Physico-chemical and Microbiological Water Quality of Bhimgoda Wetland of Garhwal Himalaya, India

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Abstract
This study aimed to assess the physical, chemical and microbial quality of water of Bhimgoda wetland located in the Hardwar city of Uttarakhand, India. This reservoir is a unique wetland that provides habitat for migratory birds from all over the Himalayan and Arctic region during the winter season specially the marsh loving migratory birds. Water samples were collected monthly over a period of two years from October, 2014 to September, 2016. Fourteen physico-chemical parameters and microbial diversity including bacteria, fungi and actinomycetes were analyzed during the study period. The results revealed the presence of colony forming units of bacteria, which was maximum during the monsoon season (92,087 CFU.ml^{-1}) and minimum during the winter season (51,842 CFU.ml^{-1}). A total of eleven species of bacteria, four species of actinomycetes and ten species of fungi were identified in the water sample. The α-diversity of microorganisms in Bhimgoda wetland was 25. This revealed the poor quality of the water of Bhimgoda wetland which was also confirmed by the results of physico-chemical parameters. These results will be used in the sustainable restoration of the lake. Sustainable restoration of the lake will boost the efforts of Indian Government to get it declared as Ramsar site.
Keywords: Bhimgoda wetland, microbiological, physico-chemical, Uttarakhand, water quality

Introduction

Water is the main source of energy and governs the evolution on the Earth. Water is considered as very important for the survival of any life on the planet "Earth". It is used in the greatest amount across the globe for various human activities 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 nutrient or very rich in nutrients. Wetlands are the most productive ecosystems and essential 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 all types of microorganisms such as bacteria, fungi and actinomycetes (Karafistan and Arik-Colakoglu 2005)

Access to safe water is a fundamental human need and therefore, it is the duty of the government to provide safe and clean 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 quality because such type of water bodies are the recipients of raw sewage from industries, households, municipal, animal farms, etc. They all directly affect or degrade the quality of water. 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 (Kavka et.al. 2006). The objective of this work is to assess the level and seasonal dynamics of physico-chemical parameters along with the microbial pollution of Bhimgoda wetland located in the Hardwar city of Uttarakhand, India.

A lot of work has been done on various lakes and rivers for various aspects that includes the works of Ramesh and Krishnaiah (2014) on Bellandur lake of Bangalore; Singh and Laura (2012) on Tilyar lake of Rohtak, Haryana; Ravi Kumar et.al. (2013) on Sankey tank and Mallathahalli lake of Bangalore, Singh et.al. (2008) on some lakes of the Western Himalayan region in India, Pinto et. al. (2012) on microbiological quality assessment of beaches of south coast, Brazil, Cucak et. al. (2016) on microbiological water quality of Nisava river, Sharma and Sharma (2016) on microbiological water quality of Alaknanda river and Kora et. al. (2017) on bacteriological screening of Hussain Sagar lake. However, a particular literature is available on the public domains specially related to the microbiological water quality of any wetland. No sincere attempt has been made to assess the microbiological, physical and chemical assessment of water quality of Bhimgoda wetland. Therefore, this study was aimed to determine the physical, chemical and microbiological water quality of the Bhimgoda wetland.

Material and Methods

Study area

Bhimgoda wetland is an artificial wetland, which is situated between the Neeldhara and other tributaries of the Ganga, in the vicinity of famous Rajaji National Park, located at Hardwar in Uttarakhand. Geographically, this wetland is located between latitude $29^0$58' N and longitude $78^0$13' E on transitional zone of Himalayan foothills and plains at an altitude of 310 m above m.s.l. The area of Bhimgoda wetland is around 2.5 km^2 (**Figure 1**). It is a standby reservoir, which is basically created for power generation after impoundment of lotic water body. It is fed by the discharge channel of river Ganga. The water level is controlled, which often fluctuates and goes up and down., due to which a marshy island become distinct and plays host to thousand of marsh loving migratory water birds. Hussain and De Roy (1993) have biogeographically categorized Indian wetlands. According to their categorization, Bhimgoda wetland lies in biogeographic province 4.8.4 (Indo-Gangetic Monsoon forests). Bhimgoda wetland belongs to water-storage reservoir or dam type wetland. The chief aquatic vegetation of this reservoir or wetland comprises *Eichhornia crassipes, Potomageton pectinatus, Typha elephantine* and *Ceratophyllum demersum*.

Figure 1: The study area (Google map of Bhimgoda wetland)

Water quality indicators

Physico-chemical and microbiological water quality indicators were selected with respect to the following properties:

Physico-Chemical Parameters: The physico-chemical parameters that were considered to assess the water quality of Bhimgoda wetland were water temperature, conductivity, transparency, pH, turbidity, total dissolved solids (TDS), dissolved oxygen (DO), free CO_2, phosphates (PO_4), nitrates (NO_3), sulphates, chlorides, sodium and potassium. Out of these water quality parameters five (pH, transparency, water temperature, dissolved oxygen and free CO_2) were analyzed at the sampling sites 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 fit 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. 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.

Sampling

The water sampling was undertaken from the Bhimgoda wetland (310 m a.s.l.). Water sample from the Bhimgoda wetland were collected during October 2014 to September 2016 by dipping the autoclaved thermosteel flask and closing the cap under 20 cm of surface water to prevent the atmospheric exposure and contamination to assess the quality of water of Bhimgoda wetland. Water sample were 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 (1991) and APHA (2005). 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 (145 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 water. All the replicates were mixed thoroughly in the sample bottle and then placed it in the ice box filled with freezed ice packs and analyzed within 24 hours.

Bacterial 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) was used for fungal species. SDA media was supplemented with $50 mg.l^{-1}$ 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 37^0C. *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 37^0C for 24 hrs for total coliforms and 44^0C (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 37^0C for 24 hrs (APHA, 2005).

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 sample were sent to Microbial Type Culture Collection and Gene Bank, MTCC (Institute of Microbial Technology), Chandigarh for identification.

Statistical analysis

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.

Results and Discussion

The physico-chemical environmental variables of habitat environment were recorded from the lower altitude lake Bhimgoda wetland of the Garhwal Himalaya. The mean seasonal data on the physicochemical variables of this wetland has been presented in **Table 1**. The water temperature in lower altitude lake ranged from a minimum of 11.63 ± 1.32 ^{0}C in winter season to a maximum of 18.20 ± 0.70 ^{0}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 (30.0+10.7 NTU) and very turbid water (197.0 ± 89.7 NTU) was recorded during monsoon season as a consequence of heavy precipitation in the upper catchment area of the reservoir. The transparency of the reservoir was found to be minimum (0.00 ± 0.01 m) during monsoon season and maximum (0.91 ± 0.47 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 (0.164 ± 0.010 µS cm^{-1}) in winter season and maximum (0.242 ± 0.015 µS cm^{-1} during monsoon season. However, the total dissolved solids (TDS) were recorded minimum (43.0 ± 4.5 µS cm^{-1}) in winter and maximum (217.0 ± 7.8 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 and nitrates. The water of this lake was recorded to be alkaline. It was minimum (7.57 ± 0.08) during summer and maximum (8.27 ± 0.21) during winter season. 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 (5.80 ± 0.29 mg l^{-1}) during monsoon and maximum (8.80 ± 0.63 mg l^{-1}) during winter season. However, free carbon dioxide was recorded to be minimum (0.42 ± 0.05 mg l^{-1}) in winter season and maximum (1.73 ± 0.19 mg l^{-1}) during monsoon season in the Bhimgoda wetland. The free CO_2 is an end product of respiration and aerobic decomposition of organic matter (Welch, 1952). 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 (0.051 ± 0.004 mg.l^{-1}) during monsoon season. The concentration of nitrates was recorded to be minimum (0.069 ± 0.004 mg.l^{-1}) during summer season and maximum (0.103 ± 0.021mg.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 ± 0.3 mg.l^{-1}) in winter season and maximum (0.11 ± 0.15 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 (8.67 ± 2.27 mg.l^{-1}) in winter season and maximum (11.14 ± 2.43 mg.l^{-1}) during monsoon season. Sodium is naturally occurring element in water. The concentration of Sodium was recorded to be minimum (12.69 ± 0.61 mg.l^{-1}) in winter season and maximum (19.15 ± 0.25 mg.l^{-1}) during monsoon season. Potassium is another important cations occurring naturally. The concentration of Potassium was recorded to be minimum (1.81 ± 0.39 mg.l^{-1}) in winter season and maximum (4.24 ± 0.59 mg.l^{-1}) during monsoon season. Bhimgoda wetland is situated at an altitude of 310 m a.s.l., which is basically a reservoir and is converted into wetland by accumulation of silt and sediments. It is favorable place for migrating birds. The Bhimgoda 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 Bhimgoda wetland. Discharge of domestic and households wastes located in the catchment area creating pollution problem in Bhimgoda wetland. Bhimgoda 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. An overall eleven species of bacteria (*Bacillus circulans, Campylobacter sp, Escherichia coli, Enterobacter aerogens, Klebsiella sp, Microbacterium schleiferi, Paenibacillus azatofixans, Pseudomonas aeruginosa, Pseudomonas fluorescens, Staphylococcus aureus, Streptococcus faecalis);* two genera of Actinomycetes *Streptomyces* sp (*Streptomyces clavifer, Streptomyces rangoon)* and *Nocardia* sp. were found in the Bhimgoda 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 Bhimgoda wetland. The α- Diversity of microbes was found to be 25 in Bhimgoda wetland (**Table 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 Bhimgoda wetland which comes from fecal matter of migratory birds. Migratory birds used to stay at the Bhimgoda wetland from October to April. Coliforms bacterial populations were found high in Bhimgoda wetland. Fecal discharge of domestic and households wastes located in the catchment area is the major contributor to coliform pollution in Bhimgoda wetland. *Enterobacter aerogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Streptocossus fecalis, Legionella sp*. and *Campylobacter sp*. were detected in large numbers in Bhimgoda wetland. The presence of colony forming units of bacteria, which was maximum during the monsoon season (92,087 CFU.ml^{-1}) and minimum during the winter season (51,842 CFU.ml^{-1}) (**Figure 2**)

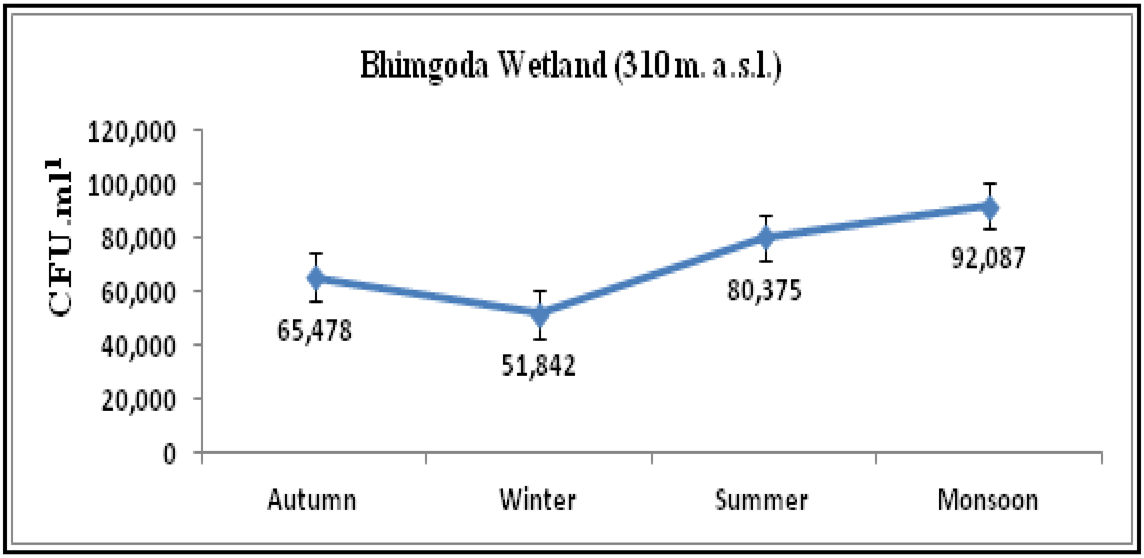

Figure 2: Mean ($\bar{x}$ ±SD) seasonal variations in colony forming units (CFU. ml^{-1}) of bacteria in Bhimgoda wetland of Garhwal Himalaya

Table 1: Mean ($\bar{x}$ ± SD) seasonal variations in physico-chemical environmental variables of Bhimgoda wetland of Garhwal Himalaya, India

Parameters	**Autumn (Oct-Nov)**	**Winter (Dec-March)**	**Summer (April-June)**	**Monsoon (July to Sept)**
Water Temperature (^{0}C)	15.00±1.42	11.63±1.32	17.38±1.87	18.20±0.70
Turbidity (NTU)	63.0±14.6	30.0±10.7	84.5±57.3	197±89.7
Transparency (m)	0.85±0.07	0.91±0.47	0.37±0.11	0.00±0.01
Conductivity (µS.cm^{-1})	0.198±0.009	0.164±0.010	0.184±0.012	0.242±0.015
TDS (mg.l^{-1})	96.3±19.7	43.0±4.5	57.0±13.2	217±7.8
pH	7.79±0.04	8.27±0.21	7.57±0.08	7.75±0.39
Dissolved Oxygen (mg.l^{-1})	8.20±0.92	8.80±0.63	7.62±0.30	5.80±0.29
Free CO_2 (mg.l^{-1})	0.58±0.11	0.42±0.05	0.64±0.08	1.73±0.19
Phosphates (mg.l^{-1})	0.04±0.008	0.05±0.006	0.036±0.005	0.051±0.004
Nitrates (mg.l^{-1})	0.074±0.005	0.087±0.011	0.069±0.004	0.103±0.021
Sulphates (mg.l^{-1})	0.06±0.007	0.01±0.3	0.08±0.01	0.11±0.15
Chlorides (mg.l^{-1})	8.95±1.13	8.67±2.27	9.17±2.31	11.14±2.43
Sodium (mg.l^{-1})	16.75±2.39	12.69±0.61	17.41±0.34	19.15±0.25
Potassium (mg.l^{-1})	2.63±0.32	1.81±0.39	2.68±0.42	4.24±0.59

Table 2: Microbial diversity in Bhimgoda wetland of Garhwal Himalaya, India

S. No.	Microbes	Bhimgoda wetland
	A. Bacteria	
1	*Bacillus circulans*	+
2	*Campylobacter sp**	+
3	*Escherichia coli*	+
4	*Enterobacter aerogens*	+
5	*Klebsiella sp**	+
6	*Microbacterium schleiferi*	+
7	*Paenibacillus azatofixans*	+
8	*Pseudomonas aeruginosa*	+
9	*Pseudomonas fluorescens*	+
10	*Staphylococcus aureus*	+
11	*Streptococcus faecalis*	+
	B. Actinomycetes	
1	*Streptomyces clavifer*	+
2	*Streptomyces rangoon*	+
3	*Streptomyces spp**	+
4	*Nocardia spp**	+
	C. Fungi	
1	*Achlya spp**	+
2	*Alternaria sp**	+
3	*Aspergillus flavus*	+
4	*Aspergillus niger*	+
5	*Cladosporium spp**	+
6	*Curvularia spp**	+
7	*Penicillum spp**	+
8	*Phoma sp**	+
9	*Rhizopus sp**	+
10	*Trichoderma sp**	+
α- Diversity		**25**

*: Unidentified at species level; +: Present

Conclusions

The results obtained during this study demonstrated poor physico-chemical and microbiological quality of the water of Bhimgoda wetland 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 the trophic levels are related by the aquatic food chain.

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