*, *Planctomycetes*, *Polynucleobacter*, *Schlesneria* and *Azohydromonas*. Planctomycetes were found in all the four districts with lower relative abundance; maximum dominance was recorded at the west and in east district it was recorded as minimum. The springs of east district showed *Emticicia* which is a gram-negative bacteria genus belonging to family Cytophagaceae and they are ubiquitous in the aquatic environment.[54,69]

A number of significant members of the genera were identified in aquatic systems viz. *Emticicia aquatica*, *Emticicia aquatilis*[57] and *Emticicia fonts*. The bacterial genera Flavobacterium was found in all the other three districts except in the south. *Flavobacterium* a gram-negative bacterium belongs to phylum Bacteroidetes and are widely distributed in a freshwater ecosystem.[23]

Rhodobacter and *Acinetobacter* were found in the south district, which was absent in spring waters of other districts. The diversity of *Rhodobacter* in an aquatic system is ubiquitous; they are photosynthetic bacteria belonging to phylum Proteobacteria.[37] Some of the Important *Rhodobacter* sp. identified from aquatic environment is *Rhodobacter adriaticus* isolated from Adriatic Sea.[37] *Rhodovulum aestuarii* isolated from brackish water was collected from an estuary.[65]

Suresh et al[80] isolated *Rhodobacter azalea* and *Rhodobacter locals* from different pond samples of Kukatpally, India. *Rhodobacter vinaykumarii* is marine phototrophic *alpha-proteobacterium* from tidal waters in Visakhapatnam, on the east coast of India.[77] *Rhodobacter changelensis* is isolated from the Indian Himalayan snow test of Changlapass.[2] Investigations of microbial diversity, particularly bacterial in Lonar Soda Lake situated in Buldhana area of Maharashtra were reported by Joshi et al.[40] About 196 strains of oxygen consuming and alkaliphilic microscopic organisms were separated utilizing different media.

Microbes mediated biogeochemical transformations in wetlands: Conrad et al[14] used a range of methods for elucidating the pathways of methane formation like isotope fractionation and molecular detection. Sun et al[79] examined the methane production rates in three peat lands. Irvine et al demonstrated that methanogens in salt marshes are N-limited. Acetate addition to these soils stimulated rates of CH_4 production by increasing the relative abundance of the *Methanosarcinaceae*.[8] Alam and Jia[1] confirmed that addition of nitrogenous fertilizers stimulated specific methane oxidizers in many different rice soils. Gu et al[33] studied the interactions between the cycles of sulphur and nitrogen in wetlands. Fifty percent of the isolates were found to be Bacillus.[45] *Rhodococcus* sp., was found to be associated with *Azotobacter* sp. nitrogen-fixing and these strains along with the nitrogen-fixing strains were found to be associated to plants symbiotically.[48,60,66]

Studies for microbial density: Density of microbes refers to a measure of overall quantity of microbes within a wetland. Plate counts are very useful for estimating a key stream of bacteria and fungi and have been found to be compatible with molecular methods while calculating living microbial diversity and density. Vacca et al[86] suggested that it is the only qualitative method used for the approximation of actual bacterial density. Staining samples using BacLight kit allowed for separation between viable and non-viable

bacteria.[18] Epifluorescent microscopy is used to determine total viable bacterial counts in wetlands.[18,52,84]

Wetland loss and degradation: Wetlands are facing the negative effects of the travel industry.[11] It began with the arrival of the European colonialists who regarded wetlands as wastelands unless there was some economic advantage. They have converted mangroves to paddy field in West Bengal and the present Bangladesh. In addition, marshes were considered particularly suitable for drainage because they harboured mosquitoes and other vectors of disease. Even today wetlands are being considered wastelands in our revenue records. All remaining wetlands were targets of major hydrological changes on a large scale.

Wetlands harbour high levels of biodiversity, hence these hotspots would receive top priority for the conservation of biodiversity. The urban wetlands are the most threatened for their existence as rapid loss of wetlands has taken place in Bengaluru, Delhi, Hyderabad and Kolkata with rapid urbanization.

Wetlands are being used as land fill sites or as for dumping solid wastes. Floods in Kashmir valley and Kedarnath valley clearly show the loss of floodplain wetlands which resulted in flooding. Long-term changes adversely affect the wetlands which are due to the disposal in the wetland of untreated domestic and indust rial wastewaters. Unless the sources and routes of their water supply are not blocked, alterations in the wetlands cannot take place.

Measures for restoration of wetland diversity: Studies have shown that many small wetlands together support more biological diversity than one large wetland. There is a real need to address the real causes of wetland loss and degradation. In the Indian context, the Ministry of Environment and Forests,[51] Government of India has given high priority to the wetland conservation. The National Environment Policy (NEP)[82] 2006 seeks to set up a legally enforceable regulatory mechanism for identified wetlands to prevent their degradation and enhance their conservation.

The Wetlands Conservation Program has funded conservati on projects since 1985 and is currently supporting 115 wetlands including Asan and Jhilmil Jheel in Uttarakhand. In 2010, the MoEF notified the Wetlands (Conservation and Management Rules, 2010[83] under the Environment (Protection) Act, 1986 to provide a much needed legal framework and to set up a national authority for the conservation and management of India's wetlands. Under rule 6, section 2 of these regulations, all State governments are required to define and classify wetlands within their respective territories in accordance with the requirements laid down in rule 3.

One of the forms of wetlands protected by this law is the High Altitude Wetlands or Wetland Complexes at or above 2,500 meters altitude which cover an area equal to or greater than 5 hectares.

Conclusion

Wetlands have to be conserved as loss of wetland directly as well as indirectly harms human habitation. Although they are known to release green house emissions, they also perform very important functions such as bioremediation, maintenance of microbial biodiversity and store water for drinking as well as agricultural purposes.

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(Received 10th April 2020, accepted 14th June 2020)