## An Assessment of Cyanobacterial Biodiversity in a Freshwater River System Associated with **Thermal Discharge from a Power Station**



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**Submitted by Leena Choubisa** VMOU/Research/Ph.D./BO/2015/78

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### **CERTIFICATE**

This is to certify that the thesis entitled **"An Assessment of Cyanobacterial Biodiversity in a Freshwater River System Associated with Thermal Discharge from a Power Station"**, embodies results of original investigation which was carried out by **Mrs. Leena Choubisa** (Reg. No. VMOU/Research/Ph.D./BO/2015/78) under my supervision in the Department of Botany, School of Science and Technology, Vardhman Mahaveer Open University, Kota, Rajasthan, India for partial fulfillment of Ph.D. degree to be awarded by Vardhman Mahaveer Open University. She has done her research work duly following UGC Regulations on Minimum Standards and Procedure for the award of M.Phil/Ph.D. Degree Regulations 2009.

Date: Place: Kota **Dr. Anuradha Dubey**

### **CERTIFICATE OF ORIGINALITY**

The research work embodied in this thesis entitled **"An Assessment of Cyanobacterial Biodiversity in a Freshwater River System Associated with Thermal Discharge from a Power Station"**, has been carried out by me duly following UGC Regulations on Minimum Standards and Procedure for the award of M.Phil/Ph.D. Degree Regulations 2009 at Department of Botany, School of Science and Technology, Vardhman Mahaveer Open University, Kota, Rajasthan, India. The work submitted for consideration for the award of Ph.D. degree is original, based upon the data collected by me. The content of this thesis neither full nor in parts, has not been submitted to other institute or University for the award of any degree or fellowship previously.

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## **CHAPTER 1 INTRODUCTION**

Free floating, unattached photosynthetic micro-organisms in water are together known as phytoplanktons. In an aquatic ecosystem phytoplanktons are the primary producers, the estimate of which can be used as water quality indicator (Gupta, 2007). Any change in water or environmental conditions can be detected by response of algae as they occur at first trophic level of food chain. Phytoplanktons possess short life cycle so they respond fast towards temperature changes (Trivedy, 1989)*.* Being easily available, cheap in use, more convincing and informative, algae are used as bioindicators (Lata Dora *et al*., 2010). Changes in water quality of aquatic system can be studied through bioassessment (Smith, 1992).

In phytoplankton community algae and blue-green algae form the important component. Blue-green algae have bluish green colour imparted by dominant phycocyanin pigment (Smith, 1994). But due to their close affinity to prokaryotic bacteria than to eukaryotic algae they are popularly known as cyanobacteria. The cells of cyanobacteria do not possess a well defined nucleus.

Cyanobacteria are the most primitive, cosmopolitan, thallophytic, photosynthetic prokaryotic algae which came into existence almost 3.5 billion years ago. The evidence of their origin can be traced through carbon isotopic data and study of microfossils which states their dominance during Precambrian period (Lee, 2008; Whitton, 2012).

They thrive well in variety of habitats from freshwater to marine, acidic to alkaline, hot water to soil, desert to snow so are cosmopolitan in nature (Smith,

1994). They grow best at temperature of 30°C to 40°C (Lata Dora *et al*., 2010). They are even able to tolerate temperatures more than 50 °C. Cyanobacteria like *Synechococcus* are even able to grow at temperature up to  $73^{\circ}C$ -74<sup>o</sup>C and are most thermophilic (Langford, 2001; Lee, 2008). These are the most successful organisms that are still in existence and are exploiting every possible extreme of habitats for billions of years since their existence (Dodds and Whiles, 2011).

Cyanobacteria show morphological diversity and may occur in variety of morphological forms that can be broadly categorized as filamentous and nonfilamentous forms. They can be unicellular like *Synechocystis*, can occur in form of trichome (chain of cells) like *Oscillatoria* or filament (chain of cells enclosed in a gelatinous sheath) like *Phormidium* which can be branched as in *Scytonema* or colonial as in *Merismopedia, Microcystis* (Lee, 2008). Under the light microscope, cyanobacteria are morphologically distinct in comparison to bacteria. Their size ranges from 1 µm in diameter to several 100 µm (Dodds and Whiles, 2011).

Central vacuole is absent in cyanobacterial cells. The main pigments present in their cells are chlorophyll- a, beta carotene, c-phycocyanin, allophycocyanin, cphycoerythrin and phycoerythrocyan in uniformly distributed in chromatoplasm as plastids or chloroplasts are absent. Their chief reserve food is glycogen or cyanophycean starch. They are closely related to prokaryotic bacteria in evolution. The membrane bound cell organelles are all together absent from their cells and the cell wall is similar to Gram negative bacteria containing amino sugars and amino acids. Cell wall is enveloped with mucilaginous sheath secreted by cell membrane in some genera.

Cyanobacteria are the only oxygenic phototrophic prokaryotes which can perform oxygen evolving photosynthesisthrough photosynthetic pigments diffused in the entire cytoplasm. In the aquatic food web, most of the organic carbon contribution available is done by phytoplanktons as they play a key role in global carbon cycle through assimilation of carbon and oxygenic photosynthesis (Whitton, 2012).

The unicellular forms show slow movements while cells with trichomes exhibit gliding or creeping movements. Most of the filamentous cyanobacteria possess thick walled cells with pale yellow colour homogenous contents called as heterocysts which may be intercalary or terminal in position. These are larger than vegetative cells and are photosynthetically inactive. Besides providing a suitable site for fragmentation of the filament, they serve as site for nitrogen fixation (Desikachary, 1959).

In aggregation some cyanobacteria can result in the formation of surface water blooms which are visible with naked eyes. They form an important component of blooms along with other algal groups. In absence of central vacuole, buoyancy is imparted by pseudovacuoles to planktonic forms. These pseudovacuoles are comprised of gas vesicles in the protoplast of cell and make them float that can result in formation of surface blooms. Bloom forming cyanobacteria can discolour the entire water body hence making the water unpotable. Major bloom forming taxa of cyanaobacteria are *Anabaena, Microcystis* and *Aphanizomenon,* others being *Oscillatoria* and *Spirulina* which makes them visible as a mat on the surface of water with naked eyes. Their growth is affected by light, temperature and nutrients (Palmer, 1980; Smith, 1992; Thajuddin and Subramanian, 2005; Lee, 2008; Moss, 2010; Sarma 2013).

Some of the cyanobacteria release a variety of toxins - cyanotoxins which may be neurotoxins, hepatotoxins or cytotoxins. These toxins act as anti herbivore chemicals for grazers. Cyanotoxins are detrimental to invertebrates, fishes and human health and can cause serious health problems when ingested with water.

(Lee, 2008).

Flagellated reproductive cells are altogether absent from their life cycle. Asexual reproduction and vegetative propagation in cyanobacteria occurs by fission or fragmentation and includes the formation of hormogones, endospores, nannocytes, planococci, exospores and akinetes (Smith, 1950; Lee, 2008).

Indian sub continent has recently been declared as one of the developed countries of Asia by United States Trade Representative (USTR) in terms of its per capita Gross National Income, share of world trade and G20 membership (The Hindu, 2020). As the nation is being considered as developed country its energy demand is of the utmost importance. Income and Energy has direct relation with each other. More and more income generating ideas are there but energy demands are also increasing and nation should be independent to fulfill this demand. The country harbours many power plants whether hydro electric, atomic, nuclear, gas based or thermal to meet her demands of energy.

Rajasthan is the largest state of the Indian sub- continent lying in its west flanked by Pakistan on the west with  $342,239$  km<sup>2</sup> area with very small area covered by rivers. Rajasthan state is basically a land of desert but possess a vivid morphology with a desert, plains, hills and plateau. Although not rich, but the state possess a big network of river system like Luni, Banas, Chambal, Mahi, Parwati, Mez, Parwan etc.

It is enriched with sunlight throughout the year. Besides solar plants, the state meets its demand of energy through gas, steam, hydro, atomic and thermal power stations. Major hydro power plants are Mahi dam situated on Mahi river, Jawahar Sagar Dam and Rana Pratap Sagar dam on Chambal river. Rajasthan generates power at different locations through various power plants functional under Rajasthan Vidyut Utpadan Nigam Limited. Suratgarh Thermal Power Station and Kota Thermal Power Station, popularly known as KTPS, are the two major thermal power plants of Rajasthan. Others are Chhabra Thermal Power Station at Chhabra (Baran), Kalisindh Thermal Power Station at Jhalawar, Giral Lignite Thermal Power Plant at Barmer, Dholpur Combined Cycle Power Station at Dholpur, Mahi Hydel Power Station at Banswara and Ramgarh Gas Thermal Power Station at Jaisalmer (https://www.energy.rajasthan.gov.in/rvunl).

One of the atomic power stations known as Rajasthan Atomic Power Station is situated in Rawatbhata of Chittorgarh district, Rajasthan and is operated by Nuclear Power Corporation of India Limited (https://www.npcil.nic.in). A gas based thermal power station is situated in Anta of Baran District, Rajasthan which is operational under National Thermal Power Corporation Limited (https://www.ntpc.co.in).

Rajasthan is rich in aquatic flora and fauna with its lakes and rivers. A thermal power plant is operational on the bank of Chambal river as its water is used for the cooling purpose by thermal plant during generation of electricity and this water is again disposed into the river. Definitely after being passed and moved through the parts of thermal power plant for cooling, the water shares some chemicals in the machinery and also in turn gets heated during the cooling process. So the discharged heated water may affect the flora of water.

#### **1.1 Study Area**

The study was conducted in Kota city of Rajasthan. The city is located at 25°10"57.1" North latitude and 75°50"20.7" East longitude coordinates in south eastern part of Rajasthan (Geographic coordinates of India). Kota district is a part of Hadauti region and is bound by Baran, Bundi, Jhalawar, Tonk and Sawai Madhopur districts (Fig.1.1).

Formerly it was known as the Industrial city due to so many factories located here and playing important role in economy of the city. The city is well known for "Kota stone" all over India. But from last 20 years, around the world it is famous as an educational city. The city is an education hub now and is known for its coaching and teaching skills for aspiring medicos and engineers. Presently economy of the city is based on coaching institutes.

Besides the number of coaching centres, the city also leads in the field of higher education. It has 4 Universities i.e. Rajasthan Technical University, University of Kota, Vardhman Mahaveer Open University and Agriculture University with variety of subjects.

Kota is also famous for "Kota Doriya" sarees and dress materials. In international market demand of Kota doriya is very high and traders have a good exposure. The city is also known for delicious heeng kachoris.

#### **Chambal River**

Rajasthan is known as the land of glory, Rajas and Maharajas. There are so many Forts which attract tourists for their architect and historical past. Tourism is an economical endeavor for Rajasthani people. The famous Thar Desert is dried area that doesn"t have plenty of water. Due to the scarcity of water local people don"t have rich agriculture practices. People of Marwar area i.e. Pali and Jodhpur also have idioms that "we only have water in our eyes and not to drink". Hence, in such a dry land a perennial river is like a boon.

Chambal river is the only perennial river of desert land of Rajasthan in India. The freshwater river is one of the major tributaries of Yamuna river originating from



**Figure 1.1: Location of Kota in Rajasthan state, India (www.mapsofindia.com)**

Janapao Hills of Vindhya range in south of Mhow town near Janapav temple in Madhya Pradesh (Encyclopedia Britannica; World Water Database). After flowing for a time in a northerly direction through Madhya Pradesh (M.P.), it enters in Rajasthan at Chaurasigarh Fort where it flows in north – east direction. It again flows through M.P. before joining the Yamuna river in Uttar Pradesh. (Kulshrestha, 2005).

From its origin to end, Chambal river flows in a stretch of 960 km. Chambal river forms various deep picturesque gorges during its course that add scenic beauty to it (Fig. 1.2  $\&$  1.3). In stretch of 96 km in Rajasthan, it flows through Chittorgarh, Kota and Dholpur districts of Rajasthan. Banas, Kali Sindh, Mez, Shipra and Parwati are the chief tributaries of Chambal river. The river water is used for industrial purpose, drinking, recreational activities, electricity generation and irrigation through four dams - Gandhi Sagar Dam, Rana Pratap Sagar Dam, Jawahar Sagar Dam and Kota Barrage constructed on it during its long course.

The river has derived its name from 'Charmanayavati' and with religious beliefs holds a sacred position for the people of Kota with a holy name "Maa Charmanayavati'. The river also connects with the holy spirits of local residents of Kota. Besides being the lifeline of Kota city, Chambal river is also rich in aquatic flora and fauna including the mugger and gharial. Due to use of its water for industrial purpose and civilization on its bank, the river gets the industrial effluents and sewage water discharged into it.



**Figure 1.2: Course of Chambal river (www.mapsofindia.com)**



**Figure 1.3: Picturesque view of Chambal river** 

The left bank of Chambal river is flanked by one of the major thermal power stations of Rajasthan i.e. Kota Thermal Power Station popularly known as KTPS (www.mapsofindia.com) in the centre of Kota city. It is the first coal based super thermal power plant of Rajasthan for electricity generation. The plant has seven units of operation and uses bituminous coal as a fuel. The total installed capacity of KTPS is 1240 MW. The scheme of power plant got sanctioned in various stages. It was first commissioned in 1983 with unit 1 and the seventh unit was commissioned in 2009. The plant is spread in an area of 204 hectares (Fig. 1.4 & 1.5).

For the production of energy thermal power plants use coal for combustion. In this process they utilize thousands of cusecs of water as coolant. Generally, the waste heat is disposed from the power stations by withdrawal of water from a nearby water source whether ocean, sea, lake, pond, canal or river, passing it through the steam condenser and then again returning to the water source. This process is known as "once-through" cooling in which the cooling water gets heated by some degrees of temperature. This hot water is discharged into the source of water which rises the temperature of ambient water and create thermal plume. The addition of undesirable heat to water causing change in water temperature is known as "thermal pollution". Some part of heat is rejected to atmosphere and some heat is lost to water and ultimately to the environment (Cairns, 1969; Hill, 1972).



**Figure 1.4: Course of Chambal river through Kota city with location of Kota Thermal Power Station (www.mapsofindia.com)**



**Figure 1.5: Kota Thermal Power Station**

As KTPS is located on the bank of the Chambal river, it uses the river water as a coolant during combustion and power generation. The method of cooling is different for a set of units. Out of seven units, only two units (Unit 6 to 7) circulate hot water through cooling towers while rest of five (Unit 1 to 5) use once through cooling system with the utilization of 1180 cusecs of water (http://energy.rajasthan.gov.in/rvunl). Rarely all the seven units operate one at the same time. Along with the heated water, thermal discharge also contains some chemicals mainly chlorine which is used as an antifouling agent (Cairns, 1975; Langford, 2001). There can be a drastic rise in temperature when cooling water is discharged back to the source of water (Snoeijs and Prentice, 1989). The phytoplanktons are exposed to this elevated temperature in water which receives the discharge.

Besides affecting biological components of aquatic ecosystem, elevated water temperature may decrease dissolved oxygen (DO) level, can increase contaminant toxicity, increase metabolism of aquatic organisms, may cause migration of animals, may accelerate in loss of primary productivity, cause shift in phytoplankton community, may lead to loss of aquatic biodiversity, can denature proteins of micro organisms, can cause decreased enzyme activity, can lead to eutrophication, cause algal bloom formation and may even cause mass mortality of fishes (Choudhary, 2009; Kaushal *et al*., 2010).

#### **Site description and Sample collection:**

Chambal river has a stretch of 960 km from its origin to end and covers 225 km across Rajasthan (www.mapsofindia.com). The present study was confined to its area covered in Kota city. Three study locations/sites were selected on a stretch of Chambal river for the collection of surface water samples in Kota, Rajasthan to study cyanobacterial diversity and for the determination of physical and chemical parameters of the river water.

The first selected site was at upstream of the river, second at thermal power station as near as the discharge site (approximately in radius of 500m from the outfall point of thermal effluents) and third one was at downstream of the river. The three stations selected are as follows (Fig. 1.6,1.7,1.8):

Station 1 (Stn.1) - Akelgarh water treatment plant (upstream)

Station 2 (Stn.2) - Kota Thermal Power Station

Station 3 (Stn.3) - Choti Samadh Shiv Temple (downstream)

Station 1 represented as the Control station during the research study.

Kota Barrage is a dam constructed in 1960 on the Chambal river that maintains the availability of water throughout the year in Kota district. It is the fourth dam in the chain of dams built by the Chambal Valley Project. It has a storage capacity of 99 Mm<sup>3</sup> with a total catchment area of 27,332 km<sup>2</sup>. It releases its excess water to areas of Madhya Pradesh for agricultural purposes (http:// www.mapsofindia.com/maps/rajasthan/rivers/). Kota Barrage is located near sampling site Stn.2 at the upstream of Chambal river. The second site (Stn.2) was selected close to the outfall site of thermal effluents' discharge (as near as possible to the power plant) to study the cyanobacterial diversity under its influence. Distance between Stn.1 and Stn.2 is 7.2 km and 5.1 km between Stn.2 and Stn.3 approximately.



**Figure 1.6: Site showing Station 1 - Akelgarh water treatment plant**



**Figure 1.7: Site showing Station 2 - Kota Thermal Power Station**



**Figure 1.8: Site showing Station 3 - Choti Samadh Shiv Temple**

#### **1.2 Study Material – Cyanobacteria**

Cyanobacteria are algae having blue-green colour with diverse habitats and a wide range of organization. They possess prokaryotic structure with incipient nucleus and lack cell organelles. They can perform photosynthesis. Sexual reproduction is known to be absent in them. Reproduction is by fission or fragmentation.

#### **1.2.1 Taxonomic position of Cyanobacteria**

According to the classification proposed by F.E. Fritsch (1935). Cyanobacteria belong to the Class- Cyanophyceae (also known as Myxophyceae) of kingdom Alga. The class is divided into five orders – Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales, Stigonematales.

The new modern system of classification of cyanobacteria based on phylogenetic approach given by Anagnostidis and Komárek (1988) & Komárek *et al*. (2014), classifies Cyanobacteria into eight orders- Gloeobacterales, Synechococcales, Spirulinales ordo nov., Chroococcales, Pleurocapsales, Oscillatoriales, Chroococcidiopsidales ordo nov and Nostocales.

#### **1.2.2 Importance of Cyanobacteria**

Cyanobacteria are the only algae that can fix atmospheric dinitrogen. This function can be attributed to specialized thick walled cells called 'heterocysts' provided with nodular thickenings present in some of the genera which provide anoxygenic environment for the action of nitrogenase enzyme. Heterocysts are found in some of the genera. Because of their nitrogen fixing capacity, *Aulosira fertillisima, Anabaena doliolu*m, *Nostoc* sp., *Aphanizomenon* sp., *Gloeotricha* sp. are

excellent biofertilizers thereby playing role in increasing and maintaining the soil fertility (Thajuddin and Subramanian, 2005; Whitton, 2012; Gupta *et al*., 2013; Ananya *et al*., 2014).

They have a capacity to form symbiotic association with a variety of eukaryotic organisms including green algae, plants and also with non-photosynthetic organisms like fungi (in form of lichens), sponges, ascidians and corals. *Aphanocapsa* sp. has been found in association with marine sponges. Some of the associations can be best exemplified by *Nostoc - Sphagnum* (bryophyte), *Anabaena azollae – Azolla* sp. (pteridophyte), *Nostoc – Cycas* (gymnosperm), *Nostoc punctiforme – Gunnera* sp. (angiosperm), *Nostoc* and *Anabaena* – in rice fields and even with other group of algae "diatoms" (Thajuddin and Subramanian, 2005; Whitton, 2012; Gupta *et al*., 2013; Ananya *et al*., 2014).

Cyanobacteria play important role in ecosystem and have multiple uses in the biotechnology. These are the first oxygenic photosynthetic organisms that made present day"s aerobic environment to live in by releasing oxygen. Cyanobacteria genera like *Nostoc* sp., *Spirulina* sp., *Arthrospira,* sp., *Aphanizomenon* sp. can be used as nutrient source, health supplement and as animal feed. Being a rich source of iron, protein and vitamin B12, *Spirulina* tablets are commonly being sold in market.

In cosmetic industry, *Arthrospira* extracts can be used in cosmetics due to moisturizing effects while phycocyanin extract of *Spirulina* sp. is used in lipsticks and eyeliners. Cyanobacteria can be used as natural colouring agents for foods like ice creams and candies. They are also used for bioremediation in sewage disposal and wastewater treatment by the supply of oxygen required by bacteria for oxidative breakdown as done by *Nostoc, Scenedesmus, Anabaena*. Cyanobacteria can also be

used as tools for heavy metal removal from wastewaters. They may be used for production of secondary metabolites, bioenergy and biofuel production (Srivastava and Sharma, 2002; Thajuddin and Subramanian, 2005; Srivastava *et al*., 2007; Whitton, 2012; Gupta *et al*., 2013; Ananya *et al*., 2014).

#### **1.3 Objectives of Research:**

The study is proposed to be undertaken with the following objectives:

- 1. To analyze the physico-chemical properties of thermal polluted river water
- 2. To find out the nature and amount of thermal effluents from the power plant in river water
- 3. To study the diversity of cyanobacteria
- 4. To assess the changes in primary producer (cyanobacteria) community structure due to thermal discharge through diversity indices

#### **1.4 Significance of research work:**

- The work will be useful in assessment of the changes in biodiversity of cyanobacteria due to thermal effluents in Chambal river water. If any loss of cyanobacterial biodiversity is detected, solutions could be search of for its conservation in time.
- The study will also be useful to determine the effect of power station discharge on water which is also used as potable water.
- It could provide an idea for predicting the effect of rising global temperature in terms of global warming in relation to elevated water temperature on primary producers.

The present work is going to cover the effects of thermal effluents on cyanobacterial population and their diversity. Hence the present work is an attempt to investigate if any changes are occurring there in the community structure of cyanobacteria so as to predict any loss in their biodiversity under the effect of discharge of thermal effluents from a thermal power station in a freshwater Chambal river by determining the physical and chemical properties of its water.

The study will be helpful to find out the effects of thermal power plants on freshwater ecosystem that use nearby source of water for cooling purpose during operational activities. The study will build a foundation for further studies on the thermal effect on cyanobacteria. It may provide some useful suggestions for the conservation of biodiversity of such valuable and the most primitive oxygen evolving cyanobacteria. Also, the information gathered can be utilized for the prevention of increasing temperature and global warming due to thermal pollution by spreading awareness among the society.

## **CHAPTER 2 REVIEW OF LITERATURE**

Cyanobacteria are the most primitive prokaryotic oxygen evolving algae which are also known as "blue-green algae". They have diverse habitats and a wide range of organization. They are able to survive at temperatures as high as 74ºC (Langford, 2001; Lee, 2008; Whitton, 2012). The algae form the base of a food chain in any water body so any change in water or environmental conditions can be detected by their response towards any change. Cyanobacteria form an important component of phytoplanktons other than green algae, diatoms and dinoflagellates. As the phytoplanktons have short generation time, they react rapidly to changes in temperature. Hence, they can be used as bioindicators for bioassessment of aquatic ecosystem to assess the changes in environment (Trivedy, 1989; Smith, 1992; Lata Dora *et al*., 2010).

Thermal power plants are generally located on the coast of a lake, pond, canal river, bay, sea or ocean so as to utilize water from nearby source for cooling of condensers during the production of energy. The water gets heated in this process and is discharged back to the source. Phytoplanktons are exposed to this elevated water temperature from thermal discharge (Cairns, 1969; Hill, 1972). The temperature of water bodies determines the kind of aquatic habitat (Cassie, 2006). Elevated water temperatures and thermal effluents impose a variety of detrimental effects on the aquatic ecosystem including lowering of DO of water and the loss of biodiversity. It can also be lethal to aquatic organisms (Poornima *et al*., 2005; Kaushal *et al*., 2010). Not only cyanobacteria have various uses in diverse areas,

they also play a key role in global carbon cycles through assimilation of carbon and oxygenic photosynthesis (Thajuddin and Subramanian, 2005; Whitton, 2012; Gupta, *et al*., 2013). So their diversity needs to be conserved.

The aim of review was to study the diversity of cyanobacteria and find the effect of thermal discharge on them through assessing the differences in the cyanobacterial community. The review was also aimed to identify a research gap that can be worked out to find out the timely solution for their conservation.

Algal diversity of aquatic ecosystem has been the subject of interest for researchers since long ago. As the microalgae have short life span, they are used for bioassessment of water bodies (Smith, 1992).

A variety of work has been done on thermal effects throughout world and in India. Thermal stress, effects of elevated temperature and discharge of thermal power plants on phytoplanktons, plants, protozoans, insects, corals, fishes, aquatic animals etc. have been studied for many years by many researchers (Clark, 1969; Cairns, 1971; Hill, 1972; Boylen and Brock, 1973; Bush *et al*., 1974; Snoeijs and Prentice, 1989; Krishnakumar,1991; Mallin, *et al*., 1994; Martinez-Arroyo *et al*, 2000; Langford, 2001; Poornima *et al*., 2005; Ilus and Keskitalo, 2008; Chuang *et al*, 2009; Nwankwo *et al*., 2010; Vinitha *et al*., 2010; Feng *et al*., 2011; Hussein *et al*., 2012; Muhammad Adlan *et al*., 2012; Ramchandra *et al*., 2012; Schabhüttl *et al*., 2013; Gingerich and Mauter, 2015; Lo *et al*, 2016).

#### **2.1 Work done worldwide**

Diversity of Cyanobacteria in different water bodies all over the world, both marine and fresh water, has been explored widely by various workers.

Kouassi *et al*. (2015) studied the diversity of cyanobacteria in raw potable water reservoir along with the physico-chemical properties of water in Adzopé's city of Côte d"Ivoire. They observed the members of order Chroococcales and Oscillatoriales and studied the seasonal variations in the composition of cyanobacterial community in their study.

Two different cyanobacterial community structures were observed by Soltani *et al*. (2012) in a drain and an Iranian river. In their comparative study between drain and river, they found drain water polluted. They reported some pollution indicator species of *Ocillatoria* and *Chroococcus* in drain water and concluded that cyanobacteria can be used as the bioindicators of eutrophication.

Rejmánková *et al*. (2004) opinioned that cyanobacteria are very important organisms in terms of ecology. They play an important role in evaluation of biodiversity of an ecosystem. They have suggested for their conservation for their optimum utilization.

Coastal thermal power stations of about 1000 MW capacity are located along the coasts of sea, ocean, lake or river. They use water to condense steam and discharge up to 50 cusecs  $(m^3/s)$  of water back into the source which gets heated during the cooling process. There can be dramatic effects on the quality of water and the aquatic ecosystem of the water body receiving the heated discharge water. When cooling water is discharged back to the source of water there can be a 10°C rise in temperature in the ambient water temperature (Snoeijs and Prentice, 1989). "Thermal pollution is waste heat released to the environment as the unavoidable byproduct of the generation of electricity in steam power plants" (Hill, 1972). The temperature of heated water discharged back into the source varies with volume of water withdrawn for cooling, local climate of the area and the capacity of power plant for energy production (Madden *et al*., 2013).

The thermal effluents have diverse unwanted ecological effects on the aquatic ecosystem affecting plants and animals (Bobat, 2015). Besides affecting biological components of aquatic ecosystem, elevated water temperature may decrease dissolved oxygen (DO) level, can be fatal to fishes or even can reduce their reproductive capacity, can increase contaminant toxicity, increase metabolism of aquatic organisms, may cause migration of animals, may accelerate in loss of primary productivity, may alter the structure of a community, can cause shift in phytoplankton community, lead to loss of aquatic biodiversity, can denature proteins of micro organisms, cause decreased enzyme activity, lead to eutrophication, cause algal bloom formation and even mass mortality of fishes (John, 1971; Hill, 1972; Choudhary, 2009; Kaushal *et al*., 2010). But according to Gallup and Hickman (1975) the thermal effluents discharged from a power station do not affect the physical and chemical properties of water.

Study on the effect of thermal effluents on environment and thermal pollution has been attempted long ago both on flora and fauna. Bush *et al*. (1974) has reviewed the influence of thermal discharges on aquatic flora and fauna. Clark

(1969) reported the effects of elevated temperature on fishes. Response of aquatic insects to high temperature has been studied by Ward and Stanford (1982). The effect of thermal discharge on macro invertebrates was studied by Worthington *et al*. (2015). Teixeira *et al*. (2012) studied the thermal effects of a nuclear power plant on fish communities in a coastal area in Southeastern Brazil. Cairns (1969) observed the response of fresh water protozoans under the effect of heated water.

Various published results are available on the effect of thermal discharge on phytoplanktons (Cairns, 1971; Hill, 1972; Boylen and Brock, 1973; Bush *et al*., 1974; Snoeijs and Prentice, 1989; Mallin, *et al*., 1994; Martinez-Arroyo *et al.*, 2000; Langford, 2001; Ilus and Keskitalo, 2008; Chuang *et al*, 2009; Nwankwo *et al*., 2010; Feng *et al*., 2011; Hussein *et al*., 2012; Muhammad Adlan *et al*., 2012; Schabhüttl *et al*., 2013; Gingerich and Mauter, 2015; Lo *et al*., 2016).

Boylen and Brock (1973) studied the effect of temperature on benthic algae in a thermal stressed river that flows through the geyser basins in Wyoming, a U.S. state. They concluded that elevated temperature increases the growth rate of algae under the thermal effect.

The temperature at the discharge site was found at higher side by Nwankwo *et al*. (2014) when they studied the phytoplankton diversity of a thermal stressed lagoon in Nigeria. According to them, the rise in temperature was because of thermal effluents from a thermal station. The lower species diversity at thermally stressed site was attributed to elevated temperature and low dissolved oxygen (Nwankwo *et al*., 2010). Eloranta (1983) also observed low DO at site receiving heated water discharge in their study on pond near a thermal power plant in Finland.
In an another study on effect of temperature on cyanobacteria by measuring the photosynthesis, Konopka and Brock (1978) observed that cyanobacteria flourish well during summer with increase in biomass when temperature increases while low temperature does not support the growth of cyanobacteria.

The community structure of phytoplanktons was studied in lakes and reservoirs in U.S. that were used for cooling purpose and their primary productivity was measured. A low occurrence of cyanobacteria in two of those reservoirs that received the power plant discharge was observed by Mallin *et al*. (1994).

During the studies conducted by many workers, it was concluded that at the outfall region of a thermal power plant, cyanobacteria show dominance and higher cyanobacteria diversity was observed at discharge site (Snoeijs and Prentice, 1989; Ilus and Keskitalo, 2008; Nwankwo *et al*., 2010; Schabhüttl *et al*., 2013). A negative effect of thermal discharge on diversity of phytoplantons was observed by Nashaat *et al*. (2019).While Hussein *et al*. (2012) noticed that the cyanobacterial diversity increases at discharge point in a canal near a electricity power station in Iraq.

Ilus and Keskitalo (2008) studied the algal biomass, primary productivity and species composition under the effect of elevated temperatures in Finland. They concluded in their research that temperature is mainly responsible for rise in algal biomass at the discharge site. A change in species composition was observed by them during summer season which was found to be dominated by cyanobacteria.

On the other hand, when species diversity of seaweeds was studied in Korea to assess the community changes under the influence of a nuclear power plant, lowest species diversity was observed at the outfall region by Kim *et al*. (2008).

Under the thermal influence, seasonal variation in algal diversity and their species composition was studied by Lo *et al*. (2016).

Some power plants use chlorine as antifouling agent for biofouling control in different concentrations which may be discharged along with heated water (Langford, 2001). There are also the chances of very small amounts of some heavy metals to be discharged along with returning cooling water due to corrosion of metallic tubes in condenser (Cairns, 1971). Oil and grease are used for the maintenance of machinery system that can also contribute to the category of thermal effluents (Nwankwo *et al*., 2014).

Besides temperature, effect of chlorination on phytoplankton has also been studied. Brooks and Liptak (1979) opinioned that chlorophyll-a gets destructed when the concentration of chlorine increases more than 1.0 mg/L. It was suggested by Taylor (2006) that residual chlorine affects the phytoplanktons in water bodies in combination with high temperature.

Chuang *et al*. (2009) noticed higher temperature and lower phytoplankton chlorophyll-a at the outlet region. They observed decrease in phytoplankton productivity under the effect of chlorination in their study on the phytoplanktons of coastal region in Taiwan. On the other hand, Martinez-Arroyo *et al*. (2000) found that algal species at outfall region receiving hot water have lower capacity of photosynthesis.

Not only rise in temperature affect algae but other environmental factors like nutrients also affect them (Squires *et al*., 1979). It is not always that cyanobacteria dominate the phytoplanktons at discharge sites of high temperature but evidence for

diatoms playing the dominant part and even the shift in phytoplankton community with change in algal species composition has also been obtained.

Higher temperature favours proliferation of cyanobacterial algal community tending to alter diversity among aquatic phytoplanktons (Schabhüttl *et al*., 2013).

As a result of thermal discharge, diatoms dominated the phytoplanktons when algal composition was studied for the impact of thermal power plant by many workers (Martinez-Arroyo *et al*., 2000; Feng *et al*., 2011; Hussein *et al*., 2012).

When response of algal community to thermal effluents was assessed on the Provo river of USA, it was found that diatoms dominated the community with rich species diversity (Squires *et al*., 1979). Muhammad Adlan *et al*., (2012) observed a low number of cyanobacteria and dominance of diatoms in a phytoplankton community at discharge site near a thermal power plant. They suggested that thermal discharge has an influence on the phytoplankton abundance in their study on marine phytoplanktons in Malaysia.

Even a shift in community structure has been observed by few workers. A shift in community structure from diatoms to cyanobacteria with rise in temperature has been observed by Konopka and Brock (1978).

Li *et al*. (2011) observed a complete shift in phytoplankton community structure from diatoms to dinoflagellates in summer season at outfall region in Daya Bay nearby South China Sea which receive the thermal effluents from a nuclear power plant in Hong Kong. They opinioned that the shift was occurred because of rise in temperature caused due to thermal discharge. On the contrary, Cuici *et al*.

(2006) had observed diatoms to be the dominant phytoplanktons during their study in the same bay in the south coast of China.

A shift in diatom dominated community to cyanobacteria dominated community was reported by Foerster *et al*. (1974) in a study of thermal effects on the Connecticut River, New Hampshire in U.S.

Change in phytoplankton community structure influenced by thermal effluents from power plants may be co-related to possible ecological effects of global warming (Li *et al*., 2011).

In a study on periphytic algae in a lake near thermoelectric power plant in China, Feng *et al*. (2011) observed that diatoms dominate the perphytic algal community. They also indicated that nutrient level also plays an important role in distribution of periphytic algae.

In case of flowing water, species diversity was found decreased under the influence of thermal discharge in comparison to stagnant water (Snoeijs and Prentice, 1989).

## **2.2 Work done in India**

Plants are the oxygen synthesizers, existence of which is of utmost importance. Being easily available, cheap in use, more convincing and informative, algae are used as bioindicators (Lata Dora *et al*., 2010). Algae have also been used as bioindicators for the determination of water quality by Kshirsagar (2013).

Occurrence and abundance of algae and their diversity in different environments in relation to water quality and its effect has been studied widely by many workers in India (Gopal *et al*., 1981; Sharma and Durve, 1985; Muthukumar *et al*., 2007; Srivastava, 2009; Devi *et al*., 2010; Makander and Bhatnagar, 2010; Sivakumar *et al*., 2012; Kumar and Sahu, 2012; Mishra *et al*., 2012; Bhatnagar and Bhardwaj, 2013a & b; Ganai and Parveen, 2014; Kharkongor and Ramanujam, 2014; Mangal and Pathania, 2015).

Muthukumar *et al*. (2007) and Sivakumar *et al*. (2012) observed various genera of cyanobacteria in their study at different freshwater ponds and coastal areas in Tamilnadu. Kumar and Sahu (2012) reported the highest occurrence of *Microcystis* and *Oscillatoria* sp. in the sites of sewage pond on addition of domestic effluents.

Devi *et al.* (2010) have explained the diversity of cyanobacteria in various types of habitats and ecological conditions of India. Physical and chemical properties of water were analyzed by Suresh *et al*. (2012) to study the microalgal diversity in regions of Western and Eastern Ghats in India.

Some trace amount of heavy metals was detected by Singh *et al*. (2010) in fly ash and groundwater near a thermal power plant in Delhi suggesting the presence of heavy metals in thermal effluent.

The majority of thermal studies have focused in southern part of India. The effect of thermal effluents on aquatic flora and fauna has been sparsely studied by some workers (Krishnakumar, 1991; Kailasam and Sivakami, 2004; Poornima *et al*., 2005; Poornima *et al*., 2006; Vinitha *et al*., 2010; Mohammed, 2012).

Kailasam and Sivakami (2004) studied the effect of thermal discharge on benthic fauna in coastal areas of India. Changes in the net productivity of seaweeds and their chlorophyll content were measured by Mohammed (2012) in south coastal region of India. Muthulakshmi *et al*. (2019) observed a negligible effect of thermal discharge from a nuclear power plant on the population of zooplanktons in the coastal region of southern India.

Phytoplankton species diversity was studied by Zargar and Ghosh (2006) in a reservoir under the influence of heated water discharge from a nuclear power plant in Karnataka. A lower diversity of phytoplanktons at the discharge site was observed by them.

Various studies have been conducted on the effect of thermal effluents on phytoplanktons (Krishnakumar *et al*., 1991; Poornima *et al*., 2005; Vinitha *et al*., 2010). Thermal discharge raises the ambient temperature of water body which generally increases the algal growth with decrease in amount of oxygen dissolved in water (Krishnakumar *et al*., 1991).

Effluents from coal based thermal power stations include heated water, fly ash handling wastewater, metal cleaning waste water and material storage run off (Ramchandra *et al*., 2012). Some phytoplanktons may be entrained along with cooling water current which may then be subjected to mechanical shock and chemical & thermal stress (Poornima *et al*., 2005).

Contamination of nearby water resources or ground water was observed by Ramchandra *et al*., (2012) due to leaching out of heavy metals from the plant when the water quality in the vicinity of a coal based thermal power plant in Udupi district of Karnataka was assessed by them. Nair (1995) has reported the high concentrations of copper and mercury in water discharged from an atomic power station in south of India during his study.

Krishnakumar (1994) stated that high temperature from thermal effluent affect the primary productivity of phytoplanktons. Thermal stress tends to decrease the primary productivity of phytoplanktons (Poornima *et al*., 2006).

Effect of temperature along with chlorination on productivity of phytoplanktons has also been studied by few workers. Poornima *et al*. (2005) indicated in their work stated that when algal composition was studied for the impact of thermal power plant as a result of thermal discharge, diatoms dominated the phytoplanktons. They also have reported that the effect of thermal power plants is localized on phytoplanktons.

Vinitha *et al*. (2010) also studied the effect of chlorination on phytoplankton in South India. They observed a decrease in chlorophyll content and primary productivity in diatoms under chlorine stress.

Kumar *et al*. (2015) studied the diversity of Chambal river in a stretch in M.P. based on phytoplanktons, zooplanktons, macrophytes and macrobenthos to assess the river health of Chambal river.

## **2.3 Work done in Rajasthan**

The water bodies of Rajasthan have been explored for research since many years by various workers. Research has been conducted on water bodies in Rajasthan in terms of physico-chemical parameters of water, algal study and phytoplankton diversity. Phytoplankton algal biodiversity has been studied in various lakes of Ajmer, Udaipur, Jaisamand, Bundi, Jaipur, Jodhpur, Kota, Sri Ganganagar and Bikaner (Sharma and Durve, 1985; Pandey and Pandey, 2002; Tiwari and Singh, 2008; Makander and Bhatnagar, 2010; Pareek, *et al.*, 2011; Singh, *et al*., 2011; Mishra *et al*., 2012; Bhatnagar and Bhardwaj, 2013 (a&b); Borase, *et al*., 2013; Balai *et al*., 2015; Bhupender and Kumar, 2015; Kumar *et al*., 2015; Maheshwari, *et al*., 2015; Mangal and Pathania, 2015).

Maheshwari *et al*. (2015) measured primary productivity of Lake Ramgarh in Jaipur and studied the species diversity of phytoplanktons and reported higher number of phytoplanktons in summer season. The green algae of Jal Mahal lake in Jaipur was studied by Sharma *et al*. (1999). Diatom diversity of water bodies has been studied by some workers in Rajasthan. In Galta kund, another water body of Jaipur, Pareek *et al*. (2011) observed some fresh water diatoms. Diatom diversity in Mawatha lake of Jaipur has also been studied by Singh, *et al*. (2011). Narayan and Barupal (2015) studied the freshwater diatoms of a lake in Jodhpur.

The biodiversity of cyanobacteria in freshwater ponds in Jodhpur was studied by Makander and Bhatnagar (2010). They reported 13 genera of cyanobacteria in their study and suggested that the richness and abundance of aquatic cyanobacteria is dependent on the physical and chemical properties of water.

A wide variety of work has been documented on water bodies of Udaipur and Jaisamand by Pandey and Pandey (2002); Mishra *et al*. (2012); Balai *et al*. (2015); Mangal and Pathania (2015). In a study on Lake Udai Sagar in Udaipur, primary productivity of lake was measured and cyanobacteria were found dominant in lake by Kumar *et al*. (2015). The Indira Gandhi Canal in Sri Ganganagar also has been selected for the study of biodiversity of cyanobacteria.

Chambal river has been the focus of study for many workers. The various available reports suggest that some workers have documented their work on the physico-chemical parameters and algal biodiversity of Chambal river in past few years (Saksena *et al*., 2008; Tiwari and Singh, 2008; Jain, 2012; Bhatnagar and Bhardwaj, 2013 a&b; Gaur *et al*., 2014; Kumar *et al*., 2014; Jain *et al*., 2015; Yadav *et al*., 2014; Grover *et al*., 2017).

Pollution status of Chambal river has been studied by few workers. Saksena *et al*., (2008) suggested that the river water is free of pollution in a stretch of river studied by them in Madhya Pradesh. When the physico-chemical parameters of Chambal river were assessed by Tiwari and Singh (2008) in Dholpur, they suggested that the Chambal river water is safe for irrigation and domestic purpose though the river water was found polluted with organic waste. Gupta *et al*. (2011) indicated that the river is moderately polluted in Kota, Rajasthan on the basis of its physicochemical analysis of water. Chauhan *et al.* (2019) studied the pollution status of Chambal river in Kota city through microbiological assessment. In a stretch of Chambal river near the National Chambal Sanctuary region, Grover *et al*. (2017) reported 102 taxa belonging to 40 genera of diatoms.

Chambal river is dominated by members of Chlorophyceae (green algae) but the members of cyanobacteria (blue-green algae) are also known to be commonly present in the river (Bhatnagar and Bhardwaj, 2013 a&b; Gaur *et al*., 2014).

Study on algal biodiversity (Bhatnagar and Bhardwaj, 2013 a&b), water quality & trophic status (Gaur *et al*., 2014) of freshwater Chambal river has been conducted by few workers. In a comparative study between Rana Pratap Sagar dam and Chambal river, Gaur *et al*. (2014) observed genera like *Oscillatoria*, *Microcystis*, *Phormidium, Nostoc* and *Anabaena* in Chambal river water.

Saksena *et al*. (2008) studied various physical and chemical parameters of the Chambal river in National Chambal Sanctuary, M.P. and observed the river water being of oligosaprobic nature and under the permissible limits of water pollution. In another study conducted, the river water is found to be moderately polluted in Kota city of Rajasthan by Gupta *et al*. (2011).

Bhatnagar and Bhardwaj (2013 b) reported total 13 genera and 21 species of cyanobacteria during their study. Cyanobacteria that were found to be very common in Chambal river are *Merismopedia, Oscillatoria* and *Microcystis*. In an another study conducted by Bhatnagar and Bhardwaj (2013 a) at Kota Barrage constructed on Chambal river, total 10 genera and 18 species were reported by them both in upstream and downstream of the river. According to authors, cyanobacteria showed dominance during summer season. They also investigated *Microcystis aeruginosa* and *Oscillatoria* sp. in downstream of river as pollution tolearant genera. Chambal river is dominated by members of Chlorophyceae (green algae) but with presence of some species of cyanobacteria like *Oscillatoria, Microcystis and Merismopedia*.

A limited research work on thermal power plants in Rajasthan is known to be documented. Air quality status around Suratgarh thermal power plant has been assessed by Yadav *et al*. (2011). They reported the pollutants were in their permissible limits in the air. Meena *et al*. (2015) investigated the air quality by measuring suspended particulate matter in air in Kota city and indicated that KTPS is the major contributor of air pollution.

#### **Research Gap**

The water bodies in the 'land of desert' have been a subject of interest for many workers. Diatoms of different water bodies of Rajasthan have been documented by many workers. Research on Chambal river has been focused on its water quality and pollution status. Algal diversity and water pollution status of Chambal river has been documented by Bhatnagar and Bhardwaj (2013 a; 2013b). The water quality of Chambal river has been determined by Gupta *et al*. (2011) while Gaur *et al.* (2014) have studied the trophic status of Chambal river.

The effect of thermal power plants on flora of Rajasthan has been sparsely studied. Research on power plants of Rajasthan, has been focused mainly on study of air quality, machinery study or use of fly ash. The air quality status around Suratgarh Thermal Power Plant has been assessed by Yadav *et al*. (2011). The quality of air around Kota Thermal Power Station has been assessed by Meena *et al*. (2015). Khandelwal and Shrivastava (2013) have studied the impact of pollution emission on growth performance of some angiosperms around the Kota Thermal Power Plant.

There exists a gap of knowledge on the study on Chambal river as the effect of thermal power plants on microalgae or phytoplanktons in a freshwater river system like Chambal river has not been attempted so far in Rajasthan state of India. Precisely in Rajasthan, the effect of thermal pollution on primary producers, particulary the cyanobacteria, has not yet been reported in any water body to the best of my knowledge.

## **CHAPTER 3 MATERIALS AND METHODS**

The present research study conducted in July 2017 to June 2018 is based on an appropriate research methodology in which the plan of research was designed, method of sampling and source of data were selected as per the requirement of the study.

### **3.1 Research Design**

The procedural plan, design and structure of investigation for the present study are experimental. It is based on cause-and-effect relationship as the effects are measured for the known cause (Kumar, 2011).

## **3.2 Sampling**

The sample unit is individual of a cyanobacterial community. Each cyanobacterium of the population has an equal and independent chance of selection so sampling done is simple random probability sampling method (Kumar, 2011). The sampling period is one year as the sampling was done from July 2017 to June 2018.

## **3.3 Source of Data**

The information for the study is collected afresh and for the first time through experiments and observations so the source and kind of data is primary data (Kumar, 2011).

## **3.4 Material**

The material used for research is Cyanobacteria. The class Cyanophyceae consists of various morphological forms belonging to five different orders: Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales and Stigonematales (Fritsch, 1935). The freshwater planktonic forms of cyanobacteria were studied in river water of Chambal. The Cyanobacteria are classified into eight orders according to new modern system of classification of cyanobacteria: Gloeobacterales, Synechococcales, Spirulinales ordo nov., Chroococcales, Pleurocapsales, Oscillatoriales, Chroococcidiopsidales ordo nov. and Nostocales (Anagnostidis and Komárek, 1988; Komárek *et al*., 2014).

Distilled water, sample tubes, test tubes, beakers, flasks, burettes, suitable chemicals and reagents were required for the determination of physico-chemical properties of water.

Slides, brush, forceps, needle, glycerin, cover slips, dropper, microscope, test tubes were required for the cyanobacterial study.

#### **3.5 Methods**

Three sampling sites were selected on the stretch of Chambal river in the Kota city for the present investigation.

To determine the physico-chemical characteristics the water was collected in triplicates from the same site in one litre capacity of clean polyethylene bottles. The bottles were first rinsed with distilled water three times and then with river water twice before taking the samples. Light intensity, temperature and pH were

determined immediately after the sample collection on the spot /collection site only. The samples were brought to the laboratory for the analysis of parameters like electrical conductivity, turbidity, total alkalinity, hardness, chlorides, fluorides, sulphates, nitrates, total dissolved solids, dissolved oxygen, BOD & COD.

The methods prescribed by APHA (1999), Maiti (2004), Gupta (2007) and Aery (2010) were followed for the determination of physico-chemical parameters of water.

For the study of diversity of cyanobacteria, the surface water samples were collected from the sampling sites in the morning time during second week of every month from July 2017 to June 2018 in the replica of three. The collected samples were studied within 48 hours with the preparation of fresh mounts in laboratory for cyanobacterial study. For further detailed examination the samples were also fixed and preserved using Lugol's solution.

With the aid of available standard keys and monographs (Smith, 1950; Prescott,1954; Desikachary,1959; Palmer,1980; Baker and Fabbro, 2002; Bellinger and Sigee, 2015) identification of phytoplanktonic Cyanobacteria was done using Metzer research microscope and imported camera MD500 as an attachment. Identified samples were also cross identified by the experts of Cyanobacterial study.

#### **Physico-chemical analysis:**

## **3.5.1 Determination of Light intensity**

The intensity of light was measured in lux units with the help of portable digital lux meter of Amor make (Figure 3.1).

#### **3.5.2 Determination of Temperature**

Temperature was observed in  $\mathrm{C}$  on the spot/ collection site by dipping a digital MEXTECH Multi- thermometer in water sample during sampling period (Figure 3.2).

#### **3.5.3 Determination of pH**

pH was measured using a portable digital HANNA pH meter on the spot/collection site during sampling period (Figure 3.3).

#### **3.5.4 Determination of Electrical Conductivity**

The conductivity or the specific conductance of water sample was observed in the laboratory by  $\mu$ C turbidity meter 135 of make Systronics. The conductance was measured in  $\mu$ S/ cm (Figure 3.4).

#### **3.5.5 Determination of Total Dissolved Salts (TDS)**

The TDS was measured using  $\mu$ C turbidity meter 135 of Systronics. For TDS measurement, the cell was rinsed with distilled water in a beaker. The mode of measurement was changed and TDS reading was observed and noted.

## **3.5.6 Determination of Turbidity**

Turbidimeter (Nephelometer) is the instrument that was used for measuring the turbidity in NTU units (Figure 3.4).

Samples were shaked thoroughly and the air bubbles were subsided. The nephelometer was set at 100 using 40 NTU standard suspension. Sample was placed in nephelometer sample tube and reading was observed on the scale.



**Figure 3.1: Digital Lux meter**



**Figure 3.2: Digital thermometer**



**Figure 3.3: Digital pH meter**



**Figure 3.4: Conductivity and turbidity meter**

The turbidity was calculated with the help of following calculation.

Calculation

Turbidity (NTU) = Nephelometer reading  $\times$  0.4 $\times$ dilution factor

## **3.5.7 Determination of Alkalinity**

Alkalinity was determined by titrating 50 mL of the water sample with a strong acid like  $H_2SO_4$  or HCl by adding 2-3 drops of methyl orange as an indicator. The reading where the orange colour appears was noted. The end point reading was used for calculating the alkalinity.

Calculation

$$
1000 \,\text{mL} \, 1 \,\text{N} \, \text{HCl} = 50 \,\text{g} \, \text{of} \, \text{CaCO}_3
$$

Phenolphthalein alkalinity (PA) as  $CaCO_3$  mg/L =  $\frac{A A V 01 IIC1 \times 30 \times 10}{Volume \text{ of Sample}}$ AxN of  $HCl \times 50 \times 1000$ 

Total alkalinity (TA) as CaCO<sub>3</sub> mg/L =  $\frac{\text{B} \times \text{N} \text{ of } \text{HCl} \times 50 \times 1000}{\text{Volume of Sample}}$ 

where

 $A =$  Volume of HCl used with phenolphthalein (mL)

 $B =$  Volume of HCl used with phenolphthalein and methyl orange (mL)

## **3.5.8 Determination of Hardness**

The property of water to form lather or precipitate soap is generally referred to as hardness. The total hardness is the sum of calcium and magnesium concentrations where both of them are expressed as calcium carbonate in milligrams per liter.

## **a. Total Hardness**

A pinch of Eriochrome black T indicator was added to 10 mL of sample resulting a wine red colour solution which was then titrated against 0.01M EDTA slowly till the appearance of blue colour. The end point reading was noted and used for calculating the total hardness.

Calculation

Hardness (EDTA) as mg CaCO<sub>3</sub>/L = 
$$
\frac{A \times B \times 1000}{mL \text{ sample}}
$$

where

 $A = mL$  titration for sample

 $B = mg CaCO<sub>3</sub>$  equivalent to 1.00 mL EDTA titrant

## **b. Calcium Hardness**

A pinch of Murexide indicator and 2 mL of NaOH were added to 50 mL of sample which was then titrated against 0.01M EDTA slowly with continous stirring till the appearance of purple colour. The end point reading was noted and used for the calculating calcium hardness.

Calculation

$$
mg Ca/L = \frac{A \times B \times 400.8}{mL \, sample}
$$
  
Calcium Hardness as mg CaCO<sub>3</sub>/L =  $\frac{A \times B \times 1000}{mL \, sample}$ 

where

 $A = mL$  titration for sample

 $B = mg CaCO<sub>3</sub>$  equivalent to 1.00 mL EDTA titrant at the calcium indicator end point  $C =$ Magnessium hardness

It is computed from the results of separate determination of calcium hardness and total hardness.

mg Mg/L = [Total hardness(as mg CaCO<sub>3</sub>/L) - Calcium hardness(as mg CaCO<sub>3</sub>/L)] $\times$ 0.243

## **3.5.9 Determination of Chloride**

2 mL of  $K_2CrO_4$  was added to 50 mL of sample in a conical flask. The content was then titrated against  $0.02$  N AgNO<sub>3</sub> until a persistent red tinge appears. The end point readings were used to place their values in the following formula to determine the chloride content.

Calculation

$$
Chloride (mg/L) = \frac{V \times N \text{ of } AgNO_3 \times 1000 \times 35.5}{Sample Amount}
$$

where

 $V =$  Volume of AgNO<sub>3</sub> (in mL)

 $N =$  Normality of AgNO<sub>3</sub>

#### **3.5.10 Determination of Nitrate**

The determination of nitrate was done by UV spectrophotometric screening method. 1 mL of 1N HCl was added to 50 mL of distilled water and mixed thoroughly. Absorbance or transmittance was read against distilled water set at zero absorbance or 100% transmittance at wavelength of 220 nm for the preparation of standard curve. Then 1 mL of 1N HCl was added to 50 mL of sample and mixed thoroughly. Absorbance or transmittance was read against distilled water set at zero absorbance or 100% transmittance at wavelength of 220 nm to determine interference due to dissolved organic matter.

#### **3.5.11 Determination of Sulphate**

20 mL of buffer solution was added to 100 mL of sample which was then stirred on a magnetic stirrer.  $BaCl<sub>2</sub>$  crystals were added while stirring. The mixture was stirred for 1 minute and after 4 minutes it was poured into the absorption cell / cuvette in a UV spectrophotometer to measure at absorbance of 420 nm. The reading was noted and sulphate was determined by making following calculation:

Calculation

$$
mg SO_4^{2.7} / L = \frac{mg SO_4^{2.7} \times 1000}{mL sample}
$$

#### **3.5.12 Determination of Fluoride**

Fluoride concentration was determined by ion selective electrode method. A fluoride detector of make Thermo Scientific model Orion Star A214 was used for this purpose.

Fluoride buffer was prepared by mixing 57 mL glacial acetic acid, 58 g NaCl and 4.0 g CDTA (1,2 cyclohexylene diamine tetra acetic acid) into 500 mL distilled water. All the contents were dissolved in it. By slowly adding 6 N NaOH with stirring pH was adjusted between 5.3 and 5.5. It was then made up to 1000 mL in a volumetric flask.

A series of standards equivalent to 0.5, 1.0 and 2.0 mg F- /L were prepared. To 25 mL of the standard 25 mL (equal volume) of buffer solution was added. The electrodes were then immersed in this solution while stirring the magnetic stirrer. Electrodes were kept in the solution for about 3 minutes and the reading was noted. The electrodes were then withdrawn from the solution, rinsed with distilled water and were gently blotted dry between the readings.

Calculation

mg F/
$$
L = \frac{\mu g F}{\text{Volume of sample (mL)}}
$$

## **3.5.13 Determination of Disssolved Oxygen (DO)**

Winkler"s method was used to estimate DO of water (Maiti, 2004).

Water sample was taken in a BOD bottle and  $1 \text{ mL of } MnSO_4$  and  $1 \text{ mL of } MnSO_4$ alkaline KI solution was added well below the surface of water with the help of separate pipettes. A precipitate was observed which was agitated by placing the stopper and shaking thoroughly by inverting the bottle repeatedly. The precipitate was dissolved by adding  $1-2$  mL of conc.  $H_2SO_4$ . The whole content was then transferred gently in a conical flask. Within 1 hr of dissolution of precipitate, the sample was titrated against 0.025 N sodium thiosulphate by adding few drops of starch indicator. The end point where the initial dark colour disappears completely was noted as reading. DO was calculated by using the end point reading value in the formula given below.

Calculation

$$
DO (mg/L) = \frac{V_1 \times N \times 8 \times 1000}{V_2 - V_3}
$$

where

 $V_1$  = Vol. of sodium thiosulphate (titrant)

 $V_2$  = Vol. of sampling bottle

 $V_3$  = Vol. of MnSO<sub>4</sub> and KI solutions

 $N =$  Normality of sodium thiosulphate (0.025)

## **3.5.14 Determination of Biochemical Oxygen Demand (BOD)**

5 day test was required for determination of BOD through incubation of water sample with the help of BOD incubator. The difference in oxygen concentration of the sample at a time and after incubating it for 5 days at 20°C was measured.

In this procedure the Winkler"s method was used for measuring the oxygen of water. In a BOD bottle the water sample was taken to which  $1 \text{ mL of MnSO}_4$  and 1 mL of alkaline KI solution was added with the help of separate pipettes well below the surface of water. This resulted in a precipitate. By placing the stopper on the mouth of the BOD bottle, the precipitate was then agitated through shaking thoroughly by inverting the bottle repeatedly. The precipitate was dissolved with addition of 1-2 mL of conc.  $H_2SO_4$  to it. Then the whole content was gently transferred in a conical flask. The sample was titrated against 0.025 N sodium thiosulphate by adding few drops of starch indicator within 1 hr of dissolution of precipitate. The reading was noted at the end point which is when the initial dark colour disappears completely. The amount of oxygen dissolved was calculated by using the value of end point reading in the formula used for calculation of DO.

The DO measured before incubation was initial DO and final DO was measured after incubation. The BOD values were calculated by placing the DO values in the formula.

Calculation

$$
BOD mg/L = \frac{D_1 - D_2}{P}
$$

where

 $D_1 = DO$  of diluted sample immediately after preparation (mg/L)

 $D_2 = DO$  of diluted sample after 5 day incubation at 20 $^{\circ}$ C (mg/L)

 $P =$  Decimal volumetric of sample used

#### **3.5.15 Determination of Chemical Oxygen Demand (COD)**

20 mL of sample was taken in a refluxing flask with a ground joint for Liebig reflux condenser. 10 mL 0.25 N potassium dichromate solution was added to it along with 1g of  $HgSO_4$  (mercuric sulphate) and a pinch of silver sulphate. Then 30 mL of conc.  $H_2SO_4$  was added slowly for mixing  $HgSO_4$ . The mixture was then refluxed for about 2 hours on a hot water plate. The mixture was then cooled down after disconnecting the reflux condenser and was diluted to about twice its volume with distilled water. 2-3 drops of ferroin indicator was added and the solution was titrated with 0.1 N ferrous ammonium sulphate (FAS) and reading was noted at the end point when blue-green colour changes to reddish brown colour.

Using the same amount of chemicals and reagents a blank containing the volume of distilled water as same as volume of sample was also refluxed and titrated to note the end point readings. The following calculation was performed using the values obtained during the end point of titration.

Calculation

$$
COD \text{ as } mg/L = \frac{(B - A) \times N \times 1000 \times 8}{Volume \text{ of sample}}
$$

where

 $A = Vol.$  of FAS used for sample (in mL)

 $B = Vol$ . of FAS used for blank (in mL)

 $N =$  Normality of FAS

## **3.6 Diversity of Cyanobacteria**

The study of diversity of cyanobacteria includes the observation, identification and enumeration of cyanobacteria so as to find their population number and study their biodiversity.

#### **Collection and preservation**

Phytoplanktons were collected in triplicates from the selected stations (Stn.1, Stn.2 & Stn.3) in 500 mL wide mouthed polyethylene bottles. The bottles were rinsed with distilled water first before collection. Surface samples were preferred to collect phytoplanktons in water. Cyanobacteria were observed fresh after collection. The samples were also preserved for further detailed study by adding 0.3 mL Lugol's solution to 100 mL water sample, brought to the laboratory and kept in dark (Maiti, 2004) (Fig. 3.5).

## **Concentration**

Sedimentation method of concentration was adopted because of its non selective and non destructive in nature. 50 mL of sample was allowed to settle in a capped wide mouth centrifuge tubes without any vibration overnight. Supernatant was then decanted and 5 mL of final volume was obtained.

#### **Observation and Identification**

With the help of dropper, needle, forceps semi permanent glass slides were prepared. Observation was done with the help of a trinocular research Metzer-M vision plus microscope (Model METZ-5000 DTM) at 100x and 400 x magnification. Various keys and monographs (Smith,1950; Prescott, 1954; Desikachary,1959; Prescott,1970; Palmer,1980; Baker and Fabbro,2002; Bellinger and Sigee, 2015)

online database [\(www.algaebase.org\)](http://www.algaebase.org/) and research publications (Anagnostidis and Komárek 1988; Komárek and Komárková, 2003; Das *et al*., 2010; Liu *et al*., 2013; Komárek *et al*., 2014, Tandon, 2016; Menezes *et al*., 2020) were used for the identification of Cyanobacteria. Microphotographs were also taken during observation with the help of an imported camera MD500 attached to the compound trinocular research microscope (Fig. 3.6, 3.7 & 3.8).

#### **Enumeration**

Counting of cyanobacteria was done by Haemocytometer. It is actually a glass slide with centrally located "H" shaped groove. The central chamber is divided into 25 sub chambers. Each sub chamber is further sub divided into 16 chambers making the total number of sub chambers as 400.

A drop of agitated sample was placed in the central cubical chamber having 400 small chambers of haemocytometer and covered by placing a glass cover. Any kind of overflow was avoided. The cyanobacteria were allowed to settle for 5 minutes. The cyanobacteria were then counted under 100x and 400x magnification. Ten drops of agitated sample were observed.

## Calculation

Concentration factor Phytoplankton units per mL  $=\frac{No.of~phytoplankton~cellsin~central~chamber \times 10^4}{2}$  $=$ 

Volume of water constituted after consitution Concentration factor =  $\frac{\text{Volume of water concentrated}}{\text{Volume of water}}$ 

## **3.7 Cyanobacteria Community Structure**

The structure of a community and the changes occurring in a community can be described by using diversity indices which are based on presence or absence of species. These indices are helpful in assessing the changes in community structure because of any change in environment.

The diversity indices consider the number of species (richness) and the number of individuals (abundance) (Aery, 2010). The following diversity indices were used to evaluate the cyanobacterial biodiversity and evaluate the cyanobacterial community structure by using online biodiversity calculator: (http://www.alyoung.com/labs/biodiversity\_calculator.html).

#### **1. Shannon- Weaver Index of diversity**

It is commonly used to calculate the diversity of aquatic ecosystem (Aery, 2010).

It was calculated by the following formula:

$$
H = 1 - \sum_{i=1}^{s} (p_i)^2 (\log 2 p_i)
$$

where

 $H =$  index of species diversity

 $s =$  number of species

 $p_i$  = proportion of total sample belonging to the i<sup>th</sup> species, or

Total no. of all individual s in the sample  $p_i = \frac{\text{Number of individual s of one species}}{\text{Total no of all initial red s in the sound}}$ 

## **2. Sorensen's Index of Similarity**

This index was given by Sorensen in 1948. It expresses the number of species common or similar between two communities compared. It measures the ratio of common to average number of species in two communities (Sørensen, 1948; Aery, 2010).

The index was calculated as follows:

$$
IS_s = \frac{C}{1/2(A+B)} \times 100
$$

$$
= \frac{2C}{A+B} \times 100
$$

where

 $C = No$ . of species common to two releves/sites

A = Total number of species in releve/site A

 $B = Total number of species in relevance/site B$ 

Statistical analysis was performed using software IBM SPSS statistical package version 25 for Microsoft.



**Figure 3.5: Samples collected for analysis of cyanobacteria**



**Figure 3.6: Semi permanent slides for identification of cyanobacteria**



**Figure 3.7 Digital Microcamera MD 500**



**Figure 3.8: Compound Trinocular Microscope attached with digital Camera to take microphotographs (Showing algal image on laptop screen)**

# **CHAPTER 4 PHYSICO-CHEMICAL PROPERTIES OF FRESHWATER RIVER SYSTEM**

Rivers are life-line for human civilization. Around the world most of the human civilizations are developed along riversides, as they provide necessary component of human survival i.e. water. There are many rivers in India and most of them like Ganga, Yamuna, Narmada, Chenab, Godawari, Chambal etc. are replenishing life of people since ages. Rivers are worshiped by Indian communities due to their necessity for living being, so that humans can learn to protect them. Scientifically rivers are one of the major dynamic lentic environments the nature of which keeps on changing from source to end because of movement and flow of water. Aquatic plants and animals are greatly influenced by the contents and properties of water (Palmer, 1980). Algae are the major autotrophs in rivers. Any kind of industrial set up situated near water bodies generally pollute them and harm their flora too. Water bodies located near the power plants are generally used as the source of water for cooling the condensers and in turn receive the discharge of thermal waste. Hence, algae are also influenced by the thermal pollution.

Present research work is attempted to study the effect of thermal effluents on Chambal river water in order to evaluate its effect on Cyanobacteria and their diversity. Here it is necessary to study the physical and chemical properties of water. It is even more important when a water body is under the influence of a nearby thermal power plant. In order to assess the impact of thermal discharge, water samples were collected every month for one whole year and various physicochemical parameters were determined to conduct the research study i.e., Light intensity, Temperature, pH, Electrical Conductivity, Total Dissolved Salts, Turbidity, Total Alkalinity, Hardness, Chloride, Nitrate, Sulphate, Fluoride, Disssolved Oxygen, Biochemical Oxygen Demand and Chemical Oxygen Demand.

Physico- chemical analysis of water depicts the cause of changes in water quality and biological indices determine the effects of change (Palmer, 1980). The physical and chemical parameters of freshwater river system were determined using the standard methods prescribed by APHA (1999), Maiti (2004), Gupta (2007) and Aery (2010).

The water samples were collected in triplicates of clean polyethylene bottles of one litre capacity from the three sampling sites during second week of every month from July 2017 to June 2018 in the morning time for the determination of the physical and chemical characteristics of the water. After the sample collection, light intensity, pH and temperature were determined on the spot only. The samples were brought to the laboratory for the analysis of other parameters like Electrical Conductivity (EC), Total Dissolved Salts (TDS), Turbidity, Total Alkalinity (TA), Hardness, Chloride, Nitrate, Sulphate, Fluoride, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD).

Samples were collected to perform the study for the whole year and results were analyzed on seasonal basis (Figure 4.1). Three seasons were studied round the year. Monsoon season was considered from July 2017 to October 2017, winter season from November 2017 to February 2018 and summer season from March 2018 to June 2018.

#### **Experimental Observations**

The main physico-chemical parameters of Chambal river water that were determined are as follows:

#### **4.1 Light Intensity**

The intensity of light ranged from 335 lux to 1167 lux in monsoon season, 707 lux to 1052 lux in winter season and 243 lux to 1231 lux in summer season round the year.

During the monsoon season, the light intensity was 440 lux at Stn.1, 517 lux at Stn.2 and 596 lux at Stn.3 in July. In the month of August it was 335 lux at Stn.1, 883 lux at Stn.2 and 659 lux at Stn.3. The light intensity of 528 lux was observed at Stn.1, 1076 lux at Stn.2 and 1121 lux at Stn.3 in the month of September. The month of October was marked with light intensity of 1167 lux at Stn.1, 1124 lux at Stn.2 and 1106 lux at Stn.3.

In the winter season, the light intensity was 1007 lux at Stn.1, 886 lux at Stn.2 and 780 lux at Stn.3 in the month of November. In the month of December, the intensity of light at Stn.1 was 707 lux, 802 lux at Stn.2 and 765 lux at Stn.3. In the month of January, light intensity was of 763 lux at Stn.1, 1052 lux at Stn.2 & 1047 lux at Stn.3. The light intensity was recorded 980 lux at Stn.1, 871 lux at Stn.2 and 918 lux at Stn.3 in the month of February.

In summers, light intensity of 1140 lux was observed at Stn.1, 837 lux at Stn.2 and 243 lux at Stn.3 in the month of March. In the month of April, the light intensity was 1113 lux at Stn.1, 1092 lux at Stn.2 and 1079 lux at Stn.3. The light

intensity of 1124 lux, 863 lux and 1014 lux was measured at Stn.1, Stn.2 & Stn.3 respectively in the month of May. In the month of June, light intensity of 1231 lux observed at Stn.1, 1151 lux at Stn.2 and 1143 lux at Stn.3 (Figure 4.3).

#### **4.2 Temperature**

The maximum surface water temperature was observed 42.2°C to minimum of 20.1°C at the three stations in all the seasons.

In monsoon season, the water temperature of  $28.9 \text{ °C}$  was recorded in the month of July at Stn.1, 31.5 °C at Stn.2 and 28.1 °C at Stn.3. The month of August was observed with temperature of 27.8 °C at Stn.1, 30.3°C at Stn.2 & 28.0°C at Stn.3. Stn.1 was at  $30.6^{\circ}$ C, Stn.2 at  $31.9^{\circ}$ C and Stn.3 was at  $30.9^{\circ}$ C in the month of September. The month of October was marked with 31.7°C at Stn.1, Stn.2 with 35.9°C and Stn.3 with 28.9°C.

During the winter season, the minimum of water temperature was recorded. The temperature was recorded 28.0°C at Stn.1, 34.3°C at Stn.2 and 25.4°C at Stn.3 in the month of November. In the month of December  $24.0^{\circ}$ C of the temperature was observed at Stn.1, 30.1°C at Stn.2 & 21.0°C at Stn.3. 21.8°C of temperature was recorded in the month of January at Stn.1, 29.5°C at Stn.2 & 20.1°C temperature at Stn.3. The temperature of 22.7°C was recorded at Stn.1, 27.5°C at Stn.2 & 21.5°C at Stn.3 in the month of February.

During the study period, the summer season exhibits the higher water temperature. The surface water temperature of 26.9°C was observed at Stn.1, 34.4°C at Stn.2 and 22.3°C at Stn.3 in the month of March. 32.2°C of temperature was recorded at Stn.1, 39.1°C at Stn.2 & 27.8°C at Stn.3 in the month of April.



**Figure 4.1: Sampling at the collection site**



**Figure 4.2: Determination of light at the sampling station**

The temperature was measured 31.1°C, 42.2°C and 28.2°C at Stn.1, Stn.2 & Stn.3 respectively in the month of May. It was observed at  $34.0^{\circ}$ C at Stn.1,  $41.2^{\circ}$ C at Stn.2 and 29.6°C at Stn.3. in the month of June (Figure 4.4).

#### **4.3 pH**

The pH of water ranged between 6.7 to 8.5 units during the study period. In the monsoon season, it was measured 7.8 at Stn.3 and 7.9 at both Stn.1&2 in the month of July. It was observed 7.8 at Stn.1, 7.5 at Stn.2 & 7.7 units at Stn.3 in August month. The pH was measured 7.9 at Stn.1, 8.1 at Stn.2 and 8.0 at Stn.3 in the September month. In the month of October, 8.3 units of pH was observed at Stn.1. Stn.2 was recorded with 8.0 and Stn.3 with 8.0 units in the same month.

During winters, the pH of water was lowered and observed at 7.8 at the Stn.1 in the month of November while the pH of 7.5 was recorded at Stn.2 and 7.1 at Stn.3 in the same month. The December month noticed almost the same pH of 8.0, 7.7 and 7.5 units at Stn.1, Stn.2 & Stn.3 respectively. In the month of January 2018, Stn.1 was noticed at the pH of 7.9, Stn.2 at 7.6 and Stn.3 at 7.3 units. pH of 7.8 was recorded at Stn.1, 8.1 at Stn.2 and 6.8 at Stn.3 in the month of February.

In summers, the pH of 7.9 was observed at Stn.1, 7.7 at Stn.2 and 7.1 at Stn.3 in March month. The pH was found consistent at Stn.2 during April, May and June at 7.9 while it was almost same at Stn.1 with 8.4, 8.3 and 8.5 units in April, May and June respectively. The pH varied at Stn.3 in April with 8.5 units, 6.7 in May and 7.1 in June 2018 (Figure 4.5).
# **4.4 Electrical Conductivity (EC)**

EC of river water ranged between 263.7  $\mu$ S/ cm to 648.3  $\mu$ S/ cm round the year during the study period.

In summer season, the electrical conductivity was measured in the range of 300 at Stn.1 during July, September and October. It was measured 333.3 µS/ cm in the month of July, 304.7 µS/ cm in September and 306.0 µS/ cm in October at Stn.1. Stn.2 was reported with EC of 364.0  $\mu$ S/ cm in July month and 379.0  $\mu$ S/ cm at Stn.3 in the same month. The month of August was recorded with  $263.7 \mu$ S/ cm at Stn.1, 297.3 µS/ cm at Stn.2 and 298.3 µS/ cm at Stn.3. EC of 304.6 µS/ cm was observed at Stn.1, 328.0 µS/ cm at St. 2 and 394.0 µS/ cm at Stn.3 in the month of September. It was measured 306.0  $\mu$ S/ cm at Stn.1, 314.0  $\mu$ S/ cm at Stn.2 and 418.7 µS/ cm at Stn3. in October.

With the arrival of winters, 294.7 of EC was measured at Stn.1, of 305.0 at Stn.2 and 406.0 at Stn.3 in November. The month of December was marked with the electrical conductance of 281.3  $\mu$ S/ cm, 302.3  $\mu$ S/ cm and 394.3  $\mu$ S/ cm at Stn.1, Stn.2 and Stn.3 respectively. EC at Stn.2 and 3 were almost found to be similar in January and February with. It was 324.0  $\mu$ S/ cm at Stn.1 and 320.3  $\mu$ S/ cm at Stn.2 in January and 329.3 µS/ cm at Stn.1 and 339.3 µS/ cm at Stn.3 in the month of February while it was measured 478.0  $\mu$ S/ cm in January and 453.7  $\mu$ S/ cm in February at Stn.3.

In summer season, EC was 327.3 µS/ cm at Stn.1, 330.3 µS/ cm at Stn.2 but 450.3 µS/ cm at Stn.3 in the month of March. The month of April got the EC of 327.0 µS/ cm at Stn.1, 322.3 µS/ cm at Stn2 and 454.7 µS/ cm at Stn.3. In May month, it was observed at 415.0  $\mu$ S/ cm at Stn.1, 400.7  $\mu$ S/ cm at Stn.2 and 648.3  $\mu$ S/ cm at Stn.3. EC of 485.7  $\mu$ S/ cm was measured at Stn.1, 486.7  $\mu$ S/ cm at Stn.2 and 554.7 µS/ cm at Stn3 in June (Figure 4.6).

# **4.5 Total Dissolved Salts (TDS)**

The maximum value of TDS was recorded 455.0 mg/L and minimum to 191.7 mg/L in river water at the three sites selected for the study period.

During summers, the TDS of water was measured 235.0 mg/L at Stn.1, 273.3 mg/L at Stn.2 and 294.7 mg/L at Stn.3 in the month of July and 191.7 mg/L at Stn.1, and similar at Stn.2 & Stn.3 with 216.7 mg/L in August month. The TDS was measured 291.7 mg/L at Stn.1, 271.7 mg/L at Stn.2 and 332.0 mg/L at Stn.3 in the September month. In the month of October, 250.3 mg/L of TDS was observed at Stn.1. Stn.2 was recorded with 246.3 mg/L and Stn.3 with 300.0 mg/L in the month of October.

With the arrival of winters, the TDS was observed at 238.3 mg/L at Stn.1 during November. The TDS of 245.7 mg/L was recorded at Stn.2 and 355.0 mg/L at Stn.3 in the same month. In the month of December, the TDS of 233.0, 240.0 and 339.0 was measured at Stn.1, Stn.2 & Stn.3 respectively. In the month of January 2018, TDS of 260.0 mg/L was noticed at the Stn.1, 256.3 mg/L at Stn.2 and 382.0 mg/L at Stn.3. TDS of 267.0 mg/L was measured at Stn.1, 272.0 mg/L at Stn.2 and 365.3 mg/L at Stn.3 during February month.

In summers, the TDS of 264.3 mg/L was observed at Stn.1, 268.3 mg/L at Stn.2 and 368.3 mg/L at Stn.3 in March month. The TDS was found 264.0 mg/L at Stn.1 during April while it was measured 257.7 mg/L at Stn.2 and 370.7 mg/L at Stn.3. In the month of May TDS of 290.0 mg/L was measured, 279.7 mg/L at Stn.2 and 455.0 mg/L at Stn.3. It was observed 337.3, 344.0 and 388.3 at Stn.1, Stn.2 & Stn.3 respectively in the month of June (Figure 4.7).

# **4.6 Turbidity**

The turbidity of river water ranged between 1.7 NTU units to 17.5 NTU units round the year.

The turbidity of water was observed 8.8 NTU units at Stn.1 in Monsoon season in month of July and 11.9 NTU units at Stn.2 and 13.7 NTU units at Stn.3. In August, it was 2.4 NTU units at Stn.1, 3.3 NTU units at Stn.2 and 1.9 NTU units at Stn.3. The turbidity of 2.4 NTU units was observed at Stn.1, 2.4 NTU units at Stn.2 and 3.0 NTU units at Stn.3 in the month of September. The month of October was observed with turbidity of 4.8 NTU units at Stn.1, of 6.7 NTU units at Stn.2 and of 10.7 NTU units at Stn.3.

In winter season, the turbidity at Stn.1 was recorded 1.7 NTU units, 2.7 NTU units at Stn.2 and 4.6 NTU units at Stn.3 in the month of November. In the month of December, 2.5 NTU units of the turbidity was observed at Stn.1, 3.4 NTU units at Stn.2 & 6.3 NTU units at Stn.3. The turbidity was measured in the month of January at Stn.1 with 3.3 NTU units, Stn.2 with 3.4 NTU units and Stn.3 with 5.6 NTU units. The turbidity of 2.4 NTU units was recorded at Stn.1, 2.9 NTU units at Stn.2  $\&$  6.8 NTU units at Stn.3 in the month of February.

In March, during the summer season, turbidity of 2.5 NTU units was observed at Stn.1, 2.5 NTU units at Stn.2 and 5.9 NTU units at Stn.3. 3.7 NTU units of turbidity was recorded at Stn.1, 3.0 NTU units at Stn.2 & 11.3 NTU units at Stn.3 in the month of April. The turbidity was measured 4.6 NTU units, 3.2 NTU units and 17.5 NTU units at Stn.1, Stn.2 & Stn.3 respectively in the month of May. In June, it was observed at 6.5 NTU units at Stn.1, 4.7 NTU units at Stn.2 and 4.1 NTU units at Stn.3 (Figure 4.8).

# **4.7 Total Alkalinity (TA)**

TA of river water ranged between 96.7 mg/L to 203.3 mg/L. The maximum value of TA was observed at Stn.3 in the month of May.

During the monsoon season, the total alkalinity at Stn.1 was measured 116.7 mg/L in the month of July. Stn.2 was reported with TA of 120.0 mg/L and Stn.3 with 133.3 mg/L in July. The month of August was reported with 100.0 mg/L at Stn.1, 106.7 mg/L at Stn.2 and 113.3 mg/L at Stn.3. TA of 140.0 mg/L was observed at Stn.1, 120.0 mg/L at Stn.2 and 150.0 mg/L at Stn.3 in September. It was measured 119.3 mg/L at Stn.1, 113.3 mg/L at Stn.2 and 133.0 mg/L at Stn.3 in October.

On the onset of winters, 96.7 mg/L of TA was measured at Stn.1, of 116.7 at Stn.2 and 153.3 at Stn.3 in November. The month of December was observed with the TA of 99.7 mg/L at Stn.1, 106.7 mg/L at Stn.2 & 156.7 mg/L at Stn.3. TA at Stn.1 was observed 116.7 mg/L, 113.3 mg/L at Stn.2 and 180.0 mg/L at Stn.3 in January. While it was measured 130.0 mg/L at Stn.1,126.7 mg/L at Stn.2 and 129.3 mg/L at Stn.3 in the month of February.

In summer season, TA at Stn.1was 123.3 mg/L but, 113.3 mg/L at Stn.2 and 133.3 mg/L at Stn.3 in March. The month of April was measured with the TA of 133.3 mg/L at Stn.1, 113.3 mg/L at Stn.2 and 196.7 mg/L at Stn.3. In May and June, it was almost similar at Stn.2. It was measured 122.7 mg/L in May and 126.7 mg/L in June while it was 140.0 mg/L at Stn.1 and 203.3 mg/L at Stn.3 in the month of May. In the month of June, it was observed at 126.7 mg/L at Stn.1 and 136.7 mg/L at Stn.3 (Figure 4.9).

# **4.8 Hardness**

Hardness includes the hardness caused by both calcium and magnesium concentrations.

#### **(a). Total Hardness (TH)**

It ranged between 96.7 mg/L to 200.0 mg/L at all the three stations.

In the monsoon season, the total hardness of water at Stn.1 was measured 123.3 mg/L, Stn.2 with TH of 136.7 mg/L and Stn.3 with 153.3 mg/L in the month of July. The month of August was observed with 100.0 mg/L at Stn.1, 106.7 mg/L at Stn.2 and 120.0 mg/L at Stn.3. TH of 140.0 mg/L was observed at St. 1 and Stn.2 but 160.0 mg/L at Stn.3 in the month of September. It was measured 130.0 mg/L at Stn.1, 129.3 mg/L at Stn.2 and 170.0 mg/L at Stn.3 in October.

During winter season, TH of 96.7 mg/L was measured at Stn.1, of 106.7 at Stn.2 and 186.7 at Stn.3 in November. In the month of December, it was observed with 103.3 mg/L at Stn.1, 110.0 mg/L at Stn.2 & 176.7 mg/L at Stn.3. TH in January was measured 150.0 mg/L, 143.3 mg/L and 196.7 mg/L at Stn.1, 2 and 3

respectively. In February, it was observed 148.3 mg/L at Stn.1, 160.0 mg/L at Stn.2 and 200.0 mg/L at Stn.3.

TH at Stn.1 was 143.3 mg/L, 150.0 mg/L at Stn.2 and 193.3 mg/L at Stn.3 in the March month of summer season. The month of April was measured with the TH of 133.3 mg/L at Stn.1, 113.3 mg/L at Stn.2 and 186.7 mg/L at Stn.3. In the month of May, it was measured 130.0 mg/L at Stn.1 and 2 while it was measured 190.0 mg/L at Stn.3. It was observed 156.7 mg/L at Stn.1, 136.7 mg/L at Stn.2 and 160.0 mg/L at Stn.3 in the month of June (Figure 4.10).

#### **(b). Calcium Hardness**

Calcium hardness varied between 60.0 mg/L to 126.7 mg/L.

During monsoons, the calcium hardness at Stn.1 was measured 73.3 mg/L, Stn.2 with 83.3 mg/L and Stn.3 with 93.3 mg/L in the month of July. The month of August was observed with 60.0 mg/L at Stn.1, 63.3 mg/L at Stn.2 and 70.0 mg/L at Stn.3. Calcium Hardness of 80.0 mg/L was observed at St. 1 and Stn.2 while 100.0 mg/L at Stn.3 in the month of September. It was measured 90.0 mg/L at Stn.1, 86.7 mg/L at Stn.2 and 100.0 mg/L at Stn.3 in October.

In winter season, calcium hardness of 60.0 mg/L was measured at Stn.1, 66.7 mg/L at Stn.2 and 116.7 mg/L at Stn.3 in November. In the month of December, it was observed with 63.3 mg/L at Stn.1, 80.0 mg/L at Stn.2 & 96.7 mg/L at Stn.3. Calcium Hardness was measured 86.7 mg/L at Stn.1 and 2 while 116.7 mg/L at Stn.3 in January. In February, it was observed 86.7 mg/L, 100.0 mg/L and 106.7 mg/L at Stn.1, 2 at 3 respectively.

Calcium Hardness at Stn.1 was measured 83.3 mg/L in March, April and May months of summer season. It was 90.0 mg/L at Stn.2 and 103.3 mg/L at Stn.3 in the month of March. The month of April was measured 73.3 mg/L at Stn.2 and 126.7 mg/L at Stn.3. It was reported 81.7 mg/L at Stn.2 and 110.0 mg/L at Stn.3 in the month of May. It was observed 96.7 mg/L at Stn.1 and 3 but 86.7 mg/L at Stn.2 in the month of June (Figure 4.11).

#### **(c). Magnesium Hardness**

The magnesium hardness was observed maximum at 93.3 mg/L and minimum at 30.0 mg/L during the study period.

During the study period, the monsoon season exhibited the magnesium hardness of 50.0 mg/L at Stn.1, 53.3 mg/L at Stn.2 and 60.0 mg/L at Stn.3 of water in the month of July. The month of August was observed with 40.0 mg/L at Stn.1, 43.3 mg/L at Stn.2 and 50.0 mg/L at Stn.3. In the month of September, magnesium hardness of 60.0 mg/L was observed at all the three stations. It was measured 40.0 mg/L at Stn.1, 42.0 mg/L at Stn2 and 70.0 mg/L at Stn.3 in October.

In winter season, magnesium hardness of 36.7 mg/L was measured at Stn.1, 40.0 mg/L at Stn.2 and 70.0 mg/L at Stn.3 in November. In the month of December, it was observed with 40.0 mg/L at Stn.1, 30.0 mg/L at Stn.2 & 80.0 mg/L at Stn.3. Magnesium hardness was measured 63.3mg/L at Stn.1, 56.7 mg/L at Stn.2 and 80.0 mg/L at Stn.3 in January. In February, it was observed 61.7 mg/L, 60.0 mg/L and 93.3 mg/L at Stn.1, 2 at 3 respectively.

In March month of summers, magnesium hardness was measured 60.0 mg/L at Stn.1 and 2 but 90.0 mg/L at Stn.3. In the month of April it was measured 50.0 mg/L at Stn.1, 40.0 mg/L at Stn.2 and 53.3 mg/L at Stn.3. It was observed 46.7 mg/L at Stn.1, 48.3 mg/L at Stn.2 and 80.0 mg/L at Stn.3 in the month of May. In June month, 60.0 mg/L of magnesium hardness was measured at Stn.1 and Stn.3 while 53.3 mg/L at Stn.2 (Figure 4.12).

#### **4.9 Chloride**

The maximum value of chloride concentration was observed 20.0 mg/L and minimum at 88.7 mg/L in water at the three sites selected for the study period.

In the monsoon time, the chloride content in water at Stn.1 was measured 33.3 mg/L in July. Stn.2 was recorded with chloride content of 46.7 mg/L and Stn.3 at 50.0 mg/L in the same month. It was observed 20.0 mg/L at Stn.1, 30.0 at Stn.2 and 33.3 mg/L at Stn.3 in August month. The chloride content was measured 30.0 mg/L at Stn.1, 40.0 mg/L at Stn.2 and 50.0 mg/L at Stn.3 in the September month. In the month of October, 31.3 mg/L of chloride content was observed at Stn.1, Stn.2 was recorded with 38.0 mg/L and Stn.3 with 44.0 mg/L.

During November, the chloride content was measured at 30.0 mg/L at the Stn.1, 36.7 mg/L at Stn.2 and 73.3 mg/L at Stn.3 in the winter season. In the month of December, it was observed 39.3 mg/L, 40.7 mg/L and 68.3 mg/L at Stn.1, Stn.2 & Stn.3 respectively. In the month of January, chloride content of 30.7 mg/L was measured at Stn.1, 31.3 mg/L at Stn.2 and 66.7 mg/L at Stn.3. During February month, chloride content of 40.0 mg/L was measured at Stn.1, 40.7 mg/L at Stn.2 and 80.0 mg/L at Stn.3.

With the arrival of summers, the chloride content of 43.3 mg/L was observed at Stn.1, 36.7 mg/L at Stn.2 and 86.7 mg/L at Stn.3 in March month. The chloride content was found 40.0 mg/L at Stn.1 during April while it was measured 33.3 mg/L at Stn.2 and 73.3 mg/L at Stn.3. In the month of May, chloride content of 49.3 mg/L was measured at Stn.1, 40.0 mg/L at Stn.2 and 88.7 mg/L at Stn.3. It was observed 46.7 mg/L, 50.0 mg/L and 60.0 mg/L at Stn.1, Stn.2 & Stn.3 respectively in the month of June (Figure 4.13).

# **4.10 Nitrate**

Nitrate content of river water varied between 1.3 mg/L to 8.0 mg/L round the year.

During monsoon season, the nitrate content at Stn.1 was measured 2.7 mg/L in the month of July while 4.3 mg/L at Stn.2 and 4.0 mg/L at Stn.3. It was found 4.7 mg/L at Stn.1, 3.0 mg/L at Stn.2 and 4.0 mg/L at Stn.3 in the month of August. 6.0 mg/L of nitrate concentration was observed at Stn.1, 7.0 mg/L at Stn.2 and 8.0 mg/L at Stn.3 in September. It was measured 5.0 mg/L at Stn.1, 6.7 mg/L at Stn.2 and 7.7 mg/L at Stn.3 in October.

During winters, 3.0 mg/L of nitrates were measured at Stn.1, of 2.3 at Stn.2 and 4.3 at Stn.3 in November. The month of December was observed with the nitrate content of 2.0 mg/L at Stn.1, 2.3 mg/L at Stn.2 & 7.7 mg/L at Stn.3. Nitrate content at Stn.1 was found to be similar in January and February with 2.0 mg/L while it was measured 2.0 mg/L and 3.0 mg/L at Stn.2 in January and February respectively. Stn.3 was observed with 8.0 mg/L of nitrate concentration in January and 4.7 mg/L in February at Stn.3.

In summer season, the nitrate content at Stn.1 was measured 2.0 mg/L, 2.3 mg/L at Stn.2 and 6.7 mg/L at Stn.3 in March. It was measured with 1.7 mg/L at Stn.1, 5.7 mg/L at Stn.2 and 1.3 mg/L at Stn.3 in the month of April. In May and June, it was almost similar at Stn.1 and Stn.2 It was measured 2.3 mg/L in May, 2.1 mg/L in June at Stn.1and it was 2.0 mg/L in the month of May and 2.2 mg/L in June at Stn.2. It was observed 7.0 mg/L in the month of May and 2.7 mg/L at Stn.3 in June (Figure 4.14).

# **4.11 Sulphate**

Sulphate concentration of water varied with minimum of 4.7 mg/L to maximum of 38.3 mg/L.

The Monsoon season started with the month of July in which sulphate content was measured 12.3 mg/L at Stn.1, 21.3 mg/L at Stn.2 and 38.3 mg/L at Stn.3. In August, it was 10.0 mg/L at Stn.1, 12.0 mg/L at Stn.2 and 15.0 mg/L at Stn.3. The sulphate content of 19.0 mg/L was observed at Stn.1, 22.0 mg/L at Stn.2 and 31.0 mg/L at Stn.3 in the month of September. The month of October was observed with sulphate content of 10.3 mg/L at Stn.1, of 8.3 mg/L at Stn.2 and of 13.7 mg/L at Stn.3.

On the onset of winters, the sulphate content at Stn.1 was recorded 4.7 mg/L at Stn.1, 6.7 mg/L at Stn.2 and 8.7 mg/L at Stn.3 in the month of November. In the month of December, 7.3 mg/L of sulphate was observed at Stn.1, 8.7 mg/L at Stn.2 & 12.3 mg/L at Stn.3. In the month of January, the sulphates were measured 14.3 mg/L at Stn.1, 17.3 mg/L at Stn.2 and 11.3 mg/L at Stn.3. The sulphate content of 9.0 mg/L was observed at Stn.1, 8.0 mg/L at Stn.2 & 12.7 mg/L at Stn.3 in the month of February.

During summer season, in March, sulphate content of 10.3 mg/L was observed at Stn.1, 9.3 mg/L at Stn.2 and 15.7 mg/L at Stn.3. In the month of April, 11.3 mg/L of sulphate content was recorded at Stn.1, 15.7 mg/L at Stn.2 & 21.3 mg/L at Stn.3. It was measured 17.7 mg/L, 20.7 mg/L and 25.0 mg/L at Stn.1, Stn.2 & Stn.3 respectively in the month of May. It was observed 14.7 mg/L at Stn.1, 12.0 mg/L at Stn.2 and 26.7 mg/L at Stn.3 in June (Figure 4.15).

#### **4.12 Fluoride**

Fluorides in water varied from a minimum of 0.11 mg/L to maximum of 0.35 mg/L at the three selected sites during the study period.

The river water in monsoon season depicted the fluoride content of 0.19 mg/L at Stn.1, 0.22 mg/L at Stn.2 and Stn.3 in the month of July. It was observed 0.11 mg/L at Stn.1, 0.13 at both Stn.2 and Stn.3 in August month. The fluoride content was measured 0.19 mg/L at Stn.1, 0.23 mg/L at Stn.2 and 0.27 mg/L at Stn.3 in the September month. In the month of October, 0.27 mg/L of fluoride content was observed at Stn.1, Stn.2 was recorded with 0.26 mg/L and Stn.3 with 0.32 mg/L.

With the arrival of winters, the fluoride content was measured at 0.21 mg/L at the Stn.1, 0.26 mg/L at Stn.2 and 0.32 mg/L at Stn.3 in November month. In the month of December it was 0.24 mg/L at Stn.1, 0.28 mg/L at Stn.2 and 0.30 mg/L at Stn.3. It was 0.22 mg/L in January month at Stn.1 and 2 while 0.29 mg/L at Stn.3. During February month, fluoride content of 0.3 mg/L was observed at all the three stations.

During summers, the fluoride content of 0.27 mg/L was observed at Stn.1, 0.28 mg/L at Stn.2 and 0.35 mg/L at Stn.3 in March month. The fluoride content was found 0.21 mg/L at Stn.1 during April while it was measured 0.20 mg/L at Stn.2 and 0.32 mg/L at Stn.3. In the month of May, fluoride content of 0.25 mg/L was measured at Stn.1, 0.23 mg/L at Stn.2 and 0.34 mg/L at Stn.3. In the month of June it was observed 0.11 mg/L at Stn.1, 0.17 mg/L at Stn.2 and 0.18 mg/L at Stn.3 (Figure 4.16).

#### **4.13 Dissolved Oxygen (DO)**

Dissolved oxygen of river water ranged between 3.9 mg/L to 8.5 mg/L round the year.

In monsoon season, the DO of water at Stn.1 was measured 6.7 mg/L in the month of July while 7.1 mg/L at Stn.2 and Stn.3. It was observed 7.0 mg/L at Stn.1, 6.5 mg/L at Stn.2 and 6.1 mg/L at Stn.3 in the month of August. 7.9 mg/L of DO was observed at Stn.1, 5.9 mg/L at Stn.2 and 6.9 mg/L at Stn.3 in September. It was measured 7.1 mg/L at Stn.1, 6.8 mg/L at Stn.2 and 6.3 mg/L at Stn.3 in the month of October.

7.5 mg/L of DO was measured at Stn.1, of 5.7mg/L at Stn.2 and 4.9 mg/L at Stn.3 in November during winters. The month of December was observed with the DO of 8.5 mg/L at Stn.1, 7.6 mg/L at Stn.2 & 5.0 mg/L at Stn.3. DO at Stn.1 was found to be almost similar with 7.5 mg/L in January and with 7.4 mg/L in February at Stn.1. DO of 7.4 mg/L was measured at Stn.2 in January and 7.2 mg/L in February. Stn.3 was observed with 6.0 mg/L DO in January and 3.9 mg/L in February.

In summers, the DO at Stn.1 was measured 7.0 mg/L, 6.9 mg/L at Stn.2 and 4.0 mg/L at Stn.3 in March. It was measured with 6.6 mg/L at Stn.1, 6.1 mg/L at Stn.2 and 6.5 mg/L at Stn.3 in the month of April. In May and June, it was similar at Stn.3 with 5.2 mg/L. It was measured 6.5 mg/L in May at Stn.1 and 6.2 at mg/L Stn.2. In the month of June it was 6.8 mg/L at Stn.1 and 6.4 mg/L at Stn.2 June (Figure 4.17).

#### **4.14 Biochemical Oxygen Demand (BOD)**

BOD values of water in river ranged between 0.2 mg/L to 5.03 mg/L during the study period.

In the July month of Monsoon season, BOD of water was measured 1.0 mg/L at Stn.1, 2.8 mg/L at Stn.2 and 5.0 mg/L at Stn.3. In August, it was 2.0 mg/L at Stn.1, 2.1 mg/L at Stn.2 and 1.4 mg/L at Stn.3. The BOD at Stn.1 was observed with 1.4 mg/L, Stn.2 with 0.5 mg/L and Stn.3 with 5.0 mg/L in the month of September. The month of October was measured with 0.9 mg/L at both Stn.1 and Stn.2 but with 4.6 mg/L of BOD at Stn.3.

During the winter season, the BOD at was observed 2.4 mg/L at Stn.1 and Stn.3, while 0.7 mg/L at Stn.2 in the month of November. In the month of December, 0.5 mg/L of BOD was observed at Stn.1, 0.9 mg/L at Stn.2 & 2.9 mg/L at Stn.3. 0.3 mg/L of BOD was measured at Stn.1 in the month of January and February. It was 0.6 mg/L at Stn.2 and 2.1 mg/L at Stn.3. BOD of 0.2 mg/L was recorded at Stn.2 & 3.6 mg/L at Stn.3 in the month of February.

In summer season, BOD of 0.9 mg/L was observed at Stn.1, 0.6 mg/L at Stn.2 and 2.9 mg/L at Stn.3 in the month of March. At Stn.1, 2.1 mg/L of BOD was measured in the month of April and May. 2.0 mg/L of BOD was observed at Stn.2 and 2.6 mg/L at Stn.3. It was measured 1.7 mg/L and 2.5 mg/L at Stn.2 & Stn.3 respectively in the month of May. In June, it was observed at 2.2 mg/L at Stn.1, 2.1 mg/L at Stn.2 and 1.7 mg/L at Stn.3 (Figure 4.18).

#### **4.15 Chemical Oxygen Demand (COD)**

COD ranged from 4.0 mg/L to 66.67 mg/L round the year in river water.

COD was measured 26.7 mg/L at Stn.1, 57.3 mg/L at Stn.2 and 66.7 mg/L at Stn.3 in water in the month of July during Monsoon season. It was 20.7 mg/L at Stn.1, 40.7 mg/L at Stn.2 and 56.7 mg/L at Stn.3 in August. The COD at Stn.1 was measured with 21.7 mg/L, Stn.2 with 48.3 mg/L and Stn.3 with 64.3 mg/L in the month of September. It was observed with 15.7 mg/L at Stn.1, 31.3 mg/L at Stn.2 and 57.7 mg/L of COD at Stn.3 in the month of October.

In the month of November, COD was measured 7.7 mg/L at Stn.1, 38.3 mg/L at Stn.2 and 61.7 mg/L at Stn.3 during the winter season. In the month of December, 9.3 mg/L of COD was observed at Stn.1, 29.3 mg/L at Stn.2 & 55.0 mg/L at Stn.3. COD of 4.0 mg/L was measured at Stn.1, 14.0 mg/L at Stn.2 and 41.3 mg/L at Stn.3 in the month of January. In the month of February, it was 14.0 mg/L at Stn.1, 11.0 mg/L at Stn.2 and 34.7 mg/L COD was recorded at Stn.3.

In the summer season, 14.7 mg/L of COD was observed at Stn.1, 12.3 mg/L at Stn.2 and 36.0 mg/L at Stn.3 in March month. In the month of April, 18.7 mg/L of COD was measured at Stn.1, 35.7 mg/L at Stn.2 and 12.7 mg/L at Stn.3. It was measured 22.0 mg/L at Stn.1, 18.3 mg/L at Stn.2 and 44.7 mg/L at Stn.3 in the month of May. In June, it was observed at 18.3 mg/L at Stn.1, 13.3 mg/L at Stn.2 and 24.3 mg/L at Stn.3 (Figure 4.19).



**Figure 4.3: Light Intensity at Three Stations**



**Figure 4.4: Temperature at three stations**



**Figure 4.5: pH values at three stations**



**Figure 4.6: EC at three stations**



**Figure 4.7: TDS at three stations**



**Figure 4.8: Turbidity at three stations**



**Figure 4.9: Total Alkalinity at Three Stations**



**Figure 4.10: Total Hardness at Three Stations**



**Figure 4.11: Calcium Hardness at Three Stations**



**Figure 4.12: Magnesium Hardness at Three Stations**



**Figure 4.13: Chloride Content at Three Stations**



**Figure 4.14: Nitrate Content at Three Stations**



**Figure 4.15: Sulphate Concentration at Three Stations**



**Figure 4.16: Fluoride Content at Three Stations**



**Figure 4.17: DO Level at Three Stations**



**Figure 4.18: BOD Level at Three Stations**



**Figure 4.19: COD at Three Stations**

# **CHAPTER 5 DIVERSITY AND COMMUNITY STRUCTURE OF CYANOBACTERIA**

Cyanobacteria are chlorophyll containing photosynthetic blue-green algae which have close affinity with bacteria as they possess prokaryotic structure with absence of sexual reproduction in them. They flourish in every possible environment with a wide range of organization. They may be unicellular, filamentous or colonial. They have multiple uses of economic importance. They can also be used as bioindicators for the bioassessment of an ecosystem.

Biodiversity, also known as biological diversity, can be defined as variation among species and habitats of living organisms be it at gene level; species level or at ecosystem level. It is basically the variety of life. Three types of biodiversity are known –  $\alpha$  diversity (local),  $\beta$  diversity (turnover) and  $\gamma$  diversity (regional). In general, α-diversity is the diversity of life forms within a habitat or within community diversity. The concept of biodiversity at species level is known as species diversity or  $\alpha$ - diversity and has two components - richness & evenness. Species richness is the number of kinds of species in a community of a particular taxon. Evenness is how the number of individuals are distributed among the species. The β-diversity is between habitat diversity and γ-diversity is the diversity of landscape-scale areas (Ricklefs and Miller, 1999; Odum and Barrett, 2010).

#### **5.1 Diversity of Cyanobacteria**

The surface water samples were collected from the three sampling sites in the morning time during second week of every month from July 2017 to June 2018

to study the diversity of cyanobacteria. The collected samples were studied within 48 hrs with the preparation of fresh mounts in laboratory for the study of diversity of cyanobacteria. The samples were also fixed and preserved using Lugol"s solution for further examination in detail. Identification of Cyanobacteria was done with the help of available standard keys, monographs (Smith, 1950; Prescott, 1954; Desikachary, 1959; Prescott, 1970; Palmer, 1980; Baker and Fabbro, 2002; Bellinger and Sigee, 2015), online database [\(www.algaebase.org\)](http://www.algaebase.org/) and research publications (Anagnostidis and Komárek, 1988; Komárek and Komárková, 2003; Das *et al*., 2010; Liu *et al*., 2013; Komárek *et al*., 2014, Tandon, 2016; Menezes *et al*., 2020). For this purpose a trinocular research Metzer-M vision plus microscope was used and microphotographs were taken by an attached imported camera MD500. The quantitative results of cyanobacteria were expressed as organisms/mL (Figure 5.1).

The diversity of Cyanobacteria was studied for twelve months during three seasons– monsoon, winter and summer round the year. Various forms of cyanobacteria were observed during the study period. They belong to Cyanophyceae class of Kingdom - Alga. The class is divided into five orders – Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales, Stigonematales (Fritsch, 1935).

Total 14 genera and 25 species of planktonic freshwater Cyanabacteria were observed during the study period that belonged to 2 orders of Fritsch"s classification (Fritsch, 1935):

Order- Chroococcales: *Chroococcus, Gloeocapsa, Synechocystis, Synechococcus, Microcystis, Aphanocapsa, Merismopedia*

Order-Nostocales: *Lyngbya, Oscillatoria, Spirulina, Cylindrospermopsis, Anabaena, Nostoc, Planktothrix*

On the basis of modern system of classification (Anagnostidis and Komárek, 1988; Komárek *et al*., 2014) the cyanobacteria observed during the study period belonged to orders as mentioned below:

Order-Synechococcales: *Synechocystis, Synechococcus, Aphanocapsa, Merismopedia* Order-Spirulinales ordo nov.: *Spirulina*

Order-Chroococcales: *Chroococcus, Gloeocapsa, Microcystis* Order-Oscillatoriales: *Lyngbya, Oscillatoria, Planktothrix* Order-Nostocales: *Anabaena, Nostoc, Cylindrospermopsis*

#### **Experimental Observations:**

#### **Seasonal Diversity of Cyanobacteria**

#### **Monsoon Season**

Various taxa of Cyanobacteria were found and studied during the rainy season at three stations. The monsoon season started from July 2017 and lasted to October 2017. *Anabaena, Nostoc, Oscillatoria, Chroococcus* and *Synechococcus* were the major Cyanobacteria found during the monsoon season at Stn.1 (control station). *Oscillatoria* was commonly found during July to September 2017 mostly at all the stations studied. The Cyanobacteria like *Chroococcus, Oscillatoria, Aphanocapsa, Planktothrix, Synechococcu*s and *Microcystis* were apparent at Stn.3 while the Stn.2 was found abundant with the presence of *Anabaena*, *Oscillatoria, Chroococcus, Planktothrix, Merismopedia* and *Aphanocapsa* during this season.

# **Winter Season**

The season of winter which was considered from November 2017 to February 2018 marked with the presence of *Anabaena, Oscillatoria, Aphanocapsa, Planktothrix* at Stn.1. The Stn.2 was flourished with *Oscillatoria, Aphanocapsa, Chroococcus, Synechococcus, Spirulina, Anabaena* and *Merismopedia*. While *Chroococcus, Oscillatoria* and *Merismopedia* were observed at Stn.3 in winter season. *Merismopedia* was dominant at Stn.3 during this season.

# **Summer Season**

The summer season started from March 2018 and remained till June 2018. This season was flourished with *Oscillatoria, Aphanocapsa, Planktothrix, Chroococcus, Spirulina, Anabaena* and *Merismopedia* at station Stn.1. *Planktothrix, Anabaena, Oscillatoria, Lyngbya, Aphanocapsa, Chroococcus, Synechococcus, Synechocystis* and *Merismopedia* were observed at Stn.2. *Microcystis* and *Cylindrospermopsis* were also observed at Stn.2 during this season. *Microcystis, Oscilllatoria* and *Chroococcus* were observed at Stn.3 in the month of June 2018 but *Merismopedia* was dominant at Stn.3 during the summer season.

Bhatnagar and Bhardwaj (2013 a&b) have reported genera viz. *Anabaena* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Gloeocapsa* sp., *Microcystis* sp., *Merismopedia* sp., *Nostoc* sp., *Phormidium* sp., *Oscillatoria* sp., *Spirulina* sp., *Arthrospira* sp. in their study in 2011-2012 at Kota Barrage.



# **from July 2017 to June 2018**

**Table 5.1: Seasonwise occurrence of Cyanobacteria at Three Stations** 

(+ present - absent)

#### **Systematic Enumeration and Description of some of the observed taxa:**

Order- Synechococcales (Komárek *et al*., 2014)

#### **Genus***- Aphanocapsa* Näg.

Two species were observed.

### *Aphanocapsa grevillei* (Hass.) Rabenh.

Desikachary, 1959, p. 134, pl. 21, fig.9

Thallus spherical, gelatinous, light blue green in colour, irregular, cells spherical and closely arranged in mucilage, individual sheaths around the cells absent, 3.2-5.6 µ diameter (Plate1 Figure a).

# *Aphanocapsa littoralis* Hansgirg

Desikachary, 1959, p. 131, pl. 21, fig.1

Thallus mucilaginous, amorphous without any definite shape, cells spherical to subspherical, single or in twos, sparsely aggregated, 4-6 µ diameter (Plate1 Figure b).

**Genus** *- Synechocystis* Sauvageau.

*Synechocystis* sp.

Desikachary, 1959, p. 144, pl. 25, fig.9&11

Cells spherical, single or two together after cell division, no distinguishable mucilage envelopes are found, rarely in colonies. (Plate1 Figure c).

# **Genus** *- Synechococcus* Näg.

#### *Synechococcus* sp.

Desikachary, 1959, p. 143, pl. 25, fig.; Prescott, 1970, p. 460. pl. 102, fig.6-8

Oblong or cylindrical unicells, longer than broad cells, or rarely in colonies of 2-4 in one plane, round apices, division transverse, highly granular, sheath absent, free-floating (Plate 1 Figure d).

#### **Genus** *- Merismopedia* Meyen

Two species were observed during the study.

# *Merismopedia elegans* A. Br.

Desikachary, 1959, p. 156, pl. 29, fig.9; Prescott, 1970, p. 459. pl. 101, fig. 1

Colonies small or big, 16-4000 celled, light blue in colour, cells spherical or oblong, more or less cells closely arranged forming a plate, free-floating or planktonic, 5-7  $\mu$  broad, 6-9  $\mu$  long (Plate 1 Figure e).

# *Merismopedia minima* Beck

Desikachary, 1959, p. 154, pl. 29, fig.11

Colony small, 4 to many celled, pale blue in colour, free-floating,  $0.5$ - $0.6 \mu$ broad (Plate 1 Figure f).

Order- Spirulinales ordo nov. (Komárek *et al*., 2014)

# **Genus** *– Spirulina* Turpin

Only one species was observed.

#### *Spirulina laxissima* forma *major* f. nov. West, G.S

Desikachary, 1959, p. 196, pl. 36, fig. 6; Prescott, 1970, p. 480. pl. 107, fig.17

Trichomes 0.7- 0.8 µ broad, unicellular, spirals regular and very loose, not tapering towards the apices, terminal cell rounded, obtuse, sheath absent, freefloating (Plate 2 Figure g). According to new classification, Komárek *et al*. (2014) has placed this genus as *Glaucospira laxissima* (Baruah *et al.*, 2014). Order- Chroococcales (Komárek *et al*., 2014)

#### **Genus** *- Chroococcus* Nag.

Three species of *Chroococcus* were found.

#### *Chroococcus* **sp.**

Desikachary, 1959, p. 98

Cells spherical or subspherical or hemispherical with round margins in group of 2-16 in mucilaginous matrix with distinct gelatinous sheath of individual cell, concentric rings in mucilaginous sheath generally absent, free swimming colony, cells not vacuolated, light to bright blue-green in colour (Plate 2 Figure h).

# *Chroococcus dispersus* (v. Keissler) Lemm.

Prescott, 1970, p. 447. pl. 100, fig.1-3; Das, Bhakta and Adhikary, 2010, p.337, pl.1, fig.7

Cells 2-16 or more in tabular mucilaginous matrix, with round margins, cells in groups isolated from each other, free swimming colony, individual envelopes not lamellated, totally gelatinised, colourless, light or brilliant blue-green in colour (Plate 2 Figure i).

*Chroococcus minor* (Kütz.) Näg.

Desikachary, 1959, p. 105, pl. 24, fig.1; Prescott, 1970, p. 449. pl. 100, fig.12

Gelatinous colony, cells spherical, singly or in pairs, seldom 4 or 8, dirty blue-green or olive green in colour, colourless sheath (Plate 2 Figure j).

# **Genus** *- Gloeocapsa* Kützing

Two species were observed

# *Gloeocapsa* **sp.**

Desikachary, 1959, p. 111 ; Prescott, 1970, p. 451

Cells spherical, surrounded by a laminated sheath, 2-8 in colonies with a number of concentric layers of mucilage, definite thicker sheaths, individual sheaths lamellated or unlamellated (Plate 2 Figure k).

# *Gloeocapsa punctata* Näg.

Desikachary, 1959, p. 115, pl. 23, fig.2; Prescott, 1970, p. 452, pl. 101, fig.7

Thallus gelatinous, cells spherical, cells 2-16 in colonies, surrounded by a colourless, unlamellated thick sheath, with sheath  $3.5\n-7$   $\mu$  broad, light blue-green in colour (Plate 2 Figure l).

#### **Genus** *- Microcystis* Küetzing

Four species of this genera were observed.

#### *Microcystis aeruginosa* Kütz.

Desikachary, 1959, p. 93, pl. 17, fig.1,2,6 and pl. 18, fig. 10 ; Prescott, 1970, p. 456. pl, 102, fig.1-4; Das, Bhakta and Adhikary, 2010, p.337, pl.1, fig.4

Colonies with numerous ovate or spherical cells, colonies when young round or slightly longer than broad, cells  $3-7 \mu$  in diameter, generally with gas vacuoles, colony solid but when old become perforated or clathrate, colonial mucilage hyaline and distinct, blue-green in colour, free-floating or planktonic. (Plate 3 Figure m).

#### *Microcystis flos-aquae* (Wittr.) Kirchner

Desikachary, 1959, p. 94, pl. 17, fig.11 and pl. 18, fig. 11; Baker and Fabbro, 2002, p. 9, photo 9; Das, Bhakta and Adhikary, 2010, p.337, pl.1, fig.5

Colonies roughly spherical, ellipsoidal or somewhat elongate often squarish in optical section, cells globose or spherical, with gas vacuoles, cells  $3-7 \mu$  in diameter, colonies non-perforate or non-clathrate, with indistinct colonial mucilage, free-floating or planktonic. (Plate 3 Figure n).

# *Microcystis wesenbergii* (Komárek) Komárek in Kondrateva

Baker and Fabbro, 2002, p. 9, photo 13; Tandon, Kesarwani, Mishra, Dikshit and Tiwari, 2016, p.6, pl. 1, fig.1 and p.7, pl. 2, fig. 8&9

Colonies irregular, micro to macroscopic, often lobed and fenestrate, boundary of the colonial mucilage sharply defined and refracrive, cells spherical, sparsely well distributed within the mucilage, packed with aerotopes,  $4.5$ -7.5  $\mu$  in diameter, planktonic. (Plate 3 Figure o).

#### *Microcystis smithii* Komárek et Anagnostidis

Tandon, Kesarwani, Mishra, Dikshit and Tiwari, 2016, p.6, pl. 1, fig.7 and p.8, pl. 3, fig. 23& 24

Colonies spherical or slightly irregular, non-clathrate, cells scattered or rarely densely arranged, cells loosely arranged within colony, cells single or in pairs after division spherical with several brownish aerotopes in each cell, 3.2-5.6µm in diameter, colourless mucilage, without refractive margin, free-floating (Plate 3 Figure p).

Order- Oscillatoriales (Anagnostidis and Komárek,1988; Komárek *et al*., 2014)

**Genus** *- Lyngbya* Ag.

# *Lyngbya* sp.

Desikachary, 1959, p. 278 ; Prescott, 1970, p. 497

Unbranched trichome of cells in a thin or very massive delicate, firm, distinct sheath, sheath mostly colourless, mostly cylindrical trichome with very slight tapering, trichomes may be solitary or form a coiled mass, cells 1-24 µm in diameter, free-floating or attached ( Plate 4 Figure q).

**Genus** *- Oscillatoria* Vaucher

Four species were observed

*Oscillatoria sp.*

Desikachary, 1959, p. 198

Trichome straight forming a free floating thallus, mucilaginous sheath absent, end of trichome distinctly marked, have oscillating movement (Plate 4 Figure r).

#### *Oscillatoria subbrevis* Schmidle

Desikachary, 1959, p. 207, pl. 37, fig.2 and pl. 40, fig. 1 ; Prescott, 1970, p. 491. pl. 107, fig.23

Trichomes single, straight, not attenuated at the apices, cells 1-2 µ long, not granulated at the cross-walls, end cell rounded, calyptra absent, 5-6 µ broad (Plate 4 Figure s).

# *Oscillatoria tenuis* Ag. ex Gomont

Desikachary, 1959, p. 222, pl. 42, fig.15 ; Prescott, 1970, p. 491, pl. 110, fig.8,9,14

Trichome straight, slightly constricted at the cross-walls, not attenuated at the apices, not capitates, end cell hemispherical with thickened outer membrane, mucilaginous sheath absent, 4-10  $\mu$  broad (Plate 4 Figure t).

#### *Oscillatoria chlorina* Kütz. ex Gomont

Desikachary, 1959, p. 215, pl. 40, fig.4

Trichome straight or curved, uncontricted or slightly constricted at the crosswalls, gas vacuoles absent, not granulated at the cross-walls, calyptra absent, 3.5-4  $\mu$ broad (Plate 4 Figure u).

# **Genus** *- Planktothrix* Anagnostidis & Komárek

# *Planktothrix* **sp***.*

Baker and Fabbro, 2002, p. 20, fig. 5

Straight cylindrical trichomes, cross walls slightly constricted, rounded apical cell, slightly tapering towards the terminal cell, sheath absent, cells isodiametric, planktonic, containing gas vesicles (Plate 5 Figure v).

Order- Nostocales (Komárek *et al*. 2014)

# **Genus** *- Nostoc* Vaucher

*Nostoc* sp.

Desikachary, 1959, p. 372 ; Prescott, 1970, p. 520

Mucilaginous thallus with a definite shape, periphery dense and darkly coloured, unbranched trichomes of bead-like cylindrical cells, curved or entangled filaments, intercalary heterocysts, cells depressed, spherical or cylindrical, trichomes with no basal-distal differentiation, colonies microscopic or macroscopic, freefloating or attached (Plate 5 Figure w).

#### **Genus** *- Anabaena* Bory

#### *Anabaena* **sp.**

Desikachary, 1959, p. 391 ; Prescott, 1970, p. 510

Straight and uniformly broad trichomes, either with sheath or without a sheath, cells cylindrical or barrel shaped, trichomes are not parallel, intercalary heterocysts, single spores are found or in series that are formed near the heterocysts, free- floating or attached on moist substrates. (Plate 5 Figure x).
### **Genus** *- Cylindrospermopsis* Seenaya & Subba Raju

#### *Cylindrospermopsis* sp.

Baker and Fabbro, 2002, p. 37, fig. 11; Komárek and Komárková, 2003, p. 14 fig. 3, table 6; Menezes, Valério, Botelho and Dias, 2020, p. 4 fig.1

Solitary straight or slightly curved trichome, mucilaginous envelope absent, vegetative cells cylindrical with little or no constriction at cross walls, conical or long ovoid terminal heterocysts one at either end or at both the ends, akinete cylindrical, solitary, intercalary, planktonic, gas vesicles present ( Plate 5 Figure y).



- a. *Aphanocapsa grevillei* b. *Aphanocapsa littoralis*
- c. *Synechocystis* sp. d. *Synechococcus* sp.
- a. *Aphanocapsa grevillei* b. *Aphanocapsa littoralis*<br>
c. *Synechocystis* sp. d. *Synechococcus* sp.<br>
e. *Merismopedia elegans* f. *Merismopedia minima* 
	-
	-
	-



- g. *Spirulina laxissima* forma *major* h. *Chroococcus* sp*.*
- i. *Chroococcus dispersus* j. *Chroococcus minor*
- 
- 
- 
- k. *Gloeocapsa* sp. l. *Gloeocapsa punctata*







- m. *Microcystis aeruginosa* n. *Microcystis flos-aquae*
	-
- o. *Microcystis wesenbergii* p. *Microcystis smithii*





- 
- s. *Oscillatoria subbrevis* t. *Oscillatoria tenuis*
- q. *Lyngbya* sp. r. *Oscillatoria* sp.
	-
- u. *Oscillatoria chlorina*
- 



v. *Planktothrix* sp. w. *Nostoc* sp.

y

- 
- 
- x. *Anabaena* sp. y. *Cylindrospermopsis* sp.

#### **5.2 Community Structure of Cyanobacteria**

The community structure of Cyanobacteria is comprised of their various forms and species. All the stations selected for study were observed with different community structure of cyanobacteria. During the study period, total 25 species of cyanobacteria belonging to 14 different genera were observed in freshwater of Chambal river at three stations selected for the study. 9 taxa with 14 species of cyanobacteria were observed at Stn.1, 6 taxa with 16 species were observed at Stn.3 and total 13 taxa with 21 species at Stn.2. The taxa observed during the study period represent the following orders: Synechococcales, Spirulinales ordo nov., Chroococcales, Oscillatoriales and Nostocales (4,1,3,3 and 3 taxa respectively).

The community structure of cyanobacteria at Stn.1 is composed of some taxa like *Anabaena*, *Aphanocapsa, Chroococcus, Merismopedia, Oscillatoria, Planktothrix, Nostoc Spirulina and Synechococcus. Nostoc* was only observed at Stn.1 in the community of cyanobacteria during the study while *Merismopedia* was represented with only one species (*M. elegans).*

Taxa like *Microcystis, Gloeocapsa, Lyngbya, Synechocystis* and *Cylindrospermopsis* form a part of the cyanobacterial community at Stn.2. *Merismopedia* is represented with both the species observed (*M*. *elegans* and *M. minima*) at Stn.2 round the year. Among 4 species of *Microcystis*, 2 species were not observed at Stn.2 (*Microcystis smithii* and *M. flos-aquae*). Similarly, *Oscillatoria chlorina* was found to be altogether absent from the community of cyanobacteria at Stn.2.

The cyanobacterial community at Stn.3 was found to be composed of taxa *Aphanocapsa, Chroococcus, Merismopedia, Microcystis, Oscillatoria, Planktothrix* only with the absence of other taxa like *Anabaena*, *Microcystis, Gloeocapsa, Lyngbya, Spirulina, Synechocystis, Nostoc*, *Cylindrospermopsis* and *Synechococcus.* Taxon *Microcystis* was represented with all the 4 species observed during the study at Stn.3.

Diversity can be evaluated by calculating various indices, commonly known as diversity indices. "Diversity indices are mathematical functions that combine richness and evenness in a single measure" (Colwell, 2009).

The diversity indices are concerned with taxa diversity and take taxa occurrence into account. They consider the number of species (richness) and the number of individuals (abundance) (Aery, 2010). The community structure is studied with the help of some diversity indices.

In order to evaluate the cyanobacterial biodiversity and evaluate the cyanobacterial community structure, the following diversity indices are used (Colwell, 2009; Aery, 2010):

#### **1. Shannon- Weaver Index of Diversity**

It is commonly used to calculate the diversity of aquatic ecosystem. It expresses both the components of taxa diversity i.e., richness and evenness. (Aery, 2010).

#### **2. Sorensen's Index of Similarity**

It measures the ratio of common to average number of taxa in two communities. It expresses the number of taxa common or similar between two communities compared. This index was given by Sorensen in 1948. (Sørensen, 1948; Aery, 2010).



**Figure 5.1: Number of cyanobacteria (organisms/mL) observed from July 2017 to June 2018 at three stations**

**Table 5.2: Diversity indices of cyanobacteria from July 2017 to June 2018**

# **A. Shannon Weaver Index (Seasonwise)**





## **B. Shannon Weaver Index (Monthwise)**

## **C. Sorensen similarity Index (Seasonwise)**



## **D. Sorensen similarity Index (Monthwise)**





**Figure 5.2: Shannon Weaver diversity index at three stations in three seasons**



**Figure 5.3: Sorensen Similarity Index at three stations in three seasons**

The average yearly values of Shannon Weaver diversity index for cyanobacteria were calculated lowest at Stn.1 and highest at station 2 round the year. It was found lowest (1.31) at Stn.1 and highest at Stn.2 (1.74) during the summer season. The highest diversity is calculated at a site near the thermal discharge point of thermal power station in the month of June while the lowest at Stn.1 in the same month. Different species of cyanobacteria tolerant to higher temperature were found of more common occurrence at station 2 (Table 5.2 A & B, Figure 5.2).

The round the year average values of similarity index (Sorensen"s) of cyanobacteria were found highest between Stn.1 & 3 and lowest between Stn.2 & 3. During summer season the highest similarity index was calculated between Stn.1 & 2 and Stn.1 & 3 while lowest during monsoon season between Stn.2 & 3. In the month of June the highest similarity index value was calculated between Stn.1 & 2 and lowest also between Stn.1 & 2 in the month of May (Table 5.2 C & D, Figure 5.3).

		<b>Sum of Squares</b>	df	<b>Mean Square</b>	F	Sig.
Light (lux)	<b>Between Groups</b>	23734.1	$\overline{2}$	11867.056	0.174	0.841
	Within Groups	2249702	33	68172.795		
	Total	2273436	35			
	<b>Between Groups</b>	407.357	$\overline{2}$	203.679	11.707	0.000
Temp (degree C)	Within Groups	574.135	33	17.398		
	Total	981.492	35			
pH (units)	<b>Between Groups</b>	2.015	$\overline{2}$	1.008	7.401	0.002
	Within Groups	4.493	33	0.136		
	Total	6.508	35			
	<b>Between Groups</b>	91448	$\overline{2}$	45723.99	9.409	0.001
$EC$ (mg/L)	Within Groups	160371	33	4859.724		
	Total	251819	35			
TDS (mg/L)	<b>Between Groups</b>	57883.5	$\overline{2}$	28941.726	15.112	0.000
	Within Groups	63200.6	33	1915.171		
	Total	121084	35			
Turbidity (NTU)	<b>Between Groups</b>	106.211	$\overline{2}$	53.105	4.725	0.016
	Within Groups	370.879	33	11.239		
	Total	477.09	35			
TA (mg/L)	<b>Between Groups</b>	8877.26	$\overline{2}$	4438.632	12.455	0.000
	Within Groups	11760.2	33	356.37		
	Total	20637.5	35			
TH(mg/L)	<b>Between Groups</b>	15880.2	$\overline{2}$	7940.078	18.945	0.000
	Within Groups	13831	33	419.121		
	Total	29711.2	35			
CaH (mg/L)	<b>Between Groups</b>	4222.16	2	2111.081	13.902	0.000
	Within Groups	5011.33	33	151.858		
	Total	9233.49	35			
$MgH$ (mg/L)	<b>Between Groups</b>	3462.38	$\mathbf{2}$	1731.189	13.049	0.000
	Within Groups	4377.98	33	132.666		
	Total	7840.36	35			

**Table 5.3: One Way ANOVA Test by Season**



(level of significance  $p < 0.05$ )

		<b>Sum of Squares</b>	df	<b>Mean Square</b>	F	Sig.
Light (lux)	<b>Between Groups</b>	1342529	11	122048.06	3.147	0.009
	Within Groups	930908	24	38787.819		
	Total	2273436	35			
Temp (degree C)	<b>Between Groups</b>	473.039	11	43.004	2.03	0.071
	Within Groups	508.453	24	21.186		
	Total	981.492	35			
pH (units)	<b>Between Groups</b>	1.968	11	0.179	0.946	0.517
	Within Groups	4.54	24	0.189		
	Total	6.508	35			
	<b>Between Groups</b>	134307	11	12209.697	2.494	0.030
$EC$ (mg/L)	Within Groups	117512	24	4896.342		
	Total	251819	35			
$TDS$ (mg/L)	<b>Between Groups</b>	47330.4	11	4302.761	1.4	0.236
	Within Groups	73753.7	24	3073.071		
	Total	121084	35			
Turbidity (NTU)	<b>Between Groups</b>	240.71	11	21.883	2.222	0.049
	Within Groups	236.38	24	9.849		
	Total	477.09	35			
	<b>Between Groups</b>	5655.91	11	514.174	0.824	0.619
$TA$ (mg/L)	Within Groups	14981.6	24	624.232		
	Total	20637.5	35			
TH(mg/L)	<b>Between Groups</b>	9306.14	11	846.013	0.995	0.478
	Within Groups	20405	24	850.209		
	Total	29711.2	35			
$CaH$ (mg/L)	<b>Between Groups</b>	2942.93	11	267.539	1.021	0.459
	Within Groups	6290.57	24	262.107		
	Total	9233.49	35			
$MgH$ (mg/L)	<b>Between Groups</b>	2677.38	11	243.398	1.131	0.381
	Within Groups	5162.97	24	215.124		
	Total	7840.36	35			

**Table 5.4: One Way ANOVA Test by Months**



(level of significance  $p < 0.05$ )

		<b>Sum of Squares</b>	df	<b>Mean Square</b>	F	Sig.
Light (lux)	<b>Between Groups</b>	23734.1	$\overline{2}$	11867.056	0.174	0.841
	Within Groups	2249702	33	68172.795		
	Total	2273436	35			
	<b>Between Groups</b>	407.357	$\overline{2}$	203.679	11.707	0.000
Temp (degree C)	Within Groups	574.135	33	17.398		
	Total	981.492	35			
pH (units)	<b>Between Groups</b>	2.015	$\overline{2}$	1.008	7.401	0.002
	Within Groups	4.493	33	0.136		
	Total	6.508	35			
	<b>Between Groups</b>	91448	$\overline{2}$	45723.99	9.409	0.001
$EC$ (mg/L)	Within Groups	160371	33	4859.724		
	Total	251819	35			
TDS (mg/L)	<b>Between Groups</b>	57883.5	$\overline{2}$	28941.726	15.112	0.000
	Within Groups	63200.6	33	1915.171		
	Total	121084	35			
Turbidity (NTU)	<b>Between Groups</b>	106.211	$\overline{2}$	53.105	4.725	0.016
	Within Groups	370.879	33	11.239		
	Total	477.09	35			
TA (mg/L)	<b>Between Groups</b>	8877.26	$\overline{2}$	4438.632	12.455	0.000
	Within Groups	11760.2	33	356.37		
	Total	20637.5	35			
TH(mg/L)	<b>Between Groups</b>	15880.2	$\overline{2}$	7940.078	18.945	0.000
	Within Groups	13831	33	419.121		
	Total	29711.2	35			
CaH (mg/L)	<b>Between Groups</b>	4222.16	2	2111.081	13.902	0.000
	Within Groups	5011.33	33	151.858		
	Total	9233.49	35			
$MgH$ (mg/L)	<b>Between Groups</b>	3462.38	$\mathbf{2}$	1731.189	13.049	0.000
	Within Groups	4377.98	33	132.666		
	Total	7840.36	35			

**Table 5.5: One Way ANOVA Test by Stations**



(level of significance  $p < 0.05$ )



# **Table 5.6: Correlation matrix between various physico-chemical parameters and the density of cyanobacteria**



# **Table 5.7: Multiple Linear Regression Analysis of physico-chemical parameters of water on the population of Cyanobacteria Coefficients<sup>a</sup>**

a. Dependent Variable: Density (no./mL)

# **CHAPTER 6 RESULTS AND DISCUSSION**

Microscopic free-floating algae in water, better known as phytoplanktons, rapidly react to any change in temperature due to their short life span and so can be used for the assessment of aquatic ecosystems. Cyanobacteria are oxygen evolving prokaryotic blue green algae which form an important component of these phytoplanktons. These algae have a wide range of organization and diverse habitats. Besides being the source of food for zooplanktons, they play an important role in oxygenic photosynthesis and carbon cycling. Being at the first trophic level, they are the more susceptible to any change in water. Phytoplanktons are exposed to the discharge of elevated water temperature in the vicinity of thermal power plants (Cairns, 1969; Hill, 1972; Trivedy, 1989; Smith, 1992; Lata Dora *et al*., 2010). A variety of negative effects are imposed on the aquatic ecosystem because of elevated water temperatures and thermal effluents that can be fatal to aquatic organisms including cyanobacteria (Poornima *et al*., 2005; Kaushal *et al*., 2010).

The study is conducted with the aim to analyze the physico-chemical properties of river water used for the cooling purpose of a thermal power plant and to study the diversity of cyanobacteria so as to assess the cyanobacterial community structure under the influence of thermal discharge using some diversity indices.

Three sampling stations were selected in Kota city during July 2017 to June 2018 to study the physical and chemical characteristics of fresh water Chambal river and its cyanobacterial diversity. Area at upstream of the river near Akelgarh water treatment plant was selected as Station 1 (Stn.1) that represents the Control station during the study period. Second sampling site Station 2 (Stn.2) was selected as near as possible (in the radius of 500 m) to the discharge site of Kota Thermal Power Station and the third site Station 3 (Stn.3) was selected at downstream of the river at Chhoti Samadh Shiv Temple.

For the determination of the physical and chemical properties of the water, the water samples were collected in the morning time from the three sampling sites in one litre capacity of clean polyethylene bottles during second week of every month from July 2017 to June 2018.

During the same period the surface water samples were collected for the study of diversity of cyanobacteria. The samples collected for cyanobacterial study were studied within 48 hrs and for further detailed examination the samples were also fixed and preserved in Lugol"s solution. With the help of available standard keys and monographs, identification of Cyanobacteria was done. Diversity indices were used to calculate the diversity for the assessment of the community structure of cyanobacteria.

The data obtained during the study is analyzed and tested statistically. The average values are calculated for the data of physical and chemical properties of water. Results were obtained separately for twelve months and the data is analyzed season-wise as well as month-wise to study the overall effect of various parameters on the density of cyanobacteria. Total thirty six observations were taken at three stations for twelve months and three seasons.

Coefficient of correlation is used to find the correlation between different properties of water and population of cyanobacteria. One-way Analysis of Variance (ANOVA) is performed to test the difference between the mean values of various physico-chemical parameters of water and density of cyanobacteria statistically ( $p<$ 0.05 at confidence level of 95%). Multiple Linear Regression is used for modeling  $(R<sup>2</sup> < 0.50)$  and to examine the effect of determined physical and chemical properties of water on the density and diversity of cyanobacteria.

The concentration and nature of different types of substances dissolved in water determine its chemical properties which in turn can determine the kind of algae present in it (Valk, 2012).

In the past 15 years almost the average global temperature has raised by about 0.76 ºC (Moss, 2010). Possible effects of changes in global temperature on freshwater can be studied through study of community structure of microbes including cyanobacteria (Sarma, 2013).

Changes in cyanobacterial diversity may have diverse effects on the food web (Dodds and Whiles, 2011). Any change in algal community structure has consequences on food chain and ecosystem dynamics. Ecosystem changes can be assessed by studying any change in a community (Lata Dora *et al*., 2010).

If any kind of change occurs in terms of growth form, pigments, biochemistry, biomass, primary productivity of primary producers, this could be used as an indication of long term effects or predictable changes that may occur or are occurring in the ecosystem. Diversity needs conservation for the different roles played by them in the ecosystem (Odum and Barett, 2010).

#### **Light and temperature**

Light intensity and temperature are the important abiotic factors for the aquatic life that affect growth of algae (Aery, 2010). Light is required for photosynthesis by all flora. High light intensity at the water surface may inhibit the process of photosynthesis in algae and plants (Welch and Lindell, 2004). Aquatic organisms have an optimum temperature for growth and limits of thermal tolerance. The changes in temperature are considered as the major environmental factor that influences the growth of the algae (Palmer, 1980).

The light intensity was observed maximum during summer season in the month of June with 1231 lux. The lowest light intensity of 335 lux was observed during monsoon season in the month of August which is may be due to cloud cover. The surface water temperature of freshwater Chambal river varied from 20.1°C to 42.2°C at three different sampling stations. Temperature differences were noticeable throughout the year between all the three stations.

The higher temperature values were observed at Stn.2 throughout the study period as compared to Stn.1 and 3. The maximum surface water temperature of 42.2ºC was recorded at Stn.2 in the month of May during the summer season. Chuang *et al*. (2009) also observed the highest temperature (41 ºC) at the outfall region near a thermal power plant in their study. The minimum temperature was measured with 20.1ºC at Stn.3 in the month of January during the winter season.

Present study is showing shifting of river water surface temperature towards higher range due to thermal activity in this part of river. On the contrary, in the study made by Gupta *et al*. (2011) and Bhatnagar and Bhardwaj (2013a) the sample collection site was near Kota barrage while in the present study the samples were collected in the vicinity of discharge point (as near as possible) of the water from KTPS. It clearly indicates that the higher water surface temperature in this area is due to thermal discharge from KTPS.

Kota thermal power plant uses once-through cooling system by passing a large volume of water through the condenser tubes which gets heated in the process and is then discharged back into the source of water. As suggested by Coutant and Brook (1970, 1973), this heated water from the thermal discharge is causing warming of the river water.

Many workers have observed an elevated temperature at the outfall region in the range of 5-10°C higher than the surrounding water and the areas distantly situated from it. A rise in temperature is observed in the present study conducted at the site closer to the discharge of warm cooling water from a thermal power station. The water temperature at Stn.2 (near the discharge point of thermal effluents) was found 4° to 10°C higher than at Stn.1 and 3 in every season throughout the year in the present study conducted on river freshwater. This is similar with the findings of Martinez-Arroyo *et al*., 2000; Poornima *et al*., 2005; Chuang *et al.,* 2009; Lo *et al.*, 2016. They have noticed the similar rise in temperature in their studies on coastal sea waters.

Most of the studies on thermal power plants have been conducted in reference to marine waters worldwide. Some of the studies suggest that there can be

a 10°C rise in temperature in the ambient water temperature when cooling water is discharged back to the source of water (Snoeijs and Prentice, 1989; Langford, 2001). During the present study almost a  $10^{\circ}$ C rise in water temperature at Stn.2 was observed as compared to other two stations. This rise in temperature is in accordance with the findings of Kailasam and Shivakami (2004), Poornima *et al*. (2006), Chuang *et al*. (2009) and Muhammad Adlan *et al*. (2012) in their studies on coastal marine waters.

Even the surface water temperature observed in Chambal river is the highest amongst till now the researches that have been performed on this river. As most of the part of Rajasthan state is a desert and it also has high light intensities and high range of annual temperature. The global warming is continuously increasing and is adding to rise in temperature. Global climate change can alter the species composition of algae in aquatic systems (Valk, 2012).

The station 2 (Stn.2) is the site nearest to the outfall region of thermal power plant. Hence, the higher surface water temperature values at the discharge site are due to discharge of thermal effluents from thermal power station at Stn.2 as the effluents contain heated water with higher temperature than the natural water (Langford, 2001, Muhammad Adlan *et al*., 2012).

In the present study, the population of Cyanobacteria is found to be dominant during the summer season and the maximum number of cyanobacteria is observed at Stn.2 in summer season when maximum temperature  $(42.2^{\circ}C)$  is measured. This is similar to the findings made by Ilus and Keskitalo (2008) that states that rise in temperature plays an important role in increasing primary productivity and biomass of phytoplanktons near the discharge area. They also observed that algal species composition was dominated by cyanobacteria at the discharge site under the effect of elevated temperature during summer season. Welch and Lindell (1992) have opinioned that cyanobacteria proliferate at high temperature with low light intensity. Kosten *et al*. (2012) also concluded that the number of cyanobacteria increases with the increase in temperature that favours the present results. The present investigation indicates a change in diversity of cyanoabacteria associated with change in temperature.

The high temperature increases the rate of photosynthesis and growth in cyanobacteria (Visser *et al*., 2016). The higher water temperature due to thermal discharge from the thermal power plant is increasing the photosynthetic rates in cyanobacteria thereby increasing their growth at Stn.2. The higher photosynthetic rates are may be the reason for the proliferation of cyanobacteria and higher diversity at Stn.2 that experiences higher water temperature round the year of study period.

The high temperature lowers the density of water (Lürling *et al*., 2013). The gas vesicles present in some taxa of cyanobacteria, provide them buoyancy and make them capable of vertical movement in water (Kim *et al*., 2019). In the present study, the water at Stn.2 is a little stagnant because of the dam. It possesses higher temperature than other two stations and is responsible for lower water density at Stn.2. It can be interpreted that may be some of the cyanobacteria move vertically making more number of cyanobacteria float on the surface of water hence enriching

the water with cyanobacteria at Stn.2. The low water density also results in sinking of other groups of algae. The higher water temperature at Stn.2 is may be responsible for making the water rich in cyanobacteria there.

Every living organism has an optimum range of temperature for survival. Cyanobacteria show dominance in the algal community in waters which receive heated waters at temperature above 35°C (Cole, 1969; Bush, 1974; Welch and Lindell, 1992). Green algae can be replaced by blue green algae or cyanobacteria with increase in temperature as suggested by Hill (1972). Previous studies prove that Cyanobacteria grow best at temperature of 30°C to 40°C (Lata Dora *et al*., 2010) and can tolerate extremes of temperature even up to 74 ºC (Langford, 2001).This was also proved in the present study that population of cyanobacteria increases when the water temperature rises due to heated water from thermal discharge and reaches its maximum at Stn.2 thereby increasing the diversity of cyanobacteria at Stn.2.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

In the present study, no significant difference is observed between the mean values of light intensity at three stations statistically season wise since the p-value of significance of ANOVA is 0.841. This is because the light was in similar range at all the three stations in every season. Difference in mean value of temperature between the groups and within the groups is significant statistically season wise as indicated by p-value (0.000). Different seasons possess a definite range of temperature which is the reason for this significant difference (Table 5.3).

#### **One-way ANOVA month wise**

The intensity of light is found to be significantly different in all the twelve months of sampling as revealed by p-value which is 0.009. The light intensity is observed in the higher range in summers at all the three stations and diffused during winters and monsoons. Though the temperature was measured different during each month but it does not show significant difference between its mean values every month statistically (Table 5.4).

#### **One-way ANOVA station wise**

Difference of light intensity is statistically not similar across the stations as the p-value is 0.841. A very significant difference is observed in the mean value of temperature between the groups and within the groups in temperature at all the three stations studied round the year. The obtained p-value of significance of ANOVA with temperature is 0.000. This indicates that thermal discharge from KTPS comprises of heater water which is rising the surface water temperature of river water possibly every year gradually (Table 5.5).

#### **Correlation**

Light intensity has a negative correlation with turbidity, magnesium hardness, nitrate, sulphate, BOD and COD. The density of cyanobacteria is found slightly positively correlated with light intensity  $(r = 0.19)$  (Table 5.6). A strong positive correlation is found between surface water temperature of river freshwater and the population of cyanobacteria in the present research study  $(r = 0.70)$  (Table 5.6). This indicates that high temperature is favouring the growth of cyanobacteria. A positive correlation was also reported by Feng *et al*. (2011) between temperature and the number of cyanobacteria. Temperature is observed to be negatively

correlated with EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, fluoride, BOD and COD.

#### **Multiple linear regression**

It is observed from the multiple linear regression analysis that light intensity has no significant effect on density of cyanobacteria round the year at all the three stations ( $p = 0.331$ ). The analysis shows that there is effect of temperature on the density of cyanobacteria which is significant  $(p=0.011)$ . It is clear from the Table 5.7 that temperature has positive impact on cyanobacteria. Hence it can be stated that with one degree rise in temperature there is increase in the number of cyanobacteria almost 500 in number. With the present study on freshwater, it can be interpreted that with the rise in water temperature due to thermal discharge the number of cyanobacteria increases.

#### **pH**

It is the hydrogen ion concentration of water. pH range of 7.5 to 9.0 units is best for the growth of cyanobacteria. They thrive well in this range of pH (Reynolds and Walsby, 1975; Palmer, 1980).

pH range between 1-7 indicates acidity and range of 7.1-14 indicates alkalinity of water. The pH values of the Chambal river in the present study ranged from 6.7 to 8.5 units at all the stations from July 2017 to June 2018 indicating that the river freshwater is slightly alkaline. The average pH of water at Stn.1is observed higher while it was low at Stn.3 round the year. The highest pH was measured in the month of April at Stn.3 and minimum in the month of May at Stn.3 during summer season. A similar pattern of pH was reported by Muhammad Adlan *et al*. (2012) in their study on sea water influenced with thermal discharge near a power station. The results for the present study are in accordance with them.

The pH range during the study was in consonant with the results obtained by Gupta *et al*. (2011), Bhatnagar and Bhardwaj (2013a), Gaur *et al*. (2014) and Chouhan *et al*. (2018) in their studies on Chambal river. There has not been a large difference in the pH values obtained during the study period and previous studies. It can be said that there has not been a drastic effect of thermal discharge on the pH of river water in these past years.

The pH was recorded highest during summer season as was the temperature. Temperature and pH are found to be directly proportional to each other throughout the study period. Cyanobacteria are found to be dominant at high pH values (Reynolds and Walsby, 1975). The pH during the summer season was found to be at higher side than in monsoon and winter seasons. Cyanobacteria also showed dominance during the summer season. pH at Stn.2 was observed higher as compared to Stn.3 and so was the density of cyanobacteria.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

A significant difference is observed between the mean values of pH of water at three stations season wise statistically in the present study, the p-value of significance of ANOVA being 0.002. It can be said that the effluents from the thermal power station are might causing a slight change in the pH of water year over year (Table 5.3).

#### **One-way ANOVA month wise**

Statistically no significant difference is observed in the mean values of pH month wise (p-value= 0.517). This is may be due to slight changes occurring in the pH of water every month which might exert a cumulative effect with the passing of years (Table 5.4).

#### **One-way ANOVA station wise**

A very significant difference is observed in the mean value of pH between all the three stations studied round the year as indicated by p-value of significance (0.002). This is clear as the river water at Stn.1 is observed with higher pH than the other two stations (Table 5.5).

#### **Correlation**

pH is observed to be in negative correlation with EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, sulphate, fluoride, BOD and COD. But it was found positively correlated with light intensity, temperature and DO. A positive correlation was observed between pH and number of cyanobacteria (r= 0.30) present in water (Table 5.6).

#### **Multiple linear regression**

Statistically the pH of water is not affecting the number of cyanobacteria as no significant effect of pH is observed on the density of cyanobacteria from the multiple linear regression analysis at all the three stations during the study period (p  $= 0.307$ ) (Table 5.7).

#### **Electrical conductivity**

It is due to the positive and negative ions of acids, bases and salts present in water (Maiti, 2004). During the study period, electrical conductivity ranged between 263.6  $\mu$ S/ cm to 648.3  $\mu$ S/ cm round the year.

The average values of EC were found similar at all the three stations in monsoon season. The minimum EC (263.7 mg/L) was measured at Stn.1 during the monsoon season in the month of August. The electrical conductivity was found lower at Stn.1 than other two stations as this is the minimally disturbed site with no addition of any kind of waste and hence lesser number of ions are present responsible for a low EC at this station.

The electrical conductivity is observed maximum at Stn.3 i.e. 648.3  $\mu$ S/ cm in the month of May during summer season. Electrical conductivity measured in the present study is two times of EC that was reported by Gupta *et al*. (2011) on the same location in summer season. The doubling of electrical conductivity in these years is may be because of increase in river pollution by dumping waste in river water year by year. As the Stn.3 is the site with maximum human interference, the higher number of ions is liberated from the decomposition of increased inorganic substances and wastes dumped in downstream of river at Stn.3. The higher amount of dissolved salts is may be the cause for higher electrical conductivity at Stn.3.

During the summer season the minimum average value of EC was measured at Stn.2 (385.0 µS/ cm) which is found lower than the other two stations studied. It can be reasoned that the lower conductivity at Stn.2 is may be due to absence of any ions liberated from thermal discharge but it is possibly only because of suspended fly ash from air in water or runoff water from the banks of the river.

According to Maiti (2004), with the higher water temperature the EC of water decreases. In the present study EC was observed lower at higher temperature at Stn.2 during the summer season in comparison to other two stations. The population of cyanobacteria is also observed greater at lower EC. It represents that lower electrical conductivity favours the growth of cyanobacteria at Stn.2.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

The mean values of electrical conductivity in water are found to be significantly different statistically season wise with 0.001 as the p-value of significance. This is also clear from the observations taken during the study period as EC is observed highest in summer season (Table 5.3).

#### **One-way ANOVA month wise**

In the month wise statistical ANOVA analysis, a significant difference is observed in the mean values of EC (p-value= 0.030) which is may be due to addition of different amount of ions on monthly basis at three stations (Table 5.4).

#### **One-way ANOVA station wise**

As indicated by the p-value of significance (0.001) a very significant difference is observed in the mean values of EC round the year at all the three stations studied. This is supported by the fact that the river water at Stn.3 is observed with higher EC than the other two stations (Table 5.5).

#### **Correlation**

Correlation matrix suggests that EC has a positive correlation with TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, sulphate, fluoride, BOD and COD. But a negative correlation is found between EC and temperature, pH and DO. It is also found to be in a slightly positive correlation with the number of cyanobacteria present in water  $(r= 0.22)$ (Table 5.6).

#### **Multiple linear regression**

Multiple linear regression analysis indicates that there is no significant effect of EC is observed on the density of cyanobacteria during the study period at all the three stations ( $p = 0.122$ ). Statistically the electrical conductance of water is not affecting the number of cyanobacteria (Table 5.7).

#### **Total dissolved salts (TDS)**

Total dissolved salts are the dissolved carbonates, bicarbonates, chlorides, sulphates, phosphates and nitrates of calcium, magnesium, sodium, potassium, iron, magnesium etc. in water (Aery, 2010).

TDS of freshwater river ranged between 191.7 mg/L to 455.0 mg/L in the study period. The lower values of TDS are obtained at Stn.1as compared to other two stations round the year. In summer season, the maximum TDS was found at Stn.3 in the month of May and minimum in monsoon season at Stn.1 in the August month.

The average value of TDS in summer season at Stn.2 is very close to that observed by Bhatnagar and Bhardwaj (2013a) at a point near Kota Barrage. This reveals that in this span of time there has not been much significant change in TDS of water at Stn.2. This indicates that thermal discharge does not contain significant amount of dissolved salts of calcium, magnesium, sodium, potassium, iron or magnesium in water.

A very low TDS value was reported by Gupta *et al*. (2011) at the same point in summers during their study. Higher amount of total dissolved salts in the present study is may be due to increased human interference at Stn.3 as the present study has been conducted after a gap of six years of that performed by Gupta *et al*. (2011). This indicates an increase in addition of dissolved salts at Stn.3 from sewage, inorganic substances and wastes dumped at Stn.3 in downstream of the river in these past years.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

Statistically a very significant difference is observed in the mean values of TDS in water in different seasons as indicated by the p-value of significance which is 0.000 as the TDS values were observed higher in summer season (Table 5.3).

#### **One-way ANOVA month wise**

Statistically no significant difference is observed in the mean values of TDS in month wise ANOVA analysis (p-value=  $0.236$ ) which is may be due to addition of different amount of dissolved salts on monthly basis at the selected sites of study (Table 5.4).
## **One-way ANOVA station wise**

The p-value of significance (0.000) in station wise ANOVA analysis indicates a very significant difference in the mean values of TDS round the year study. This is clear from the highest values of TDS at Stn.3 and lowest at Stn.1 (Table 5.5).

# **Correlation**

A positive correlation is observed between TDS and light intensity, EC, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, sulphate, fluoride, BOD and COD while a negative correlation is observed between TDS and temperature, pH and DO. TDS and the density of Cyanobacteria are not found to be correlated with other (Table 5.6). It can be interpreted that population of cyanobacteria is not dependent on total solids dissolved in water.

#### **Multiple linear regression**

Multiple linear regression analysis shows no significant effect of EC is on the density of cyanobacteria ( $p = 0.122$ ) during the study period at all the three stations (Table 5.7).

# **Turbidity**

It is the cloudiness of water and is caused by suspended materials, silt, clay, phytoplanktons, zooplanktons, colloidal matter and suspended fine particles of organic and inorganic matter (Aery, 2010; Pal and Choudhury, 2014).

The turbidity of river water varied between minimum of 1.7 NTU units to maximum of 17.5 NTU units during the study period. Monsoon season is observed with high turbidity at all the stations which is possibly due to run off waters during rains when land surface is washed off into the river.

Stn.1was observed with minimum turbidity while the most turbid water was observed at Stn.3. The water was found to be less turbid at Stn.1 due to less human activities. Turbidity increased at sites distant from the control station. The reason for this high turbidity at Stn.3 is due to presence of more concentration of total dissolved salts, colloidal dispersion and suspended particles in the river water. Anthropogenic activities are also responsible for higher turbidity at Stn.3. A pattern of increased turbidity of river water from Stn.1 to Stn.3 i.e. from upstream to downstream was noticed in the present study.

Annual average value of turbidity is observed higher at Stn.2 than that at Stn.1. The presence of different forms of phytoplanktonic cyanobacteria and their high density is responsible for the turbidity at Stn.2. The suspended particles of fly ash in air from the thermal power plant may also be the reason for this turbidity.

#### **Statistical analysis**

## **One-way ANOVA season wise**

A significant difference in the mean values of turbidity of water is observed statistically season wise (p-value= 0.016). As during the monsoon season the turbidity of river water increases there is observed detectable difference in the turbidity of water (Table 5.3).

#### **One-way ANOVA month wise**

A significant difference is observed statistically in the mean values of turbidity in the month wise ANOVA analysis (p-value= 0.049). A difference is observed in the turbidity of water every month due to changes in presence of total dissolved salts, colloidal suspension, suspended particles and planktonic cyanobacteria (Table 5.4).

#### **One-way ANOVA station wise**

A statistical significant difference is observed in the mean values of turbidity at all the three stations studied indicated by the p-value of significance (0.016). Highest turbidity was observed at Stn.3 round the year during the study period than the other two stations (Table 5.5).

#### **Correlation**

Turbidity is found to have a positive correlation with EC, TDS, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, sulphate, fluoride, BOD and COD. It is found to be negatively correlated with light intensity, temperature, pH and DO. Correlation matrix indicates a slight positive relation between turbidity and the total number of cyanobacteria  $(r=0.01)$  (Table 5.6).

## **Multiple linear regression**

The turbidity of water is not affecting the number of cyanobacteria statistically as indicated by Multiple linear regression analysis. There is no significant effect of turbidity is observed on the density of cyanobacteria ( $p = 0.297$ ). (Table 5.7).

## **Total Alkalinity (TA)**

Alkalinity is an indication of concentration of carbonate, bicarbonate and hydroxide content of water (APHA, 1999). It is useful in regulating the pH of a water body (Maiti, 2004).

The TA at all the three stations varied from 96.7 mg/L to 203.3 mg/L during the present study. TA was observed maximum at Stn.3 in May month of summer season while minimum in November month of winter season at Stn.1. Low values of total alkalinity were observed at Stn.1 in winter season among three stations. But TA at Stn.1 was observed higher than at Stn.2. The high values of TA at Stn.1 are may be due to dissolution of carbonate minerals from rocks and from atmospheric carbon dioxide dissolved into the water.

Bhatnagar and Bhardwaj (2013a) calculated the average value of total alkalinity with 119 mg/L in Chambal river at Kota barrage in summers. Similar results were also obtained in the present study. In this reference it can be said that in a gap of these years there is not much effect of thermal discharge on total alkalinity of river water.

Stn.3 was observed with highest values of total alkalinity amongst all stations studied in all the three seasons. This is may be due to higher concentration of carbonates and bicarbonates as a result of addition of industrial waste, domestic sewage and residues of food in water.

pH does not measure the total alkalinity (Maiti, 2004) at Stn.3. It is also observed in correlation matrix that pH and TA were not found to be positively correlated (Table 5.6).

#### **Statistical analysis**

#### **One-way ANOVA season wise**

Statistically it was revealed that there is observed a very significant difference in the mean values of TA in all the seasons studied with the p-value of significance as 0.000. Higher values of TA are observed during the summer season (Table 5.3).

#### **One-way ANOVA month wise**

There is not observed a statistical significant difference in the mean values of TA every month (p-value= 0.619) as the changes in the total alkalinity of water are not detectable on monthly basis (Table 5.4).

## **One-way ANOVA station wise**

A very statistical significant difference is observed in the mean values of TA at all the three stations studied as indicated by the p-value of significance being 0.000. This is because the highest total alkalinity was observed at Stn.3 than the other two stations round the year during the study period (Table 5.5).

## **Correlation**

TA is found to be positively correlated with light intensity, EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, sulphate, fluoride, BOD and COD. A negative correlation is observed between TA and temperature, pH and DO. No correlation is measured between total alkalinity and the number of cyanobacteria (Table 5.6).

## **Multiple linear regression**

The analysis indicates no significant effect of total alkalinity on the density of cyanobacteria ( $p = 0.223$ ) (Table 5.7). It can be said that total alkalinity of water does not affect the population of cyanobacteria.

# **Hardness**

The hardness of water is mainly contributed by calcium and magnesium ions. The minimum value of total hardness was measured at Stn.1 in the month of November in winter season. A range of 88 mg/L to 414 mg/L was noted by Yadav *et al*. (2014) and 122 mg/L to 203 mg/L by Gaur *et al*. (2014) during their study in Chambal river. Similar results were obtained for the present work. The total hardness ranged from 96.7- 200.0 mg/L during the study period.

Hardness of water shows a pattern of gradual rise from Stn.1 to Stn.3. The river water at Stn.1 was found to be softer than Stn.2 & 3. The total hardness of water was observed greater at Stn.3 making it harder than the water at other two stations. The reason of more hardness at Stn.3 is may be due to more anthropogenic activities and continuous disposal of waste & sewage from the nearby localities. The calcium and magnesium hardness were also observed higher at Stn.3 and lower at Stn.1 round the year. They were observed low at Stn.2 during the summer season. The possible reason for the hardness of water at Stn.2 is due to the leaching of calcium and magnesium minerals from rocks and their dissolution into the river water.

According to Palmer (1980) the water is found rich in algae at lower hardness. This is also reported in the present study as a higher density and diversity of cyanobacteria is observed at Stn.2 with lower hardness during the summer season.

## **Statistical analysis**

## **One-way ANOVA season wise**

A very significant difference is observed statistically in the mean values of total hardness, calcium hardness and calcium hardness in all the seasons studied. The p-value of significance for the hardness is 0.000. Higher values of hardness are observed during the summer season as compared to other seasons studied (Table 5.3).

## **One-way ANOVA month wise**

In month wise statistical analysis no statistical significant difference is observed in the mean values of total hardness (p-value= 0.475), calcium hardness (pvalue= 0.459) and magnesium hardness (p-value= 0.381) of water. This is may be because the changes in the hardness of water are not majorly detectable every month (Table 5.4).

#### **One-way ANOVA station wise**

As indicated by the p-value of significance being 0.000, a very statistical significant difference is observed in the mean values of total hardness, calcium hardness and magnesium hardness at all the three stations studied. The high concentration of hardness at Stn.3 and low at Stn.1 is the possible reason for the significant differences during the study period (Table 5.5).

# **Correlation**

Total hardness, calcium hardness and magnesium hardness are found to be positively correlated with EC, TDS, turbidity, total alkalinity, chloride, nitrate, sulphate, fluoride, BOD and COD. Total hardness and calcium hardness are observed to be in a positive relation with light intensity while magnesium hardness is found to be negatively related with light intensity. Hardness shows a negative correlation with temperature, pH and DO. The hardness and the number of cyanobacteria are observed in a negative correlation ( $r = -0.20$  for TH,  $r = -0.13$  for CaH and  $r = -0.24$  for MgH) (Table 5.6).

## **Multiple linear regression**

The regression analysis indicates a significant effect of hardness on the population of cyanobacteria. Total hardness of water shows a significant effect on the number of cyanobacteria ( $p = 0.030$ ) but indicates a negative impact on their population. Statistically, it can be stated that with increase in one mg/L of total hardness there is a decline in the number of cyanobacteria by 1304 in number. Similarly, calcium hardness and magnesium hardness also have a statistical significant effect on the number of cyanobacteria present in water and possess a positive impact on their population ( $p= 0.031$  and  $p= 0.021$  respectively). This analysis suggests that with increase in one mg/L of calcium hardness, the number of cyanobacteria increases by 1378 and with increase in one mg/L of magnesium hardness, their number increases by 1267 approximately (Table 5.7).

# **Chloride**

Chloride is the anionic form of chlorine. Chlorine is used in the condenser tubes as an antifouling agent for power generating plants because of its biocidal properties (Adams, 1969; Cairns, 1975). It may be fatal to phytoplanktons and inhibit metabolism in them (Langford, 2001).

Under the effect of chlorine, a reduction in growth and primary productivity of diatoms has been reported by Vinitha *et al*. (2010). Brooks and Liptak (1979) and Poornima *et al.* (2006) have suggested that phytoplankton productivity decreases with increase in chlorination.

The chloride content of the freshwater river was measured in the range of 20.0 – 88.7 mg/L round the year during the study period. The highest content was observed at Stn.3 in the month of May during summer season and lowest at Stn.1during monsoon season in August month. A major difference was observed in chloride concentration values at Stn.2 & 3 in the present study as that was reported by Gupta *et al*. (2011). In the present study, Stn.3 was observed with high chloride content round the year due to industrial and domestic pollution.

The chloride content at Stn.2 was found higher than the control station Stn.1. In a study Bhatnagar and Bhardwaj (2013a) reported low values of chloride content in winter season at Kota Barrage. Gaur *et al*. (2014) also calculated low chloride content in Chambal river water in their study. This is in contradiction to the results obtained for Stn.2 in the present study as higher chloride concentration was observed at Stn.2 particularly in summer season. As the sample collection site Stn.2 in the

present study is very near to the thermal discharge site of KTPS and there is no addition of domestic and industrial sewage at this site, the possible reason behind the high chloride concentration at Stn.2 is probably due to use and discharge of chlorine used as an antifouling agent in the thermal power plant. It is observed that chloride content of river water has increased in past few years as the previous studies on Chambal river suggest. This increase in chloride concentration in this span of time indicates the use of chlorine by thermal power plant and therefore its discharge along with thermal effluents.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

A very significant difference is observed statistically in the mean values of chlorides in water in different seasons (p-value  $= 0.000$ ). The chloride content was observed higher in summer season than the other two seasons studied (Table 5.3).

#### **One-way ANOVA month wise**

A statistically significant difference is not observed in the mean values of chlorides in month wise ANOVA analysis (p-value= 0.731). This is possibly due to addition of different amount of chlorides at the selected sites of study. This is also may be due to different concentration of chlorine used and discharged by thermal power plant during its operation every month (Table 5.4).

#### **One-way ANOVA station wise**

A very significant difference is observed in the mean values of chlorides statistically at all the three stations indicated by p-value of significance (0.000) during the study period. The concentration of chlorides is observed different at the three stations round the year (Table 5.5).

# **Correlation**

The chloride content of water is observed to be positively correlated with light intensity, EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, nitrate, sulphate, fluoride, BOD and COD while it has a negative correlation is observed between temperature, pH and DO. No correlation is observed between chloride content and density of cyanobacteria in correlation matrix (Table 5.6). This is contradictory to the results of Ganai and Parveen (2014) who observed a positive correlation between chloride and density of cyanobacteria. But they worked on marine waters which already has different chemical composition in comparison to freshwater. The residual chlorine may affect the chlorophyll concentration, abundance and productivity of phytoplanktons irrespective of the elevated temperatures (Poornima *et al*., 2005; Chuang *et al*., 2009). The present work suggests that chloride content has not much effect on the population of cyanobacteria.

# **Multiple linear regression**

There is observed no significant effect of chorides on the density of cyanobacteria ( $p = 0.204$ ) as indicated by Multiple linear regression analysis. (Table 5.7).

#### **Nitrate**

It is the oxidized form of nitrogen. The highest concentration of nitrate was observed at Stn.3 round the year during the study period making the water nutrient rich at downstream. This is evident from the observation of more bloom forming cyanobacteria at Stn.3. Yadav *et al*. (2014) reported nitrate concentration in the range of 0.258 mg/L to 3.48 mg/L in their study on Chambal river which are lower than the maximum value of 8.0 mg/L value calculated during this course of investigation. As there is more colonization on the bank of river at downstream the increase in nitrate concentration at Stn.3 is due to biological oxidation of compounds from sewage and waste disposed in large quantities from residents in nearby localities. The rock dissolution, more mineralization of river and agriculture land run offs containing nitrogen fertilizers into the river at downstream also contribute to the higher nitrate content at Stn.3.

Nitrate content at Stn.2 was observed higher than Stn.1 in all the three seasons. This increase is may be due to absorption from atmosphere and mineralization in river at this site as the water is almost stagnant due to Kota barrage. This indicates that thermal discharge does not affect nitrate concentration of water.

The nitrate concentration was observed highest during the winter season at Stn.3 and lowest during winter season at Stn.1. Gaur *et al*. (2014) observed a high nitrate content in Chambal river during summer season in their study. On the contrary, a high nitrate concentration was observed during the present investigation in monsoon season at all the three stations than summers and winters. This is may be due to rain water run offs into the river carrying waste and sewage with it.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

It is revealed statistically that there is observed a significant difference in the mean values of nitrates of water in all the seasons studied with the p-value of significance being 0.007. This is also shown in the nitrate content values as higher concentration was observed during the monsoon season (Table 5.3).

#### **One-way ANOVA month wise**

There is not observed a significant difference statistically in the mean values of nitrates every month (p-value= 0.264). This is may be due to little changes in the concentration of nitates in water every month (Table 5.4).

## **One-way ANOVA station wise**

Statistically a very significant difference is observed in the mean values of nitrate at all the three stations studied. The p-value of significance in ANOVA analysis calculated is 0.007. This is also revealed in the observation as the highest nitrate content was observed at Stn.3 than the other two stations during the study period round the year (Table 5.5).

## **Correlation**

Nitrate content is found in a positive correlation with EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, sulphate, fluoride, BOD and COD while it is found to be in a negative correlation with light intensity, temperature, pH and DO. The density of cyanobacteria are found negatively correlated ( $r = -0.26$ ) with nitrate concentration (Table 5.6).

#### **Multiple linear regression**

The nitrate content of water possess no significant effect on the density of cyanobacteria ( $p = 0.970$ ) as suggested by regression analysis (Table 5.7). It can be concluded that nitrate content of water does not affect the population of cyanobacteria.

# **Sulphate**

It is one of the main anions of natural water and is present in the ion form in water (Maiti, 2004). The sulphate concentration was measured higher during monsoon season than summer and winter seasons. Among the three stations of sampling it was calculated highest at Stn.3 round the year during the study period. This is may be due to addition of domestic sewage, industrial effluents, farmland run offs and human interference. Lower values of sulphate were noted at Stn.1. Sulphate content at Stn.2 was measured lower than Stn.3 but higher than at Stn.1. This could be because of erosion of sulphide containing rocks and atmospheric fallout to soils and then to water by land run offs. The different chemicals used to control the algae and bacteria in condenser tubes comprise of sulphur containing chemicals. These chemicals are being discharged into the river at Stn.2. According to Moreno *et al*. (2009) algae can accumulate sulphur compounds. The density of cyanobacteria is also observed highest at Stn.2.

Bhatnagar and Bhardwaj (2013a) showed the range of 13.5 mg/L to 37.0 mg/L at Kota Barrage in their study in 2011-2012. The sulphate concentration at all the three stations varied from 4.7 mg/L to 38.3 mg/L during the study period. The sulphate content is measured in the same range even in the present investigation

after a gap of few years which indicates that thermal effluents have not much effect on the sulpate concentration of river water.

#### **Statistical analysis**

## **One-way ANOVA season wise**

A significant difference statistically is observed in the mean values of sulphates of water in all the seasons studied (p-value  $= 0.023$ ) as higher concentration of sulphate was observed during the monsoon season (Table 5.3).

## **One-way ANOVA month wise**

A significant difference is also observed statistically in the mean values of sulphates every month as indicated by the p-value of significance which is 0.007. A detectable amount of suphate is observed every month which is the reason for this significant difference (Table 5.4).

## **One-way ANOVA station wise**

Statistically a significant difference is observed in the mean values of sulphate (p-value 0.023) at all the three stations during the study as the highest concentration of sulphate was observed at Stn.3 than the other two stations (Table 5.5).

## **Correlation**

A positive correlation is found between sulphate and temperature, EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, BOD and COD. On the other hand, a negative correlation is observed between sulphate and light intensity, pH, fluoride and DO. The population

of cyanobacteria is found to be in slight positive correlation  $(r= 0.15)$  with sulphate concentration during the study period (Table 5.6).

## **Multiple linear regression**

The sulphate content of water do not possess a significant effect on the density of cyanobacteria ( $p = 0.490$ ) as indicated by Table 5.7 in regression analysis. This suggests that sulphate content of water does not affect the population of cyanobacteria.

#### **Fluoride**

It is an important parameter for the potability of water. Chambal river water is used for drinking purpose for the residents of Kota city. High fluoride more than 1.0 mg/L concentration in water makes it unfit for drinking purpose causing tooth decay and health disorders (APHA, 1999). The fluoride content of river water varied from  $0.11 - 0.35$  mg/L round the year during the study period.

Highest values of fluorides were observed in summer season and lowest in monsoon season. Fluoride concentration was observed lower at upstream and higher at downstream of the river. It was more or less similar at Stn.1 and 2. It exhibits a pattern of rise from Stn.1 towards Stn.3. Minimum concentration of fluorine was observed at Stn.1. This is may be due to water filtration process done by Akelgarh water filtration plant. The highest fluoride concentration was measured at Stn.3. The high fluoride content values were measured at Stn.3 which was similar to the findings made by Gupta *et al*. (2011) in their study. This is because the water at downstream is not flowing regularly and gets in contact with fluoride bearing rocks.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

A statistical significant difference is not observed in the mean values of fluorides of water in all the seasons studied (p-value  $= 0.023$ ). But in terms of observation lowest fluoride content was observed in monsoon season and highest in summer season (Table 5.3).

#### **One-way ANOVA month wise**

Statistically a very significant difference is observed in the mean values of fluorides every month (p-value  $= 0.000$ ) (Table 5.4).

#### **One-way ANOVA station wise**

During the study a significant difference is not observed statistically in the mean values of fluoride (p-value 0.088) at all the three stations as the fluoride content was observed more or less similar at the three stations (Table 5.5).

# **Correlation**

Fluoride content in water has a positive correlation with light intensity, EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, BOD and COD. But has a negative correlation with temperature, pH, sulphate and DO. A slight negative correlation is found between fluoride concentration and number of cyanobacteria (r= -0.38) (Table 5.6). This suggests that fluoride content does not affect much the growth of cyanobacteria.

## **Multiple linear regression**

There is not observed a significant effect of fluoride content of water possess on the density of cyanobacteria ( $p = 0.131$ ) as shown in regression analysis (Table 5.7). This indicates that the population of cyanobacteria is not affected by the fluoride content of water.

#### **Dissolved Oxygen (DO)**

DO is the amount of oxygen dissolved in water which is necessary for aquatic plants and animals. It is an important parameter of water. It is essential to maintain aquatic life. DO is dependent on the water temperature and the type of vegetation of water body. Generally, algae maintain dissolved oxygen (DO) and aerobic environment of water body through photosynthesis necessary for bacterial decomposition of organic matter and respiration of aquatic fauna (Palmer, 1980).

The annual range of DO is observed highest at Stn.1 and lowest at Stn.3 round the year. Low levels of DO at Stn.3 are may be due to formation of algal blooms due to introduction of sewage and organic waste. The summer season was observed with low values of DO than winters.

DO values in freshwater river ranged from 3.9 to 8.5 mg/L during the study. This is similar to the examination done by Jain *et al*. (2015) in Chambal river in Kota whereas DO values in the range of 4.3-14.59 mg/L have been reported by Saksena *et al*. (2008), Gupta *et al*. (2011), Bhatnagar and Bhardwaj (2013a), Gaur *et al*. (2014), Chouhan *et al*. (2018).

A low level of DO was also observed by Eloranta (1983) during his study in a pond receiving heated water discharge from a thermal power plant. Nwankwo *et al.* (2010) observed lower levels of dissolved oxygen with highest water temperature in their study at a site affected by waste heat discharge from a power plant at Egbin,

Nigeria. Kailasam and Sivakami (2004) also observed low DO at the thermal discharge site at higher surface water temperature. These all studies were conducted on sea waters.

As the temperature of water increases the amount of oxygen dissolved in water decreases. The range of DO observed in freshwater in the present study is lower than the previous researches. This is because the elevated water temperature is might decreasing dissolved oxygen level in water as suggested by Schwoerbel (1970) and Hill (1972). According to Krishnakumar (1994), with the 10 ºC rise in temperature, the solubility of oxygen in water decreases up to 20%. The lowering of the amount of oxygen dissolved in water in the present study is due to rise in temperature of river water in the past few years. The increase in surface water temperature is due to thermal discharge with elevated water temperature into the Chambal river. As the rise in water temperature decreases dissolved oxygen, the cyanobacterial members not able to survive in low oxygen concentration gets eliminated with surviving members of cyanobacteria (Marshall, 1971).

The dissolved oxygen concentrations at stations Stn.1 and 2 are more or less similar in range of  $5.7 - 8.5$  mg/L in three seasons. This is in accordance to the observation made by Smith and Manoylov (2013) in their study on diatoms in lake near a coal fired thermal power plant.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

In season wise analysis, a very significant difference is observed in the mean values of dissolved oxygen of water statistically (p-value= 0.000). This is clear from

the observations made during the study period as DO was measured low in summer season and higher in winter season (Table 5.3).

## **One-way ANOVA month wise**

A significant difference is not observed statistically in the mean values of DO in every month (p-value= 0.930) as clearly indicated by the monthly observations also (Table 5.4).

#### **One-way ANOVA station wise**

Statistically a very significant difference is observed in the mean values of DO studied at the three stations. This is indicated by the p-value of significance (0.000). DO at Stn.2 is found to be higher than at Stn.3 (Table 5.5).

# **Correlation**

Dissolved oxygen is found to have a positive correlation with light intensity, temperature, pH only and have a negative correlation with EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, sulphate, fluoride, BOD and COD. There is no correlation observed between DO and the population of cyanobacteria (Table 5.6).

#### **Multiple linear regression**

Statistically the number of cyanobacteria are not affected by the oxygen dissolved in water as indicated by regression analysis ( $p = 0.575$ ). It can be said that there is no significant effect of amount of oxygen dissolved in water statistically on the density of cyanobacteria though more number of cyanobacteria were observed at low DO (Table 5.7).

#### **Biochemical oxygen demand (BOD)**

"BOD is defined as the amount of oxygen required by bacteria in decomposing organic material in a sample under aerobic condition at 20 ºC over a period of 5 days" (Maiti,2004).

The lowest BOD was measured for Stn.1 (0.2 mg/L) in winter season and maximum at Stn.3 (5.03 mg/L) in monsoon season. The results obtained are similar to the findings of Bhatnagar and Bhardwaj (2013a). The BOD range of 4.2-6.5 mg/L in Chambal river water has been observed by Jain *et al*. (2015) and Chouhan *et al*. (2018).

The BOD values are found greater at Stn.3 than other two stations round the year. Higher BOD values suggest that there is more amount of oxidizable matter present at Stn.3 causing low levels of DO. This may be due to the human interference, disposal of waste and sewage.

A low BOD value of 1.43 mg/L was reported by Muhammad Adlan *et al*. (2012) at a station near the outlet from a power station. In the present study BOD values ranged from 0.2 to 2.77 mg/L at Stn.2 which are near to their observations.

## **Statistical analysis**

#### **One-way ANOVA season wise**

Statistically a very significant difference is observed in the mean values of BOD of water as suggested by p-value of significance being 0.000 in all seasons. This is because of different concentration of BOD in all the three seasons (Table 5.3).

## **One-way ANOVA month wise**

Though the observation made for BOD indicates a detectable amount of BOD in every month, there is no statistically significant difference observed in the mean values of BOD each month (p-value= 0.938) (Table 5.4).

## **One-way ANOVA station wise**

Statistically a very significant difference is observed in the mean values of BOD studied at the three stations as indicated by the p-value of significance (0.000). BOD at Stn.3 is found to be higher than at Stn.2 (Table 5.5).

# **Correlation**

A positive correlation is observed between BOD and EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, sulphate, fluoride and COD. A negative correlation of BOD is observed with light intensity, temperature, pH and DO. BOD is found to be slightly positively correlated with the number of cyanobacteria ( $r = 0.09$ ) (Table 5.6). Although the BOD is measured highest at Stn.3 but the greater number of cyanobacteria is not observed as this site the bloom forming algae is may inhibiting the growth of other cyanobacteria.

## **Multiple linear regression**

Regression analysis indicates that the BOD statistically does not affect the number of cyanobacteria present in water ( $p = 0.142$ ) (Table 5.7).

#### **Chemical Oxygen Demand (COD)**

COD is the measure of oxygen required for chemical oxidation of organic compounds with the help of strong chemical oxidant (Aery, 2010). It indicates the toxic conditions of a water body (Maiti, 2004).

COD ranged between 4.0 mg/L to 66.67 mg/L during the study period. COD values are always greater than BOD values (Maiti, 2004). The lowest values of COD were calculated at Stn.1 and highest at Stn.3 round the year during the study period.

Chouhan *et al*. (2018) observed the COD value of 12.5 mg/L at Stn.2 and value of 13.2 mg/L at Stn.3 in their study. Gupta *et al*. (2011) measured 13.8 mg/L of COD at Kota Barrage and 22.5 mg/L at Chhoti Samadh which is Stn.3.

The maximum COD value was obtained at Stn.3 during monsoons. The high values of COD indicate high amount of organic matter and chemically oxidizable inorganic substances present in water. This is may be due to addition of domestic waste, cropland waste, industrial waste and sewage at Stn.3.

The COD values at Stn.2 are higher than Stn.1 but lower than at Stn.3. The reason for the higher COD value at Stn.2 can be attributed to the content of thermal discharge which may include oil and grease or some other chemicals used for the maintenance of machinery of the coal fired thermal power plant.

The population of cyanobacteria is higher at Stn.2 as compared to Stn.3 as indicated by the lower values of COD.

#### **Statistical analysis**

#### **One-way ANOVA season wise**

A very significant difference is observed statistically in the mean values of COD of water as indicated by p-value of significance in all seasons (0.000). This is may be due to difference in concentration of COD in all the three seasons (Table 5.3).

#### **One-way ANOVA month wise**

The monthly observation taken for COD clearly indicates a difference in the amount of COD in each month but statistically a significant difference is not observed in the mean values of COD in ANOVA analysis month wise (p-value= 0.461) (Table 5.4).

## **One-way ANOVA station wise**

Statistically a very significant difference is observed in the mean values of COD station wise as indicated by the p-value of significance (0.000). COD at Stn.3 is found to be higher than at Stn.2 (Table 5.5).

#### **Correlation**

COD is observed to be in a positive correlation with EC, TDS, turbidity, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, nitrate, sulphate, fluoride and BOD. A negative correlation of COD is observed with light intensity, temperature, pH and DO. The population of cyanobacteria is found to be negatively correlated with COD ( $r = -0.18$ ) (Table 5.6).

#### **Multiple linear regression**

Statistically there is no effect of COD on the number of cyanobacteria present in water as regression analysis indicates ( $p = 0.064$ ) (Table 5.7).

## **Density of cyanobacteria**

Statistically a significant difference is observed in the mean values of total number of cyanobacteria in one-way ANOVA analysis month wise (p-value= 0.016). Though the difference in their mean values is not significant statistically in season wise and station wise analysis (Table 5.3, 5.4, 5.5).

In Multiple Linear Regression analysis the Coefficient of determination  $(R^2 =$ 0.819) shows that the variability in the density of cyanobacteria is defined by the independent variable which is about 81.9% (Table 5.7).

# **Biodiversity of Cyanobacteria**

Diversity of the cyanobacterial community in the present study is studied at the genera level diversity of a population of the individual genera of cyanobacteria.

Total 25 species belonging to 14 genera of cyanobacteria were observed in Chambal river freshwater at three stations selected for the study, representing orders: Synechococcales, Spirulinales ordo nov., Chroococcales, Oscillatoriales and Nostocales (4,1,3,3 and 3 taxa respectively) (Anagnostidis and Komárek, 1988; Komárek *et al*., 2014). The highest species richness was observed at Stn.2 (13 taxa) and lowest at Stn.3 (7 taxa). The order Synechococcales was represented with highest number of genera at the three stations round the year of study period.

The community structure of cyanobacteria at Stn.2 was rich with cyanobacteria belonging to order Synechococcales, Spirulinales ordo nov., Chroococcales, Oscillatoriales and Nostocales during the study period. Though the cyanobacterial community structure at Stn.1 was comprised of members belonging to same orders as observed at Stn.2 but order Chroococcales is represented with only 1 taxa *Chroococcus*. The Stn.3 was observed with cyanobacteria belonging to orders Synechococcales, Chroococcales and Oscillatoriales only.

The highest abundance of cyanobacteria was found at Stn.2 in May and June months of summer season while lowest was observed at Stn.1 in winter season (November). Station Stn.1 was dominated with the representatives of Chroococcales. The total share in the abundance of cyanobacteria at Stn.1 is 59%. This similarity of share by representatives of order Chroococcales is also found to be higher at Stn.2 (56%) and 3 (46%). But at Stn.2 there is observed an increase in the share of total abundance by representatives of order Synechococcales (23%) while by order Oscillatoriales (33%) at Stn.3.

The maximum density of *Chroococcus* was observed at Stn.2 throughout the study period. The highest number of cyanobacteria was observed in summer season at Stn.2 and lowest at Stn.1 in winter season. The orders Chroococclaes represented by *Chroococcus* and Synechococcales represented by *Merismopedia* and *Synechococcus* dominated at Stn.2 round the year. On the other hand Stn.1 and 3 were dominated by Chroococclaes and Oscillatoriales with *Chroococcus* and *Oscillatoria* at Stn.1 while *Microcystis* and *Oscillatoria* at Stn.3 respectively.

Cyanabacterial taxa like *Lyngbya, Gloeocapsa, Cylindrospermopsis, Microcystis, Synechocystis* were found to be absent from the cyanobacterial community at Stn.1 while these genera were a part of the community at Stn.2. The community structure of cyanobacteria is also found to be changing at Stn.3 from other two sampling stations as *Oscillatoria* and *Microcystis* were found to be dominant at Stn.3.

13 genera of cyanobacteria with 21 species were observed at Stn.2, 9 genera with 14 species at Stn.1 and 6 were observed at Stn.3. *Oscillatoria* was observed at all the stations round the year. *Merismopedia* is a temperature tolerant taxa and is found dominant at Stn.2. *Chroococcus* is another taxa found with maximum number of individuals at Stn.2.

For community structure study and analytical measurement of biodiversity, various indices are used, commonly known as diversity indices. The diversity indices are concerned with diversity and consider individual occurrence for evaluation. The richness and evenness of taxa are integrated into diversity indices which give information about ecological conditions and are used to monitor changes in environment (Lata Dora *et al*., 2010).

Diversity indices are helpful in assessing the changes in community structure when used to determine the water quality by use of a biological component (Palmer, 1980). More the degree of environmental stress, greater the change in the community structure will be (Aery, 2010).

Stress imposed by temperature varies on organisms in terms of duration and magnitude (Poornima *et al*., 2006). Changes in temperature can alter the composition and structure of algal community.

The heated water is the main component of thermal discharge. This heated water when discharged into the source of water creates a change in temperature that affects the diversity of phytoplanktons.

The computation of cyanobacterial diversity was done by Shannon Weaver diversity index. Shannon diversity index values at Stn.1 ranged from 1.11 to 1.92.They ranged from 1.10 to 2.07 at Stn.2 and from 1.26 to 1.79 at Stn.3.

The Shannon Weaver diversity index 'H' values of cyanobacteria was more or less similar at three stations in monsoon and winter season but it was measured maximum in summer season.

Lowest Shannon Weaver diversity index was calculated at Stn.1 for cyanobacteria. Higher value of "H" indicates greater diversity of cyanobacteria. It was calculated maximum at Stn.2 during the summer season. This station is nearest to the discharge site of thermal power station as compared to other two stations. Hence the higher diversity index is may be due occurrence of diverse forms and different species of cyanobacteria at Stn.2.

An increase in the abundance of cyanobacteria is observed in the present study at Stn.2 with the highest diversity of cyanobacteria. Stn.2 receives thermal discharge in form of heated water and experiences highest surface water temperature

in the summer season. This is the reason for the richness of cyanobacteria at Stn.2. Thermal tolerant taxa are found dominant at Stn.2 in the present study. Hence, it can be stated in the present study that heated water with elevated temperature associated with a thermal power plant supports the growth of cyanobacteria by increasing their population and have a positive impact on their density and diversity. Heated water from the thermal discharge is altering the cyanobacterial community by shifting the sensitive taxa to heat tolerant taxa with high diversity at Stn.2.

Some of the studies revealed decreased growth of aquatic flora near power plants and show that there is not any significant effect of thermal discharge on community of cyanobacteria. Previous studies conducted on the influence of thermal discharge showed loss of biodiversity but most of them considered seaweeds or phytoplanktons in marine water which get negatively affected with the rise in temperature (Cuici *et al*., 2006; Kim *et al*., 2008; Nwankwo *et al*,. 2014). But Mallin *et al*. (2014) suggested that marine phytoplankton community is not much affected under the effect of thermal discharge. Study of Nashaat *et al*. (2019) also make a point that thermal discharge in river water imposes a negative effect on the diversity but their work is on phytoplanktons.

In the present study, it is revealed that freshwater river cyanobacterial population is strongly positively correlated with the rise in water temperature caused due to thermal discharge. Cyanobacteria are considered as blue green algae, have higher range of thermal tolerance, they also show optimal growth at high range of temperature (Welch and Lindell, 1992). A common observation made by Brock (1975) showed blue-green algal (cyanobacterial) dominance of aquatic communities

explaining a shift in phytoplankton community.

is greater when water temperatures are warmer due to discharge from power plants. Even the studies of Konopka and Brock (1978) and Welch and Lindell (1992) revealed that as temperature increases, the algal groups with the maximum growth rates changed from diatoms to green algae to blue-green algae (cyanobacteria)

Sorensen's similarity index expresses the number of common taxa between two communities or sites compared along with the total number of individuals on both the sides. It predicts the community composition of cyanobacteria. Sorensen"s similarity index values are found higher in summer season and lower in winter season between different stations compared. The similarity index values indicate that there are present more number of common representatives of cyanobacteria on an average among Stn.1 and Stn.3. It can be stated that 45% of the cyanobacterial members are commonly present between the two sites. The lesser number of common taxa are found to be present between Stn.2 and 3 having 38% of similarity. On the other hand, sites Stn.1 and Stn.2 have 44% of common members between them.

This suggests that the cyanobacteria are more common between Stn.2 and 1 than between Stn.2 and 3. The 45% similarity between Stn.1 and 3 indicates almost a similar community structure of cyanobacteria. A decrease in similarity is observed from Stn.2 to Stn.3. This is may be due to more human interference and addition of organic content at Stn.3. Bloom forming cyanobacteria are of more common occurrence at Stn.3. Higher similarity between Stn.1 & 3 indicates a change in community structure at Stn.2. It can be concluded that there is a difference between their composition at Stn.2 from Stn.1 and 3. This difference in composition of cyanobacteria is due to thermal impact from the thermal power plant. The heated water increases the water temperature of the river receiving the thermal discharge. This rise in temperature favours the growth of thermal tolerant members of cyanobacteria at Stn.2.

Some new members have also been reported during this study which have not been reported earlier near Kota Barrage. *Planktothrix, Synechocystis*, *Cylindrospermopsis* and *Synechococcus* are reported for the first time in Chambal river water.

However, a higher diversity of phytoplanktons in marine waters was reported by Lo *et al.* (2004) and the results obtained for the present study on freshwater are in coincidence with their findings.

The diversity may be altered with rise in temperature. Change in climate or temperature can result in a shift in distribution and abundance of species (IPCC, 2007). Some of the species of the algal community may be eliminated with the arrival of new species in an algal community (Coutant and Brook, 1973). The high temperature favours the proliferation of cyanobacterial algal community among phytoplanktons and tends to alter the algal diversity (Schabhüttl *et al.*, 2013).

The present work indicates that thermal discharge has significant influence on surface water temperature of river. Among all the physical and chemical properties of river water determined during the study, surface water temperature has a strong impact on the diversity of cyanobacteria. It revealed that a significant increase in surface water temperature is reported in river water and it is highly positively correlated with the cyanobacterial population and their diversity at Stn.2

i.e. near the discharge point of thermal power plant. It can be proved strongly that even may be due to rise in temperature arrival of new opportunistic members, particularly heat tolerant taxa, has also been recorded at Stn.2 in the present study. As a result of which the diversity at Stn.2 is higher as indicated by H values. Cold water tolerant taxa are being replaced by thermal tolerant taxa of cyanobacteria at Stn.2 causing a shift in the density and diversity of cyanobacteria, hence changing the cyanobacterial community structure as indicated by the Similarity index of Sorenson.

The thermal discharge from the thermal power plant is rising the water temperature which thereby is responsible for high rates of photosynthesis and increased growth in cyanobacteria at Stn.2. As high temperature lowers the density of water so may be some of the cyanobacteria move vertically making more number of cyanobacteria floating on the surface of water hence enriching the water with cyanobacteria at Stn.2. The higher water temperature at Stn.2 is may be responsible for making the water rich in cyanobacteria.

The present research work stated that in the density of cyanobacteria, at all the three stations in different seasons, statistically not such significant difference was observed. Seasonal study included an average result of four months. This is due to different climatic and weather conditions every year which is definitely causing a gradual shifting of onset and duration of seasons resulting in a change in seasonal cycle. There is a significant difference observed statistically in the density of cyanobacteria at the three stations, every month during the study period. Maximum diversity reported in the month of June while minimum diversity reported in the month of May.

It can be stated that particular physico-chemical parameters of water like light intensity, temperature, pH and electrical conductivity have a significant effect on the density of cyanobacteria each month at each station during round the year study. As a result of which the population of cyanobacteria is observed to be different in every month thereby expressing a change in diversity.

The heated water from thermal discharge is responsible for the increase in diversity of cyanobacteria thereby causing a possible change in cyanobacterial community composition though a gradual one but which may affect the other trophic levels and in the turn the aquatic ecosystem dynamics.

# **CHAPTER 7 SUMMARY**

Cyanobacteria are the blue-green algae with morphological diversity and diverse habitats. They have prokaryotic structure so nucleus and other cell organelles are all together absent in them. Cyanobacteria lack sexual reproduction and reproduce only by fragmentation or vegetative propagation. Gas vesicles in the protoplast of their cells provide buoyancy to the floating forms. Some of them can also perform the function of nitrogen fixation.

Cyanobacteria can survive in variety of habitats both land and water. In Rajasthan state, a land of desert with few water bodies, Chambal river is a boon for the people of the state. The river is a tributary of Yamuna river and is a perennial river of Rajasthan. It is rich in flora and fauna. The river is also a home for different kinds of algae. As cyanobacteria can perform oxygenic photosynthesis they play an important role in global carbon cycle through assimilation of carbon. They play an important role in the ecosystem as 98% of carbon is fixed by these algae. They also have an important role in the biotechnology for the various uses they have. Cyanobacteria form an important component of phytoplanktons which form the base of a food web where these are the first organisms to be affected first if any change in aquatic environment occurs.

The river flows through Rajasthan and M.P. states in India. The river flows in a stretch of 960 km and its water is used for drinking, irrigation, electricity generation, industrial purpose and recreational activities.

The river serves as the life line for the residents of Kota city as the river flows from the heart of the city. Kota Thermal Power Station (KTPS) is located on the left bank of the Chambal river. It is a coal-fired plant which is functioning since 1983. The total installed capacity of the plant is 1240 MW and has seven operating units. Thermal power plants use water for the cooling purpose which in turn gets heated and is discharged back into the source of water. Heated water is the main thermal effluent discharged into the water body. The hot or warm water impose various ecological effects including lowering the amount of oxygen dissolved in water and even in mass mortality of fishes. KTPS uses Chambal river water for "the once through cooling" purpose of its five units out of seven which may affect its aquatic cyanobacteria. The water discharged from the KTPS transfers its heat energy to the Chambal river water where cyanobacteria are exposed to this heated water.

With the objective to study the diversity of cyanobacteria in Chambal river and to assess the changes in community structure of cyanobacteria due to thermal discharge the physico-chemical properties of river water were analyzed.

Three sites were selected for the present investigation on the stretch of Chambal river in Kota city. Station 1 was selected at upstream of river at Akelgarh water treatment plant. Station 2 was selected as near as possible to the thermal discharge site of Kota Thermal Power Station and Station 3 was selected at the downstream of Chambal river behind the Chhoti Samadh Shiv Temple. Stn.1 was treated as control station. Random sampling method was used for sampling for the period of one year from July 2017 to June 2018. The results were analysed season wise as well as month wise.

Total 15 physico-chemical parameters were studied during the study period. The physical and chemical characteristics of the river water were determined by collecting the water samples in triplicates from selected sites in one litre capacity of clean polyethylene bottles. After the collection of sample, light intensity, temperature and pH were determined immediately on the spot at collection sites. For the analysis of parameters like electrical conductivity, turbidity, total alkalinity, hardness, chlorides, fluorides, sulphates, nitrates, total dissolved solids, dissolved oxygen, BOD & COD the samples were brought to the laboratory. The standard prescribed methods were followed for the determination of physico-chemical parameters of water.

The surface water samples were collected from the sampling sites in the morning time during second week of every month during the study period in the replica of three to study the diversity of cyanobacteria. The collected samples were studied within 48 hours with the preparation of fresh mounts in laboratory for cyanobacterial study. The samples were also fixed and preserved using Lugol"s solution for further detailed examination. Observation and identification of phytoplanktonic Cyanobacteria was done with the help of available literature and monographs.

The physico-chemical parameters of water were determined and the average values were calculated for the data. Cyanobacteria were observed to study their diversity in freshwater. The diversity of cyanobacteria was calculated using different diversity indices for the assessment of the community structure of cyanobacteria. Data obtained was analyzed season-wise as well as month-wise to study the overall
effect of various parameters of water on the diversity of cyanobacteria. The data was also tested statistically using statistical tools of One-way ANOVA, Coefficient of correlation and Multiple linear regression.

In can be concluded in the present study that among the various physicochemical parameters of water studied, temperature, pH and EC were significantly affecting the diversity of cyanobacteria.

It revealed that the higher surface water temperature was observed at Stn.2 which was almost 10 °C higher than the control station and station at downstream. It can be concluded that the thermal power plant discharges heated water into the Chambal river which rises the ambient water temperature at the site nearest to the discharge site. The density of cyanobacteria was found highest at Stn.2 during the summer season which indicates that elevated water temperature favours the growth of cyanobacteria thereby increasing their diversity. A strong positive correlation between temperature and density of cyanobacteria was observed making it evident that temperature favours the proliferation of cyanobacteria. Mutiple linear regression analysis clearly indicates that water temperature has a significant positive impact on the number of cyanobacteria.

pH was also measured higher during summer season like the temperature. As cyanobacteria thrive well in alkaline pH of 7.5 to 9 units, the pH at Stn.2 provides favourable conditions for growth of cyanobacteria with higher diversity. Low EC also favours the growth of cyanobacteria. There is no major change observed in the concentration of total dissolved salts, total alkalinity, sulphate and nitrate in river water from the past years of research indicating that thermal discharge has no effect on these chemical parameters of water.

Correlation matrix reveals that the number of cyanobacteria is highly positively correlated with water temperature. The population of cyanobacteria is also found to be in positive correlation with light intensity and pH. Hence, the higher water temperature favours the growth of cyanobacteria thereby an increase in population and diversity of cyanobacteria is observed at Stn.2. The thermal discharge from the thermal power plant is causing increase in the temperature of water which in turn is responsible for high rates of photosynthesis and increased growth in cyanobacteria at Stn.2. As the temperature rises the density of water gets lowered so may be some of the cyanobacteria move vertically. With the presence of gas vesicles such cyanobacteria float on the surface of water leading to increase in number of cyanobacteria at Stn.2. The higher water temperature at Stn.2 is may be responsible for making the water rich in cyanobacteria.

Total 25 species belonging to 14 genera of cyanobacteria were observed in Chambal river freshwater at three stations selected for the study, representing orders: Synechococcales, Spirulinales ordo nov., Chroococcales, Oscillatoriales and Nostocales (4,1,3,3 and 3 taxa respectively). 9 genera with 14 species of cyanobacteria were observed at Stn.1, 6 genera with 16 species were observed at Stn.3 and total 13 genera with 21 species at Stn.2. *Oscillatoria* was observed round the year at all the stations in three seasons. Cyanobacteria like *Cylindrospermopsis*, *Planktothrix*, *Synechococcus* and *Synechocystis* are reported for the first time in Chambal river water in this study.

*Chroococcus* was found with maximum number of individuals at Stn.2. Heat tolerant cyanobacteria like *Merismopedia* and *Synechococcus* were also observed at Stn.2 that is marked with a higher range of temperature. Presence of 13 genera with 21 different species at Stn.2 establishes the fact that elevated water temperature from thermal discharge is favouring the population of cyanobacteria and hence increasing the diversity of cyanobacteria as indicated by Shannon Weaver diversity index.

Thermal tolerant cyanobacteria flourish well at Stn.2 receiving thermal effluents so it can be emphasized that cold or normal water tolerant members are being replaced by temperature tolerant members of cyanobacteria causing a change in the density of cyanobacteria. This in turn is responsible for the increasing diversity and more growth of opportunistic species at Stn.2 hence changing the cyanobacterial community structure.

It can be conclude that the freshwater Chambal river supports the high diversity of cyanobacteria under the thermal influence during the summer season at Stn.2.

## **Scope for further research**

Concentration of heavy metals in thermal discharge further can be analyzed to study their effect on cyanobacteria. As Chambal is a river is a perennial river, both the sides of river could have been chosen for consideration sampling.

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# **Course Work Certificate**



# **Registration Letter**

## VARDHAMAN MAHAVEER OPEN UNIVERSITY, KOTA (Department of Research)

No.: VMOU/R&D//2016/5024-5028

Date:  $4/5/16$ 

The Head/Convener/Incharge Department of Botany Vardhaman Mahaveer Open University, Kota

Registration No: VMOU/Research/Ph.D/BO/2015/78

Dear sir,

With reference to your endorsement on the application and approved synopsis of Leena Choubisa registration as a Research Scholar to supplicate for the Ph.D. Degree of the University as per UGC guideline-2009. I am to inform you that he/she has been permitted by the Vice-Chancellor on behalf of the Research Board to carry on Research on the title "An Assessment of Cyanobacterial Biodiversity in a Freshwater River System Associated with Thermal Discharge from a Power Station" under the supervision of Dr. Anuradha Sharma (Dubey), Assistant Professor Department of Botany, VMOU, Kota.

#### **Please intimate:**

- (a) Date of commencement of Ph.D. work and;
- (b) Medium of writing of thesis.
- (c) Hindi/English Transcription of title of your proposed research work.

The date of commencement of research work may be the date on which the candidate commences work or an earlier date but it should not be earlier than the date of the meeting of the Research Board in which the proposal of the candidate was submitted and approved.

The candidate has to follow the general instructions for Ph.D. and act as per guidelines issued by the University as per the UGC Regulations-2009.

Yours truly

 $-sct$ **Director** 

Copy forwarded to:

 $2.$ 

- Dr. Anuradha Sharma (Dubey), Assistant Professor Department of Botany, VMOU, Kota
	-
- Director, Academic, VMOU, Kota 3.
- Incharge, Central Library, VMOU, Kota.  $\overline{4}$ .
- Leena Choubisa D/O R K Choubisa Rajasthan State Seed And Organic Production Certification Agency 5. Karkhana Bagh Bajrang Nagar Kota-324001

6. File



# **PUBLICATIONS AND CONFERENCE/ SEMINAR/ WORKSHOP**

# **Publications**

- Choubisa, L. and Dubey, A. (2016). Cyanobacterial biodiversity of Chambal river in Rajasthan. *Journal of Phytological Research*, 29 (1 & 2), 49-54.
- Choubisa,L. and Dubey, A. (2017). Phytoplanktonic Diversity and Physicochemical Characteristics of Kishore Sagar, Kota, Rajasthan. *Journal of Phytological Research*, 30 (2), 31-39.
- Choubisa, L. and Dubey, A. (2019). Freshwater Cyanobacterial Diversity under the influence of a Thermal Power Plant. International Journal of Research and Analytical Reviews, 6(2), 321-330.
- "Influence of Thermal Power Plant on Diversity of Cyanobacteria in Rajasthan, India" by Leena Choubisa and Anuradha Dubey has been accepted for publication in journal Plant Archives. The paper will appear in Vol. 20 No.2 October 2020.

# **Conference/ Seminar/Workshop**

- XXXVIII All India Conference of the Indian Botanical Society & National Symposium on "Emerging Trends in Plant Sciences", October 26-28, 2015, University of Rajasthan, Jaipur
- National Conference on Recent Advances in Biological Sciences, Biotechnology & Sustainable Development "RABBSD-2016", March 18-19, 2016, Mohanlal Sukhadia University, Udaipur
- National Symposium on Global Environmental Challenges: Present Scenario, 21<sup>st</sup> January, 2017, University of Rajasthan, Jaipur
- National Seminar on Role of Plants in Conventional Medicine, September 30, 2019, University of Rajasthan, Jaipur
- National Conference on Biodiversity Conservation for a Sustainable Future, 11-12 October 2019, IIS University, Jaipur
- National Conference on Climate Change: The Impact on Biodiversity and Global Health Security, December 20-22, 2019, University of Rajasthan, Jaipur
- International Online Workshop on Environment and Wildlife of Rajasthan: Challenges in Conservation (IWEWRCC-2020), 5 June 2020, Shri Shraddha Nath PG College, Todi, Jhunjhunu.
- One day Online National Symposium on Behavioural and Molecular Aspects of Fauna & Flora in Present Scenario, 12<sup>th</sup> June, 2020, Vardhman Mahaveer Open University, Kota.
- National Online Faculty Development Program (FDP) & Management Development Program (MDP) on Academic Enhancement on Research Methodology, 26-29 June, 2020, Inspira Research Associaation (IRA), Jaipur.



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#### CYANOBACTERIAL BIODIVERSITY OF **CHAMBAL RIVER** IN **RAJASTHAN**

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Chambal river is the only perennial river of Rajasthan which is used for drinking, irrigation and power generation. The river hosts excellent flora and fauna of which freshwater flora is of much importance because of its basic position in the food chain. Only few research reports are available on cyanobacteria of Chambal River in Rajasthan. In present scenario some more documentation of cyanobacterial biodiversity is required for further research.

Keywords: Bioassessment; biodiversity; cyanobacteria; phytoplanktons.

#### Introduction

Rajasthan is a land of desert with very small area covered by rivers. Chambal river, a tributary of Yamuna River, is the only perennial river that originates at Mhow near Janapav temple in Madhya Pradesh. Flowing in a northerly direction through M.P., it enters Rajasthan at Chaurasigarh Fort where it flow in north – east direction<sup>1</sup>. It again flows through M.P. before joining the Yamuna river in U.P. Chambal river forms various deep picturesque gorges during its course that add scenic beauty to it. In stretch of 96 km in Rajasthan, it flows through Chittorgarh, Kota and Dholpur districts. The river water is used for industrial purpose, drinking, recreational activities, electricity generation and irrigation through four dams - Gandhi Sagar Dam, Rana Pratap Sagar Dam, Jawahar Sagar Dam and Kota Barrage constructed on it during its long course (Fig. 1 & 2)<sup>2</sup>. The river has derived its name from 'Charmanayavati' and with religious beliefs

holds a sacred position for the people of Kota with a holy name 'Maa Charmanayavati'  $2$  (Fig3)<sup>2</sup>. Besides being the lifeline of Kota city, Chambal River is also rich in aquatic flora and fauna including the mugger and gharial.

Due to use of its water for industrial purpose and colonization on its bank, the river gets the industrial effluents and sewage water discharged into it. Most of the research work has been targeted on water pollution and water quality of Chambal River. But a very stray literature is available on phytoplankton diversity of Chambal River, hence this paper is an attempt to review the works done on cyanobacterial biodiversity of Chambal river.

Cyanobacteria- Cyanobacteria are the most primitive. cosmopolitan, thallophytic, photosynthetic, prokaryotic organisms, classified under phylum alga which came into existence almost 3.5 billion years ago.



Fig. 1 Course of Chambal River

Fig. 2 Various dams constructed on Chambal river



Fig. 3 Chambal River

The evidence of their origin can be traced through carbon isotopic data and study of microfossils which states their dominance<br>during Precambrian period<sup>3,4</sup>. These are the most successful organisms that are in existence for billions of years and are still exploiting every possible extreme of Cyanobacterial cells habitats<sup>5</sup>. contain chlorophylla, beta carotene.  $C$ phycocyanin, allophycocyanin and  $C$ phycoerythrin with membrane bound nucleus and plastids lacking (prokaryotic cell structure). Motile reproductive cells are

never found in their life cycle<sup>6</sup>. They show morphological diversity and may occur in variety of morphological forms. They can be unicellular like Synechocystis, can occur in form of trichome (chain of cells) like Oscillatoria, or filament ( chain of cells enclosed in a gelatinous sheath) like Phormidium which can be branched as in Scytonema or colonial as in Merismopedia, Microcystis<sup>4</sup>. They are also known as 'bluegreen algae' because of the bluish green colour imparted by dominant phycocyanin pigment to them<sup>7</sup>.



Because of their cosmopolitan nature, cyanobacteria are found in variety of habitats from freshwater to marine, planktonic to benthic, acidic to alkaline, hot water to soil, desert to snow<sup>7</sup>. Cyanobacteria like Synechococcus are even able to grow at temperature up to 73 °C -74 °C  $^{8,4}$ 

## **Unique in its Kind**

Cyanobacteria possess some characteristic features that make them unique<sup>4,9,10,3</sup>.

- a) They are the only oxygenic phototrophic prokaryotes which can perform oxygen photosynthesis evolving through photosynthetic pigments diffused in the entire cytoplasm (chloroplasts being absent). They play a key role in global carbon cycles through assimilation of carbon and oxygenic photosynthesis. Their chief reserve food  $is$ cyanophycean starch.
- b) They are closely related to bacteria in evolution. Their cell wall is similar to Gram negative bacteria containing amino sugars and amino acids. Cell wall is enveloped with mucilaginous sheath secreted by cell membrane in some genera
- c) They are the only algae that can fix atmospheric dinitrogen. This function can be attributed to specialized thick cells called 'heterocysts' walled provided with nodular thickenings present in some of the genera which provide anoxygenic environment for the action of nitrogenase enzyme, eg. Nostoc, Anabaena, Aphanizomenon, Gloeotricha.
- d) They have a capacity to form symbiotic association with a variety of eukaryotic organisms including plants and also with non photosynthetic organisms like fungi (in form of lichens), sponges, ascidians and corals. These associations can be best exemplified by Nostoc - Sphagnum

(bryophyte), Anabaena azollae - Azolla Nostoc (pteridophyte). Cycas Nostoc (gymnosperm). Gunnera (angiosperm), Nostoc & Anabaena - rice fields and even with other group of algae 'diatoms'.

Central vacuole is absent in cells but to planktonic forms buoyancy is provided by pseudovacuoles comprised of gas vesicles hence making them float that can result in formation of surface blooms. Bloom forming cyanobacteria can discolour the entire water body and release a variety of toxins which may be neurotoxins. hepatotoxins or cytotoxins detrimental to human health and fishes hence making the water unpotable. Major bloom forming taxa are Anabaena. Microcystis and Aphanizomenon, others being Oscillatoria<br>and Spirulina<sup>11,9,12</sup>.

#### **Importance**

Cyanobacteria play important role in ecosystem and have multiple uses in the biotechnology. These are the first oxygenic photosynthetic organisms that made today's aerobic environment to live in by releasing oxygen. Cyanobacteria like **Nostoc** commune. Spirulina, Arthrospira, Aphanizomenon can be used as nutrient source, health supplement and as animal feed. Being a rich source of protein and vitamin B  $_{12}$  Spirulina tablets are commonly being sold in market. Arthrospira extracts can be used in cosmetics due to moisturizing effects. They are also used in sewage disposal and wastewater treatment by the supply of oxygen required by bacteria for oxidative breakdown as done by Nostoc, Scenedesmus, Anabaena. Because of their nitrogen fixing capacity, Aulosira fertillisima, Nostoc, Anabaena doliolum are excellent biofertilizers thereby playing role in increasing the soil fertility. Cyanobacteria may be used for production

of secondary metabolites and bioenergy production<sup>12,3</sup>

Free floating. unattached photosynthetic micro-organisms are together known as phytoplanktons. Their growth is affected by temperature, light and<br>nutrients<sup>13,14</sup>. In an aquatic ecosystem phytoplanktons are the primary producers, the estimate of which can be used as water quality indicator<sup>15</sup>. Being easily available, cheap in use, more convincing and informative, algae are used as bioindicators<sup>16</sup>. Changes in water quality of aquatic system can be studied through bioassessment<sup>14</sup>. Most of the contribution to organic carbon made available to in the aquatic food web is done by phytoplanktons as they play a key role in global carbon cycle through assimilation of carbon and oxygenic photosynthesis<sup>3</sup>.

Generally, an alga maintains dissolved oxygen (DO) and aerobic environment of through photosynthesis water body necessary for bacterial decomposition of organic matter and respiration of aquatic fauna<sup>13</sup>. DO values of  $4.3 - 14.59$  mg/L have been reported in Chambal river by<br>many workers<sup>17, 18, 19, 20</sup>.

#### **Cyanobacterial Biodiversity**

Biodiversity, also known as biological diversity, can be defined as variation among species and habitats of living organisms be it at gene level; species level or at ecosystem level. Three types of biodiversity are known  $-\alpha$  diversity (local),  $\beta$  diversity (turnover) and  $\gamma$  diversity (regional). In general  $\alpha$ diversity is the diversity of life forms within a habitat<sup>21</sup>. Some workers have studied the cyanobacterial biodiversity of Chambal River in past years along with its water quality.

Table 1. Cyanobacterial Biodiversity of Chambal River in Rajasthan



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Bhatnagar and Bhardwai  $(2013 b)^{17}$ reported total 13 genera and 21 species of cyanobacteria during their study. Genera that were very common are Merismopedia. Oscillatoria and Microcystis. In an another study conducted by Bhatnagar and Bhardwai  $(2013 a)^{22}$  at Kota Barrage constructed on Chambal river, total 10 genera and 18 species were reported by them both in upstream and downstream of the river. According to authors, cyanobacteria showed dominance during summer season. They also investigated Microcystis aeruginosa and Osillatoria sp. in downstream of river as pollution tolearant genera.

Cyanobacterial diversity was also studied at Rana Pratap Sagar Dam and Chambal river by Gaur et al. (2014)<sup>18</sup>. They reported 5 genera- Oscillatoria sp., Microcystis sp., Phormidium sp., Nostoc sp., Anabaena sp. of common occurrence.

On the basis of research work done by these authors, all the reported genera and species of cyanobacteria are enlisted in table 1.

A wide variety of taxa of cyanobacteria have been reported in Chambal River. On the basis of the available literature studied 21 species of 13 different genera are known to occur in such an elaborate freshwater river system of Rajasthan. These versatile photosynthesizers are important economically and may be utilized for SCP, biofuel production etc. There is a requirement of their further documentation to be used commercially. With the rising effects of global warming and temperature changes, there is a possible risk of loss of biodiversity. Therefore, there is a need to study cyanobacterial flora of protect River Chambal and the cyanobacterial biodiversity in the present scenario.

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#### **PHYTOPLANKTONIC DIVERSITY AND PHYSICOCHEMICAL CHARACTERISTICS OF KISHORE SAGAR, KOTA, RAJASTHAN**

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Wetlands cover the major area of the earth. Aquatic systems are greatly affected by the environmental conditions and the organisms residing within. Kishore Sagar is a artificial lake located in the centre of the city and its water is mainly used for agricultural purposes through a connecting canal system to Rajasthan and Madhya Pradesh. The lake is at a risk by many anthropogenic activities which affect the water quality. It harbours many species of phytoplantons which are mainly responsible for the productivity of any wetland. The present study is an attempt to investigate the water quality of Kishore Sagar in reference of its phytoplankton diversity. The present paper reveals that the phyoplanktonic diversity comprises of 33 species. Total 8 species belonging to 8 genera of Class Chlorophyceae, 17 species belonging to 8 genera of Class Cyanophyceae and 8 species belonging to 6 genera of Class Bacillariophyceae were identified during the study period.

Keywords: Carbon cycle; Diversity; Phytoplankton; Pollution; Water quality.

#### **Introduction**

Water forms the basis of life and forms the part essential  $in$ urbanization. industrialization and agricultural activities. Sources of pollution may be natural and runoff, agricultural, industrial and domestic wastes. Wetlands cover the major part of the earth. On the basis of current and stratification, fresh waters can be divided as standing waters and running waters<sup>1</sup>. Freshwater aquatic systems sequester a good amount of global carbon through carbon  $cycle<sup>2,3</sup>$ . Greatest values of ecosystem goods and services per unit area of all habitats have been assigned to freshwater aquatic ecosystems<sup>4</sup>. Health of an aquatic ecosystem depends on the water quality and the living content residing within including both plants and animals. Phytoplanktons are the important components of the aquatic ecosystems which are free floating, unattached photosynthetic micro-organisms. They are the primary producers which occupy the first trophic level in a food chain. Their abundance is affected by the water quality of an aquatic system. Estimation of phytoplankton diversity can be used as a water pollution indicator<sup>5</sup>. Phytoplanktonic diversity and the physicochemical parameters have been studied at various places of different lakes by many workers<sup>6,7,8,9,10,11,12</sup>. Work done by Kumar et al. on Kishore Sagar deals with diversity of diatoms only<sup>13</sup>. This study is an attempt to

investigate the phytoplankton diversity of Kishore Sagar Lake in relation to its water quality.

## **Study Area**

The small lake in Kota better known as Kishore Sagar Talab is an artificial lake which was constructed in 1946 by Prince

Dher, Deh of Bundi. It is located in the centre of the city with location 24°25.675" N latitude & 76°37.348" E longitude<sup>13</sup>. It is walled all over with a beautiful seven wonders park at one side and talab ki paal at the opposite side  $(Fig. 1)$ .

Fig. 3. Inlet point with showing

human interference



Fig. 1. View of Kishore Sagar Fig. 2. Dam constructed on lake Lake showing Jagmandir in the centre

A round of the lake can be taken by road all over. Jagmandir is situated in the middle of the lake which was constructed in 1745 by then queen of Kota for entertainment. It is accessible by boat. A dam is constructed on the lake which is 1500 m long and 5.6 m high (Fig. 2) and is used for distribution of water for crops during Kharif and Rabi seasons through a canal to Rajasthan and Madhya Pradesh. The lake has inlet and outlet of Chambal river water on regular basis<sup>13</sup> (Fig. 3). The talab is almost irregular rectangular in shape. Water is mainly used for agricultural purposes and recreational activities. The lake went under renovation after 2013 to keep the water level up to the mark in such a way that it neither makes lake occupied by green herbs nor it becomes an open land for cattle grazing as was the condition twelve years back in  $2005^{13}$ . Deity idols are also immersed every year after ganesh chaturthi and navratri which adds pollution through organic matters and chemicals. Anthropogenic activities are one of the sources

of its pollution.

#### **Materials and Methods**

Three sampling sites were selected for the present study in Kishore Sagar Talab in April 2017. Surface water samples were collected in one litre capacity plastic bottles between 9 to 11 am in the morning for water quality analysis. Water temperature and pH were determined on the spot. The samples were brought collected to laboratory for the analysis of other parameters like alkalinity, dissolved oxygen, nitrate, sulphate etc. Physicochemical parameters of water were analyzed within 24 and we<br>methods<sup>5,14,15,16</sup> using standard

The sampling for phytoplankton was done from the study site on the same day of water sampling. The collected samples were fixed and preserved in Lugol's Iodine solution until further analysis in laboratory. For taxonomic identification, a research microscope (Metzer) and a imported camera MD500 was used. The identification of algal samples was done with the help of standard
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references<sup>17,18,19,20,21,22,23</sup> **Result and Discussion Physicochemical characteristics:** Results of physicochemical parameters are summarized in Table 1. Temperature

Water temperature varies with the environmental conditions. The surface water temperature was observed 28.3°C which is almost same as was observed by Kumar et  $al.^{13}$  They observed temperature of 29 $\degree$ C in the same month during 2005.

Table 1. Mean values of physicochemical parameters of Kishore Sagar for the month April 2017

S.No.	Parameter (Unit)	Month-April 2017
1.	Temperature $(C)$	28.30
2.	pH (Units)	8.67
3.	$EC (\mu S/cm)$	444.33
4.	Turbidity (NTU)	10.91
5.	Total Alakalinity (mg/L)	126.67
6.	Total Hardness (as CaCO3) (mg/L)	173.33
7.	Calcium Hardness (as CaCO3) (mg/L)	100.00
8.	Magnessium Hardness (as CacO3) (mg/L)	73.33
9.	Chloride (as Cl) (mg/L)	60.00
10.	Nitrate (as NO3) (mg/L)	4.33
11.	Sulphate (as $SO4$ ) (mg/L)	18.00
12.	Fluoride (mg/L)	0.67
13.	Total Dissolved Solids (mg/L)	311.00
14.	Total Suspended Solids (mg/L)	34.33
15.	DO(mg/L)	6.93
16.	$COD$ (mg/L)	130.00
17.	BOD (mg/L)	4.13

## **Turbidity**

Along with suspended and colloidal matter, silt and clay, phytoplanktons also increase turbidity of water<sup>15</sup>. It can be used as an index of productivity. Turbidity was measured 10.91 NTU during the study period which is almost a unit higher beyond permissible limit of 10.

## pH

The pH value was measured 8.67 units. The

reason for higher range of pH can be attributed to presence of bicarbonates and carbonates of alkali and alkaline earth metals, sewage addition and through human interference. The lake water can be said to be 'slightly alkaline' as was investigated by<br>Kumar et al. in  $2005^{13}$ .

## **Electrical Conductivity (EC)**

Electrical conductivity is directly proportional to dissolved ions present in

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water<sup>4</sup>. This parameter is affected by the total dissolved salts. It was measured 444.33 µS/cm which is contradictory to what was observed by Kumar et  $al^{13}$ . They measured EC of  $293 \mu S/cm$ . High conductivity also indicates high productivity. With this high value, the lake can be classified as mesotrophic<sup>10</sup>.

## **Total Alkalinity**

In the study period, mean value of 126.67 mg/L was measured for total alkalinity which is on lower side from that was found in 2005 by Kumar et al.<sup>13</sup>. Mostly total alkalinity is caused due to dissolved bicarbonates. High alkalinity of water indicates high productivity of water. Richness of phytoplanktons increases the productivity of water<sup>15.</sup>

#### **Total Hardness**

Property of hardness of water is due to a complex of mixture of ions. The hardness is due to mainly calcium and magnesium ions. Total hardness is due to both calcium hardness and magnesium hardness. In wetlands, soil characteristics influence total hardness of water<sup>12</sup>. Kumar et al.<sup>13</sup> has categorized the Kishore Sagar Lake as 'nutrient - rich' as total hardness of more than 60 was observed by them with the result of 94 mg/L. In the present study, total hardness of 173.33 mg/L was observed. Hence with this reading, it can still be categorized as 'nutrient  $-$  rich'<sup>1</sup>

Kumar et al. observed calcium hardness of 60 mg/L in 2005 while the calcium hardness of 100 mg/L was observed in the present study. The data reveals a jump in the calcium hardness of water in this span of time from 2005 to 2017.

The magnesium hardness was recorded as 73.33 mg/L during the study period which is almost the double of that observed by Kumar et  $al$ <sup>13</sup>. In this leap of twelve years, the parameter has raised from 34 mg/L

 $(in 2005) to 73.33 mg/L (in 2017).$ 

The total hardness of water comprising of both calcium  $\&$  magnesium hardness depicts that the water is hard in terms of hardness<sup>16</sup>

## **Chloride and Fluoride**

High chlorine concentration is a pollution indicator. Chloride was estimated as 60 mg/L and fluoride as 0.67 mg/L in the present water body which are both below the permissible limits. As the water is supplied for crops, the water is safe for agricultural purposes being below 250 mg/L.

## **Nitrate and Sulphate**

Nitrate is the highly oxidized form of nitrogen. Nitrate – Nitrogen was found to be 4.33 mg/L and sulphate as 18 mg/L. Values for nitrate content higher than 10mg/L are toxic for human consumption. The nitrate and sulphate concentrations have been increased from 2005 to 2017 when compared to the work of Kumar et  $al$ <sup>13</sup>. They observed  $0.106$  mg /L of nitrate nitrogen and 3.2 mg/L of sulphate. Higher values of nitrogen as nitrate tend to increase net productivity of aquatic ecosystems. The attributed this reason to increased concentration of nitrate is increased disposal of domestic effluents, sewage disposal, decayed vegetables, animal matter and anthropogenic activities which has increased from 2005 to 2017 with increasing population and hence indicate polluted status of the lake. Sulphates add hardness to water. Sulphates may be added from sedimentary rocks through leaching from their weathering, being converted  $t<sub>O</sub>$ sulphate compounds adding in water<sup>16</sup>

## Total Dissolved Solids (TDS) and Total **Suspended Solids (TSS)**

TDS is a parameter that is due to various salts like chlorides, sulphates, nitrates, phosphates, carbonates and bicarbonates of elements like sodium, magnesium, iron etc.

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dissolved in water. It is an estimate of dissolved organic and inorganic waste<sup>5</sup>. TDS value of 311 was observed during the study period. Higher TDS value may be the reason for higher EC. Mean TSS value found was 34.33. TSS is amount of suspended salts of metals.

## **Dissolved Oxygen (DO)**

DO is one of the most important parameters for water quality. Chemical, physical and biological processes of a water body have an influential effect on the DO level. DO levels are important for the maintaining aquatic life. The mean value of dissolved oxygen was estimated 6.93 mg/L in Kishore Sagar in the present study which is suitable for the fishes. In the past years, the DO levels have raised from  $3.6$  mg/L (2005) to  $6.93$ mg/L (2017). As there is regular periodic inlet and outlet of water through dam, there remains a fluctuation in the organic matter addition to the lake water. This may be the reason for higher DO level during the study period. Due to higher temperatures in summer months. DO levels remain on lower side due to increased biological oxidation<sup>16</sup>

## **Biological Oxvgen Demand (BOD)**

BOD value of 4.13 mg/L was estimated during the study period. The BOD is directly proportional to the amount of oxidizable organic matter present in water and is an important parameter for pollution detection<sup>15</sup>. The BOD values lie somewhere between reasonable and tolerable category<sup>16</sup> of water. BOD values suggest the capacity of a water body for self purification.

#### **Chemical Oxygen Demand (COD)**

COD is a major parameter for pollution measurement. Like BOD, it is also a measure of oxygen requirement. COD values are always higher than BOD values as found in this period of study also. COD

value of 130 mg/L was measured during the study period.

#### **Algal diversity**

The phytoplankton diversity of Kishore Sagar Lake comprises of various types of single-celled, colonial and filamentous forms of algae belonging to different classes viz. Chlorophyceae, bacillariophyceae (both belonging to centrales and pennales) and cyanophyceae as listed in table 2.

## **Systematic Enumeration and Description** of some of the studied species Chlorophyceae

## Coelastrum microporum Nagaeli

Cells spherical or polygonal arranged to form hollow spherical or many sided coenobium with 2-64 cells, with the narrow end outwardly directed, cells 3.75-15.00 µm long, cells adjoined laterally by very short interconnecting protuberances  $\alpha$ f the mucilaginous sheath<sup>17,20,23</sup>

## **Eudorina elegans Ehrenberg**

Cells spherical and evenly spaced out within the colonial mucilaginous matrix to the edge leaving a clear zone at the centre, cells arranged in transverse bands or tiers<sup>17,18,2</sup>

## Pandorina morum Bory.

Colony spherical or oval, cells pear shaped, 8-32 densely/ compactly packed cells embedded in mucilage that extends beyond the cells at the colony edge, two equal length flagella present, cells 8-20 µm  $long<sup>17,22</sup>$ 

## Pediastrum duplex Meyen var. duplex

Colony 16-32 celled, walls smooth with lens shaped space between the inner cells, cells quadrate, the inner side of marginal cells distinctly concave, peripheral cells quadrate, the outer margin extended into two tapering truncate, blunt-tipped processes, colony upto 50-55 um in diameter, cells 8-10 um in<br>diameter<sup>17,22.23</sup>

Scenedesmus quadricauda (Turp.) de Breb

 $35$ 

Cells ovoid or ellipsoid, adjoined by their series, colony linear, two outer cells of the lateral wall to form a row of 4 in a single Choubisa & Dubey 36

Table 2. Phytoplankton diversity observed in Kishore Sagar during study period of April 2017



series bear a long curved spine at their poles  $17,18,20$ .

#### Staurastrum rotula Nordst

Cell not compressed, apex of the cell extended, poles bearing 3 or more arms or lobes so that the cell appears radiating when<br>seen from the top<sup>17,18,20</sup>.

**Tetraedron** trilobulatum (Reinsch) Hansgirg

Cells triangular, 19.5-21.4 um in diameter, angles of cells broadly rounded<sup>23</sup>.

#### Treubaria crassipina G.M. Smith

Cell tetrahedral, each apex with a long stout spine, spines broader at the base and stout, decidedly tapering, processes are not toothed at the tip<sup>17,20</sup>

#### Cyanophyceae

Aphanocapsa roeseana de Barry

Colony irregular and embedded with mucilage, cells spherical to sub-spherical, light green to yellowish and cells 5.26  $\mu$ broad and 7.89  $\mu$  long<sup>19</sup>

Arthrospira platensis (Nordst.) Gomont

Trichomes slightly constricted at the cross walls, not attenuated at the ends or only a little attenuated, more or less regularly spirally coiled, end cells broadly rounded, distance between the spirals  $43-57 \mu$ , spirals 2-6µ long and  $26-36\mu$  broad<sup>19</sup>.

Chroococcus dispersus (v. Keissler) Lemm.

Cells 4-8, 16 or more in a tabular mucilaginous free swimming colony, with round margins, without sheath, 3-4 µ broad, with sheath 5-6 µ diam., individual envelopes often totally gelatinized, not lamellated, colourless<sup>19</sup>

## Chroococcus tenax (Kirchn.) Hieron

Cells mostly in groups of 2-16, blue green, sheath colourless to brown, distinctly lamellated<sup>1</sup>

#### Chroococcus turgidus (Kutz.) Nag

Cells spherical or ellipsoidal single or in groups of 2-4, sheath colourless and distinctly lamellated<sup>19</sup>

Merismopedia elegans A. Br.

## **FRESHWATER CYANOBACTERIAL DIVERSITY UNDER THE INFLUENCE OF A THERMAL POWER PLANT**

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#### Abstract:

Cyanobacteria are the most primitive, prokaryotic photosynthetic organisms that evolved almost 3.5 billion years ago and are still in existence. They are also known as blue green algae because of the dominant phycocyanin pigment which imparts bluish colour to them. They can occur in various habitats and forms. Being autotrophs, cyanobacteria occur at the first trophic level in any aquatic food chain and are affected first if any change is detected in an aquatic ecosystem. Aquatic ecosystems play major role in global carbon cycle by photosynthesizers. Coal fired thermal power plants use water as a coolant from a nearby source of water which can be a river, lake or artificial pond. This heated water is then discharged back into that source of water. The discharge of heated water may have various ecological effects of variable degree on aquatic ecosystems affecting both animals and plants. This high temperature of water can lead to changes in different physico-chemical parameters of water, may alter the structure of a community, can cause eutrophication and may even lead to loss of biodiversity. In the present study total 19 species of cyanabacteria were observed during June 2017 to July 2018 at a site under the influence of a thermal power plant.

#### Keywords: cyanobacteria, biodiversity, prokaryotic, ecosystem, power plant

#### **L INTRODUCTION**

Cyanobacteria, also known as blue green algae, are the most primitive plants similar to bacteria which are found throughout the world in tropical, temperate and even polar regions in various forms. Cyanobacteria are the only photosynthetic pigment containing organisms that can survive high temparatures of 82°C (Smith, 1994).

Rajasthan is the largest state of India. Besides solar plants, it generates power through seven power plants at different locations. Thermal power of plant of Kota is one of the oldest thermal power plants of Rajasthan having total installed capacity of 1240 MW with seven units in operation (www.rvunl.com). Thermal power plants use coal as combustible substance for the production of energy. Generally, the waste heat is disposed from the power stations by withdrawal of water from a nearby water source passing it through the steam condenser and then again returning to the water source. Thousands of cusecs of water is utilized as coolant in 'once --through cooling' process (Hill, 1972). Kota thermal power plant uses Chambal river water for this

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purpose. The thermal effluents have diverse unwanted ecological effects, of variable degree, on the aquatic ecosystem affecting plants and animals (Bobat, 2015). This high temperature of water can lead to changes in different physico- chemical parameters of water, may alter the structure of a community, can cause eutrophication and may even lead to loss of biodiversity.

Water quality and its biotic life determine the status of an aquatic ecosystem. Physico-chemical analysis of Chambal river water has been studied by many workers (Bhatnagar and Bhardwaj, 2013; Chouhan et al., 2018; Gupta et al., 2011; Jain, 2012). Bhatnagar and Bhardwaj (2013) have reported 18 species of 10 genera of cyanobacteria at Kota Barrage. The present study is an attempt to study water quality parameters of the river freshwater and the diversity of cyanobacteria under the influence of thermal power plant from June 2017 to July 2018.

#### **II. RESEARCH METHODOLOGY**

2.1 Study area: The study was conducted in Kota city of Rajasthan. The city is located at 25°10'57.1" N latitude and 75°50'20.7" E longitude in south eastern part of Rajasthan (Geographic coordinates of India). Chambal river is the only perennial river of desert land of Rajasthan in India. The river is one of the major tributaries of Yamuna river originating from Janapao Hills of Vindhya range in south of Mhow town of Madhya Pradesh (Encyclopedia Britannica; World Water Database). The river also connects with the holy spirits of local residents of Kota. The left bank of Chambal river in the centre of Kota city is flanked by one of the thermal power stations of Rajasthan that uses the river water as coolant (www.mapsofindia.com). The site is selected as near as possible to the power plant to study the cyanobacterial diversity under its influence.



Fig 1: Course of Chambal river through Kota city with location of Kota Thermal Power Station

2.2 Research Design: The design for the present study is experimental.

2.3 Sample Design: The sample unit is individual of a cyanobacterial community and sampling is done by simple random probability sampling method.

2.4 Data: The primary data is collected through experiments and observations during the study period.

#### 2.4 Materials & Methods:

One sampling site was selected for the present investigation in the vicinity of the thermal power plant situated near Kota Barrage. The Kota Barrage is a dam constructed on the Chambal river that divides the flow of river into upstream and downstream. For the study of cyanobacterial diversity the surface water samples were collected from the sampling site from June 2017 to July 2018 between 9 to 11 am in the morning. The collected samples were studied within 48 hrs with the preparation of fresh mounts in laboratory. For further detailed examination the samples were also fixed and preserved using Lugol's Iodine solution. With the aid of available standard keys and monograph (Bellinger & Sigee, 2015; Desikachary, 1959; Palmer, 1980; Prescott, 1954; Smith, 1950) identification of phytoplanktonic Cyanobacteria was done using Metzer research microscope and imported camera MD500

To determine the physico-chemical characteristics the water was collected from the same site in one liter capacity plastic bottles. pH and temperature were determined immediately after the sample collection on the spot only. The samples were brought to the laboratory for the analysis of parameters like total alkalinity, total hardness, chlorides, total dissolved solids, dissolved oxygen, BOD & COD. For the determination of physicochemical parameters of water the methods prescribed by APHA (1999), Maiti (2004) and Gupta (2007) were followed

#### **III. RESULTS AND DISCUSSION**

#### A. Physico-chemical characteristics

#### Temperature

It is a vital factor that regulates aquatic environment. The surface water temperature was observed 30.1°C at its lowest in Dec and highest in the month of May at 42.2°C. The mean value for all seasons was observed almost 10 degrees higher than what was observed by Bhatnagar and Bhardwaj (2013). Pre-monsoon study done by Chouhan (2018) during the same time period suggests the same data of  $30^{\circ}$  C as observed in Winter season. With the discharge of heated effluents the water temperature also changes.

#### **nH**

pH is one of the most important properties of water. The pH value of the sampling site ranged between 7.5 to 8.1 units during the study period This is may be due to the presence of bicarbonates and carbonates of alkali and alkaline earth metals, sewage addition and through human interference. Similar results were also observed by Bhatnagar and Bhardwaj (2013). The water can be said to be 'slightly alkaline'

#### **Electrical Conductivity (EC)**

Electrical conductivity is related to dissolved ions present in water. The maximum value of EC was recorded 487 µS/cm in June month of summer while minimum in rainy season of August with 297 µS/cm (Annual average  $342.5 \mu S/cm$ ). High conductivity also indicates high productivity. With this high value, the river water can be classified as mesotrophic.

#### **Total Alkalinity**

In the study period annual average value of 116.6 mg/L was measured for total alkalinity. The mean values for all the seasons did not show much variation throughout the study period but it is contradictory to what was recorded by Bhatnagar and Bhardwaj (2013). Mostly total alkalinity is caused due to dissolved bicarbonates. High alkalinity of water indicates high productivity of water.

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#### **Total Hardness**

This property of water is ability to precipitate soap and is mainly attributed to complex of mixture of calcium and magnesium ions. In wetlands, soil characteristics influence total hardness of water. In the present study annual total hardness of 130.2 mg/L was observed which indicates that water is quite hard being more than 120 mg/L. Bhatnagar and Bhardwaj (2013) observed the total hardness of 99.5 mg/L in monsoon season which is contradictory to present studied monsoon season observation of 128 mg/L.

#### Chloride

High chlorine concentration is a pollution indicator. During June Chloride was estimated maximum at 50 mg/L. As the water is supplied for crops, the water is safe for agricultural purposes being below 250 mg/L.

#### **Total Dissolved Solids (TDS)**

TDS is an estimate of dissolved organic and inorganic waste. It is a parameter that is due to various salts like chlorides, sulphates, nitrates, phosphates, carbonates and bicarbonates of elements like sodium, magnesium, iron etc. dissolved in water.. Highest TDS value of 287.4 mg/L was observed during the summer season. Higher TDS value may be the reason for higher EC.

#### Dissolved Oxygen (DO)

DO levels are important for maintaining aquatic life. It is also one of the most important parameters for water quality determination. DO level of a water body is influenced by chemical, physical and biological processes of a water body. The annual mean value of dissolved oxygen was estimated 6.6 mg/L in the present study which is suitable for the fishes. DO levels are inversely proportional to temperature (Aery, 2010). Summer season has lower DO levels with rise in temperature. Due to increased biological oxidation in summer months, lower level of DO was observed.

#### **Biological Oxygen Demand (BOD)**

BOD is an important parameter for water quality and pollution detection. It is directly proportional to the amount of oxidizable organic matter present in water. It depends on DO and various types of micro organisms present in the water body. BOD value of 1.6 mg/L was estimated highest in summers and lowest in winters with 0.6 mg/L during the study period. Annual average BOD value of 1.2 mg/L suggest lower level of organic matter in water as was also concluded by Murulidhar and Murthy (2015) in their study. BOD values suggest the capacity of a water body for self purification. BOD values are always lower than COD values as also found in the study period (Aery ,2010). Higher BOD values in summer season indicates richness in cyanobacterial population.

## **Chemical Oxygen Demand (COD)**

COD is also a major parameter for pollution measurement. Like BOD, it is also a measure of oxygen requirement. Highest values of COD 44.3 mg/L was observed in the monsoon months and lowest in summer season during the study period clearly indicating the abundance of cyanobacteria in summer season. Bhatnagar and Bhardwaj (2013) also reported the maximum taxa of cyanobacteria in summer season.

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Table 1: Physico-chemical characteristics of water during July 2017 to June 2018



## **B.** Cyanobacterial Diversity

Table 2 represents the cyanaobacteria that were observed at the sampling site under the influence of thermal power plant during the study period.







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PlateA: a. Anabaena b. Merismopedia c. Aphanocapsa d. Microcystis e. Gloeocapsa f. Oscillatoria g. Chroococcus i. Spirulina

#### Systematic Enumeration and Description of some of the studied species:

#### Anabaena sp. Bory

Uniformly broad trichomes, sheath absent, intercalary heterocysts.

#### Aphanocapsa sp. Nag.

Colony many celled, irregular and embedded within a galatinous mass, cells spherical to sub-spherical, individual sheaths around the cells absent.

#### Chroococcus sp. Nag.

Cells spherical or subspherical or hemispherical, in group of 2-16 , mucilaginous free swimming colony, with round margins.

#### Chroococcus tenax (Kirchn.) Hieron

Cells mostly in groups of 2-16, blue green, sheath colourless to brown, very distinctly lamellated with 3-4 lamellae, without sheath 16-21 µ.

#### Chroococcus turgidus (Kutz.) Nag

Cells spherical or ellipsoidal single or in groups of 2-4, sheath colourless and not distinctly lamellated,  $13-25\mu$ diameter with sheath.

#### Gloeocapsa sp. Kutzing

Cells spherical, 2-8 in colonies with a number of concentric special envelopes, individual sheaths lamellated or unlamellated, arranged irregularly.

#### Lyngbya Ag.

Trichome single or free in a thin or very massive thick, firm sheath, mostly colourless sheath

#### Merismopedia elegans A. Br.

Colonies small or big, 16-4000 celled, light blue, cells spherical or oblong, more or less closely arranged, light blue, cell 5-7 µ broad and 6-9µ long.

#### Merismopedia glauca (Ehrenb.) Nag.

Colonies mostly small with 16-64 cells, rarely more, cells oval to spherical, closely arranged, 45-150 µ diameter, pale blue-green, cell 3-6 µ broad and 4.5 µ long.

#### Merismopedia minima Beck

Free swimming, cells 4 to many in small colonies, pale blue-green ,0.5-0.6 µ broad.

#### Merismopedia tenuissima Lemm.

Cells subspherical and closely packed in colonies of  $16-100$  cells,  $1.3-2$   $\mu$  broad, sometimes individual cells with distinct mucilaginous envelopes, pale blue-green.

#### Microcystis aeruginosa Kutz.

Colonies when young round or slightly longer than broad, cells spherical, generally with gas vacuoles, cell 3-7 µ diameter, colony solid but when old become clathrate , with distinct hyaline colonial mucilage.

#### Microcystis flos-aquae (Wittr.) Kirchner

Colonies roughly spherical, ellipsoidal or somewhat elongate often squarish in optical section, cells spherical, with gas vacuoles, cell  $3-7$   $\mu$  in diameter, not clathrate, with indistinct colonial mucilage.

#### Nostoc Vaucher

Mucilaginous thallus, free or attached, periphery dense and darkly coloured, filaments curved or entangled, intercalary heterocysts, cells depressed, spherical or cylindrical.

#### Oscillatoria limosa Ag. ex Gomont

Trichome straight, not constricted at the cross walls or only slightly constricted, commonly 13-16µ broad, 2-5 µ long, cross walls frequently granulated, end cell flatly rounded with slightly thickened membrane.

#### Oscillatoria princeps Vaucher ex Gomont

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Trichome mostly straight, not constricted at the cross walls, slightly capitate, rounded calyptras present, endcells thick walled,  $16-50 \mu$  broad and 3.5-7  $\mu$  long.

#### Oscillatoria tenuis Ag. ex Gomont

Trichome straight, slightly constricted at the end-walls, not attenuated at the ends, cap absent, thallus thin and slimy, pale blue green or olive green, end cell more or less hemispherical, septa mostly granulated, 2.6-5 µ long and  $4-10 \mu$  broad.

#### Spirulina sp. Turpin em. Gardner

Trichomes unicellular, sheath absent, loosely or tightly coiled into a more or less regular spiral, terminal cell rounded, without calyptra.

#### Synechocystis sp. Sauvageau

Cells spherical, single or two together after cell division, without distinguishable mucilage envelopes, rarely in colonies.

#### **IV. CONCLUSION**

Higher temperature and BOD values clearly indicate the abundance of Cyanobacteria. Anabaena, Merismopedia and Oscillatoria were very common during the study period. Different species of Merismopedia dominated in summer season even at temperature of 42.2°C. Total 11 genera with19 species were observed at the sampling site. Further studies on water parameters and cyanobacterial diversity can be used to gain some more information which can help in deciding the biodiversity status of the water body to conserve the biodiversity.

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