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Physico-Chemical and Microbiological Water Quality of Asan Wetland of Garhwal Himalaya, India

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Abstract: Asan wetland is an artificial lake that is located about 40 km west of Dehradun the capital city of Uttarakhand in India. This wetland came into existence during the construction of Asan barrage in the year 1967. This lake is a unique wetland that provides habitat for various migratory birds from all over the Himalayan and Arctic region during the winter season. The main objective of the current study was to assess the water quality of the lake that was affected by increasing level of various human activities at the site. For this purpose, the various physico-chemical and microbiological characteristics of the lake were recorded monthly for two continuous years from October, 2014 to September, 2016. After the assessment of various water quality parameters, the results represented the poor quality of the lake water. These results will be used in the sustainable restoration of the lake that will boost the efforts of Indian Government to get it declared as Ramsar site.

Keywords: Water Quality, Asan Wetland, Ramsar Site, Uttarakhand, India

1. Introduction

Water is considered as the main source of energy and governs the evolution on the Earth. Around 71% of the earth surface is overlaid by water in the form of rivers, lakes, seas, oceans, glaciers and ice caps. Water is also considered as very crucial for the survival of any life on the planet "Earth". Water is used in the greatest amount across the globe for various purposes such as drinking, bathing, washing, recreation, irrigation and aquaculture. Rivers, ponds, lakes, wetlands are the major sources of surface water or freshwater. Wetland is the surface of land that is covered with water either for the whole or part of the year. Wetlands are also known as "Biological Super Market". Wetlands are the depthless or shallow ecosystems having abundant nutrients. Wetlands are the most productive ecosystems and essential for life supporting systems that provide a wide array of species of Plants and Animals.

India has various natural wetlands that occupied an area of around 1.5 million hectares or 18.4% of countries geographical area. India has both inland as well as coastal wetland ecosystems. These wetlands are also the best source of water for human consumption in various ways including drinking, washing, bathing etc. for drinking purpose. This water must be clean and free from any type of contamination including the harmful microbes [1].

Access to safe drinking water is a fundamental right of every human being and therefore it is the duty of the government to provide safe and clean access of water to every citizen. For this we have to assess the water quality of each and every water body whose water can be used by the human beings for drinking purpose. Surface water pollution in the freshwater bodies with various organic chemicals and substances of human origin is a global issue. Due to different anthropogenic activities most of the rivers, lakes, streams and ponds are of poor water quality because such type of water bodies are the recipients of raw sewage from industries, households, municipal, animal farms, etc. They all are affecting the water quality of a water body. In order to monitor and achieve a better quality of surface water it is of urgent need to conduct a microbiological analysis that should define the water quality of a water body. Due to their metabolic diversity and ability to respond quickly to environmental changes, bacteria are the ideal indicators of the pollution of surface water [2]. The objective of this work is to assess the level and seasonal dynamics of physico-chemical parameters along with organic and fecal pollution of Asan wetland located in the Dehradun district of Uttarakhand, India. This wetland is of national importance to assess the microbial water quality because Indian government made an effort to get it declared as Ramsar site.

Asan wetland came into existence during the construction of Asan barrage at the confluence of Asan River and the outlet drain channel from Kulhul powerhouse in the year 1967. It is a standby reservoir and is mainly fed with water of the River Asan and the discharge channel of Yamuna River. In this reservoir, the water level is controlled, sometimes the water level fluctuates and goes down due to which a swampy or marshy island has been created and attracts a variety of marsh loving birds. Asan wetland belongs to water-storage reservoir or dam type and it lies in Indo-Gangetic Monsoon forests biogeographic province [3].

A lot of work has been done on various lakes and for various aspects that includes the work of Gupta and Hamid [4] on water quality of Lake Quran, Singh *et al*. [5] on some lakes of the Western Himalayan region in India; Martinez and Galera [6] on Taal lake, Philippines; Singh and Laura [7] on Tilyar lake of Rohtak, Haryana; Ravi Kumar *et al*. [8] on Sankey tank and Mallathahalli lake of Bangalore; Ramesh and Krishnaiah [9] on Bellandur lake of Bangalore; Mushtaq *et al*. [10] on Kashmir Himalayan lake; Bhateria and Jain [11] on water quality assessment of lake water; Sharma *et al*. [12] on physico-chemical and microbial water quality of Bhimgoda wetland and Rana *et al*. [13] on the assessment of surface water quality of the Himalayan Lake Beni Tal but a less amount of literature is available on the public domain specially related to the microbiological water quality of any lake. No sincere attempt has been made to assess the microbiological, physical and chemical water quality of a wetland and this has been proven by the amount of literature available on the public domain. Therefore, the aim of this study was to determining the physical, chemical and microbiological water quality of the Asan wetland.

2. Materials and Methods

2.1. Study Area

Asan wetland is an artificial wetland or in simple words we can say that this wetland is made by human beings. Asan wetland is about 3.8 km^2 in area, which is located 40 km west of Dehradun the capital city of Uttarakhand in India on the way to Ponta Sahib (Figure 1). Geographically, this wetland is situated at an altitude of 389 m a.s.l. between latitude 30°24' - 30°28' N and Longitude 77°40' - 77°44' E at the confluence of the River Asan and Yamuna. The Asan wetland was created due to regulation of river for power generation at Kulhul power station. Now, it has become a famous bird reserve of Uttarakhand for attracting the Himalayan and Arctic birds during winter season. The wetland is 287.5 m long with the minimum and maximum of water level respectively at 396 m and 402 m a.s.l. The main aquatic vegetations of this reservoir are *Eichhorniacrassipes, Potomagetonpectinatus, Typhaelephantina* and *Ceratophyllumdemersum.*

2.2. Water Quality Indicators

Physical, chemical and microbiological water quality indicators were selected with respect to the following properties:

Physical: The physical parameters that were considered to assess the water quality of Asan wetland were water temperature, conductivity, transparency, pH, turbidity and total dissolved solids (TDS). Out of these six physical quality parameters, three (pH, transparency and water temperature) were analyzed at the sampling sites.

Nutrients and Chemical Parameters: Nutrients are most responsible for the degradation to water quality. The nutrients and chemical parameters analyzed to assess the quality of water sample were dissolved oxygen (DO), free CO_2, phosphates (PO_4), nitrates (NO_3), sulphates, chlorides, sodium and potassium. Out of these eight parameters, two (DO and free CO_2) were analyzed at the sampling site and the remaining parameters were analyzed in the laboratory.

Microbiological: Microbiological assessment means the assessment of microorganisms present in the water sample to evaluate the quality of water, whether this water is fir for human consumption or not. The microbiological assessment involves the presence of any bacteria, fungi, actinomycetes, fecal coliform and total coliform. Presence of fecal coliform confirms the contamination of fecal material is an indirect source of decomposed organic matter. Recognized bacterial indicators for assessing water quality are bacteria of the Enterobacteriaceae family defined as total coliform bacteria and the fecal coliform bacteria. The coliform bacteria are gram- negative rod shaped bacteria usually found in the human and animal waste. Higher is the concentration of such bacteria higher is the pollution in water and higher is the health risk.

2.3. Sample Analysis

The water sample was undertaken from the Asan wetland (389 m a.s.l.). Water samples from the above mentioned site were collected during October 2013 to September 2015 by dipping the autoclaved thermosteel flask and closing the cap under water surface to prevent the atmospheric exposure and contamination to assess the quality of water of Asan wetland. The water sample was collected during the morning time between 8:00 am to 10:00 am. Physico-chemical parameters of the water sample were analyzed by following the standard method outlined in Wetzel and likens [14] and APHA [15]. Some of the physico-chemical parameters such as pH, DO, free CO_2, temperature were measured at the sampling site. For the remaining parameters, the water sample was transferred to the laboratory (180 km) away. For the microbiological sampling, three replicates of water sample were collected. First replicate was the surface water, second replicate was taken 10 cm below the surface water and the third replicate was taken 20 cm below the surface, mixed all the samples thoroughly in the sample bottle and then placed it in the ice box filled with frizzed ice packs and analyzed within 24 hours.

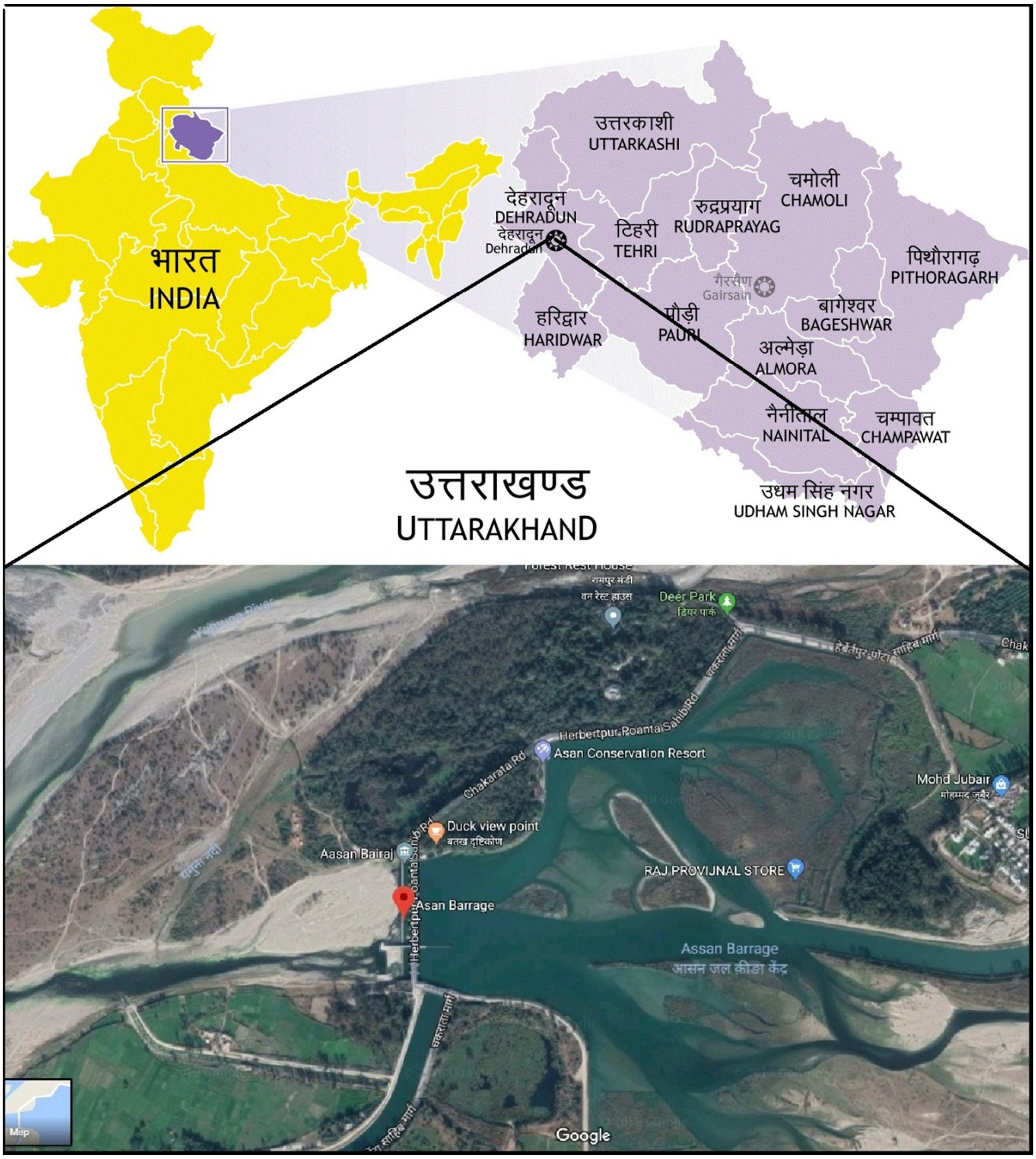

Figure 1. *Location map of the site Asan wetland.*

2.4. Microbial Enumeration

Nutrient Agar media (HiMEDIA) was used for the estimation of the numbers of colony forming units (CFUs) of bacteria. Media pH for bacterial isolation was set according to the pH of sampling sites. Sabaroud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) were used for fungal species. Both media (SDA and PDA) were supplemented with 50mg.l^{-1} of each Streptomycin and Ampicilin to prevent bacterial contamination. Actinomycetes Isolation Agar (AIA) was used for actinomycetes isolation. Specific media, Eosin Methylene Blue Agar (EMB) medium was used for the detection of the members of the family, *Enterobacteriaceae* and plates (EMB) were incubated for 24 hrs at 37°C. *Vibrio* was detected using Trypticase Citric Bile Salts (TCBS) as a selective plating medium. The medium contains sucrose and therefore allows the differentiation of *Vibrio* species such as *Vibrio cholera* (sucrose positive) and *Vibrio haemolyticus* (sucrose negative). To enrich sample for *Vibrio* growth, 1% of Alkaline Peptone Water (APW) was added to the water samples. *Vibrio* species grew on the agar plates as yellowish, round colonies. The numbers of total and fecal coliforms were determined using Most Probable Number (MPN) method. Statistical tables were used to interpret the results of

Most Probable Number (MPN) of the bacteria. From each dilution 1ml was added to each of triplicate tubes containing 5 ml of MacConkey broth. The tubes were incubated at 37°C for 24 hrs for total coliforms and 44°C (in water bath) for 24 hrs for fecal coliforms. The positive tubes were streaked on the Eosin Methylene Blue (EMB) agar plates and incubated at 37°C for 24 hrs [15].

2.5. Microbial Identification

To study the morphological characteristics, the purified selected bacterial isolates were Gram stained and observed under Phase Contrast Microscope (Nikon Eclipse TS100). Moreover, detailed biochemical characterizations were carried out to identify the bacterial isolates up to possible genus or species level. Identification of all the fungal isolates was made by microscopic analysis by using taxonomic keys and standard procedures. Some of the bacterial cultures isolated from the Asan wetland were sent to Microbial Type Culture Collection and Gene Bank, MTCC (Institute of Microbial Technology), Chandigarh for identification by using the MALDI-TOF.

Statistical treatment (mean; standard deviation) of the physico-chemical parameters of water was conducted for presenting the mean seasonal variations in bacterial CFU of the lake.

3. Results and Discussion

3.1. Physico-chemical Characteristics

The physico-chemical environmental variables of habitat environment were recorded from the lower altitude lake Asan wetland of the Garhwal Himalaya. The mean seasonal data of this wetland has been presented in Table 1. The water temperature in lower altitude lake ranged from a minimum of 11.9 ± 1.6°C in winter season to a maximum of 21.5 ± 4.3°C during monsoon season. Turbidity of water body is due to the wide range of suspended solids present or dissolved in the water. Almost clear water was recorded during the winter season (33.0+10.9 NTU) and very turbid water (228.0 ± 2.5 NTU) was recorded during monsoon season as a consequence of heavy precipitation in the upper catchment area of the lake. The transparency of the lake was found to be minimum (0.00 ± 0.00 m) during monsoon season and maximum (1.14 ± 0.13 m) in winter season during the entire period of study. The minimum and maximum transparency was due to the absence and presence of rain fall and melting of snow at higher reaches. Conductivity of water was recorded minimum (0.185 ± 0.013 µS cm^{-1}) in winter season and maximum (0.267 ± 0.018 µS cm^{-1} during monsoon season. However, the total dissolved solids (TDS) were recorded minimum (50 ± 5.6 µS cm^{-1}) in winter and maximum (238 ± 8.5 µS cm^{-1}) in monsoon season during the entire period of study. Total dissolved Solids (TDS) represents mainly the various kinds of minerals present in water. That may be composed mainly of carbonates, bicarbonates, chlorides, sulphates, phosphates, nitrates, etc. The water of this lake was recorded to be alkaline (7.64 ± 0.07 to 8.07 ± 0.08) throughout the period of study. The dissolved oxygen is one of the most important chemical parameters, which reflects the physical and biological processes prevailing in the water. The dissolved oxygen was found to be minimum (6.00 ± 0.75 mg l^{-1}) during monsoon and maximum (9.12 ± 0.36 mg l^{-1}) during winter season. However, free carbondioxide was recorded to be minimum (0.48 ± 0.40 mg l^{-1}) in winter season and maximum (1.12 ± 0.58 mg l^{-1}) during monsoon season in the Asan wetland. The free CO_2 is an end product of respiration and aerobic decomposition of organic matter [16]. Phosphates enter fresh water from atmospheric precipitation and from groundwater and surface run-off. The concentration of phosphates was recorded to be minimum (0.036 ± 0.005 mg.l^{-1}) in summer season and maximum (0.049 ± 0.003 mg.l^{-1}) during monsoon season. The concentration of nitrates was recorded to be minimum (0.077 ± 0.003 mg.l^{-1}) during summer season and maximum (0.109 ± 0.018mg.l^{-1}) during monsoon season. Nitrates represent the highest oxidized form of nitrogen. It is most predominant form of inorganic nitrogen entering freshwater, groundwater and precipitation. The concentration of sulphates was found to be minimum (0.01 ± 0.1 mg.l^{-1}) in winter season and maximum (0.08 ± 0.13 mg.l^{-1}) during monsoon season. Sulphate is a naturally occurring anion in all kinds of natural water. The concentration of chlorides was recorded to be minimum (8.98 ± 2.35 mg.l^{-1}) in winter season and maximum (12.05 ± 2.54 mg.l^{-1}) during monsoon season. Sodium is naturally occurring element in water. The concentration of Sodium was recorded to be minimum (13.00 ± 0.65 mg.l^{-1}) in winter season and maximum (19.45 ± 0.27 mg.l^{-1}) during monsoon season. Potassium is another important cations occurring naturally. The concentration of Potassium was recorded to be minimum (1.85 ± 0.43 mg.l^{-1}) in winter season and maximum (4.38 ±0.68 mg.l^{-1}) during monsoon season. Asan wetland is situated at north-west portion of Doon Valley and north-eastern slopes of Shivaliks at an altitude of 389 m a.s.l. Initially, the Asan wetland was constructed for power generation at Kulhul Power House, later it is converted into wetland by accumulation of silt and sediments which is carried out by Yamuna and Asan rivers. It is favorable place for migrating birds. The Asan wetland receives pollution from a variety of sources. Human interference in terms of tourist influx, oil leakage, gas discharge from water boat activities had adverse impact on Asan wetland. Discharge of domestic and households wastes located in the catchment area creating pollution problem in Asan wetland. There are many pharmaceuticals factories located in the catchment area of Asan. They are dumping their effluents into the Asan River which are carried ultimately into the Asan wetland. Asan wetland has received some non-point sources of pollution such as pesticides and fertilizers run-off from both agricultural and urban areas. An influx of excess nutrients has a profound effect on the wetland. Excess phosphates and nitrates routinely enter into the water of wetland from agricultural fields and run-off from sub-urban areas and affect its water quality.

Table 1. *Mean ($\overline{x}$ ± SD) seasonal variations in physico-chemical environmental variables of Asan wetland of Garhwal Himalaya, India.*

Parameters	Autumn (Oct-Nov)	Winter (Dec-March)	Summer (April-June)	Monsoon (July to Sept)
Water Temperature (°C)	15.6±1.8	11.9±1.6	21.5±4.3	21.2±2.5
Turbidity (NTU)	95±17.8	33±10.9	68±48.2	228±2.5
Transparency (m)	1.0±0.48	1.14±0.13	0.74±0.54	0.00±0.00
Conductivity ($\mu S.cm^{-1}$)	0.22±0.014	0.19±0.013	0.20±0.016	0.27±0.018
TDS ($mg.l^{-1}$)	112±31.1	50±5.6	62±13.2	238±8.5
pH	7.97±0.03	8.07±0.08	7.76±0.19	7.64±0.07
Dissolved Oxygen ($mg.l^{-1}$)	8.20±0.07	9.12±0.36	7.2±0.95	6.0±0.75
Free CO_2 ($mg.l^{-1}$)	0.62±0.15	0.48±0.40	0.98±0.49	1.12±0.58
Phosphate ($mg.l^{-1}$)	0.05±0.001	0.045±0.008	0.036±0.005	0.049±0.003
Nitrite ($mg.l^{-1}$)	0.086±0.006	0.091±0.014	0.077±0.003	0.109±0.018
Sulphate ($mg.l^{-1}$)	0.04±0.005	0.01±0.1	0.07±0.01	0.08±0.13
Chloride ($mg.l^{-1}$)	9.65±1.35	8.98±2.35	10.07±2.37	12.05±2.54
Sodium ($mg.l^{-1}$)	17.85±2.42	13.00±0.65	18.45±0.38	19.45±0.27
Potassium ($mg.l^{-1}$)	2.87±0.32	1.85±0.43	2.74±0.42	4.38±0.68

3.2. Microbiological Characteristics

An overall nine species of bacteria (*Campylobacter sp, Escherichia coli, Enterobacter aerogens, Klebsiella sp, Legionella sp, Pseudomonas aeruginosa, Pseudomonas fluorescens, Staphylococcus aureus, Streptococcus faecalis)* and two genera of Actinomycetes Streptomyces sp (*Streptomyces clavifer, Streptomyces rangoon)* and Nocardia sp. were found in the Asan wetland. However, ten genera of fungi (*Achlya spp, Alternaria sp, Aspergillus flavus, Aspergillus niger, Cladosporium spp, Curvularia spp, Penicillum spp, Phoma sp, Rhizopus sp* and *Trichoderma sp)* were recorded in Asan wetland. The α- Diversity of microbes wasfound to be 23 in Asan wetland (Table 2). The minimum CFU count was during winters, which was 55,450 $CFU.ml^{-1}$ and maximum was during monsoon season, which was 91,245 $CFU.ml^{-1}$ (Figure 2). Besides the nitrates level, high concentration of phosphates were also recorded caused by human activities. Nitrates and phosphates showed a positive correlation with bacterial growth. Nitrogen was found to be very high in Asan wetland which comes from fecal matter of migratory birds. Migratory birds used to stay at the Asan wetland from October to April. During this period, densities of Escherichia coli and Campylobacter sp. were found to be very high. Coliforms bacterial populations were found high in Asan wetland. Fecal discharge of domestic and households wastes located in the catchment area is the major contributor to coliform pollution in Asan wetland. *Enterobacter aerogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Streptocossus fecalis, Legionella sp*. and *Campylobacter sp*. were detected in large numbers in Asan wetland.

Table 2. *Microbial diversity in Asan wetland of Garhwal Himalaya, India.*

S. No.	Microbes	Asan wetland
A	Bacteria	
1	*Campylobacter sp**	+
2	*Escherichia coli*	+
3	*Enterobacter aerogens*	+
4	*Klebsiella sp**	+
5	*Legionella sp**	+
6	*Pseudomonas aeruginosa*	+
7	*Pseudomonas fluorescens*	+
8	*Staphylococcus aureus*	+
9	*Streptococcus faecalis*	+
B	Actinomycetes	
1	*Streptomyces clavifer*	+
2	*Streptomyces Rangoon*	+
3	*Streptomyces sp**	+
4	*Nocardia sp**	+
C	Fungi	
1	*Achlya spp**	+
2	*Alternaria sp**	+
3	*Aspergillus flavus*	+
4	*Aspergillus niger*	+
5	*Cladosporium sp**	+
6	*Curvularia sp**	+
7	*Penicillum sp**	+
8	*Phoma sp**	+
9	*Rhizopus sp**	+
10	*Trichoderma sp**	+
α- Diversity		23

Abbreviations: *: Unidentified at species level; +: Present

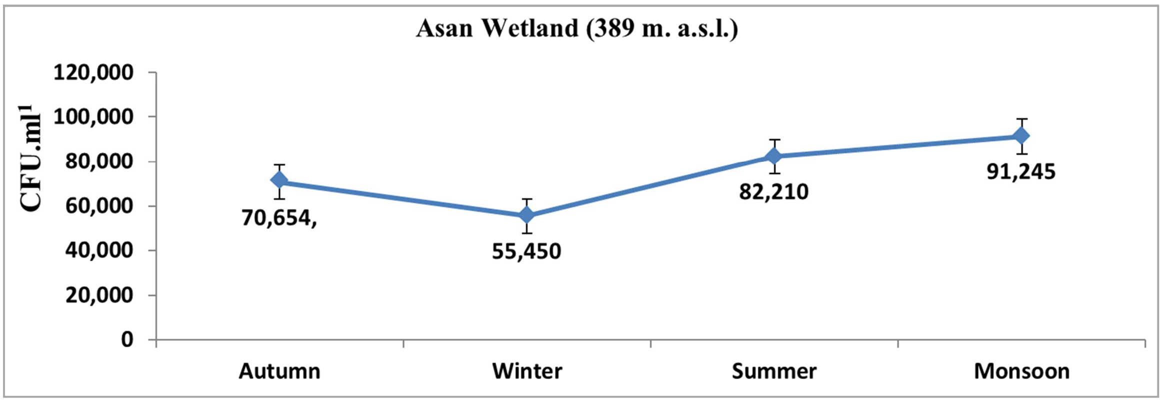

***Figure 2.** Mean ($\overline{x}$ ±SD) seasonal variations in colony forming units (CFU.ml^{-1}) of bacteria in Asan wetland of Garhwal Himalaya.*

4. Ecosystem Management

1. An efficient and effective sewage or wastewater treatment system has to be designed for the 'Asan wetland' to reduce the suspended solid, organic matter, bacterial activity and nutrients which degrade its water quality.
2. A program should be launched by the government for the farmers to aware them, to reduce the indiscriminate use of chemical pesticides and chemical fertilizers and encourage them to adopt the concept of organic agriculture and use biopesticides and biofertilizers in the nearby agriculture field to the wetland.
3. A permanent water quality program should be installed at the site to detect the fluctuation in some important water quality parameters such as water transparency, total dissolved solids, organic matter, dissolved oxygen and pH. This monitoring program would be used in the management and conservation of Asan wetland and its water quality.

5. Conclusions

The results presented in this work were observed after analyzing the water samples for two continuous years during October, 2014 to September, 2016. The data represents the poor health of the lake and also the factors which are responsible for its poor health. Many human activities have been observed that deteriorate the water quality of the lake very quickly for the last many decades. It is well known that natural water level fluctuations are vital for the aquatic ecosystem health from the microscopic to the macroscopic trophic level, since all are related by the aquatic food chain.

In this study, it was suggested that the Asan wetland is an artificial lake, that is threatened by all types of anthropogenic activities and pollution which was caused by these activities. Studies have shown that wetlands are nutrient purifies of waste water due to their ability to remove or reduce contaminants including organic matter and pathogens. The reduction is due to sedimentation, filtration, precipitation, adsorption, microbial interactions and uptake vegetation [17]. The present study on this low altitude lake of Garhwal Himalaya has revealed that this lake is much more polluted and threatened by high influx of inorganic and organic nutrients from agricultural pesticides run-off, boating activities and enteric pathogenic bacterial contamination.

This study on the Asan wetland has also revealed that the seasonal fluctuations of various physico-chemical environmental variables have important role in the distribution, periodicity and composition of microorganisms. It may be concluded that the growth, density, periodicity, dominance and diversity of microbes is influenced to the greater extent by interaction with biotic and abiotic factors, seasonal variations and various physico-chemical environmental variables in the Asan wetland.

Conflict of Interest

The authors declare that there is no conflict of interest.

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