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Influence of Physico-chemical Parameters on Mycoflora of Fish Pond in Bhadra Reservoir Project at Shivamogga district, Karnataka, India

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ABSTRACT: Physico-chemical parameters play a key role in determining the diversity and occurrence of fungal species in a freshwater ecosystem. The present work aimed to study the influence of some Physico-chemical parameters on fungal distribution, seasonally from freshwater carp ponds in the Bhadra reservoir project fish pond, Shivamogga, Karnataka, which is one of the main fish seed production farms. A study of eleven different parameters was carried out in three different seasons. The water temperature (18.75±1.57 to 28.45±3.29ºC), atmospheric air temperature (20.60±0.36 to 31.83±0.58ºC), pH (7.23±0.37 to 8.09±0.33), watercolor (light green-light brown), total dissolved solids (182.67±2.08 to 291.00±1.00 mg/L), dissolved oxygen (4.45±0.44 to 8.74±0.23 mg/L), biochemical oxygen demand (1.62±0.01 to 3.22±0.03 mg/L), carbon dioxide (1.07±0.06 to 8.60±0.35 mg/L), chloride (15.57±0.09 to 30.27±0.23 mg/L), alkalinity (0.67±1.15 to 46.33±0.58 mg/L), hardness (47.07±0.12 to193.00±1.73 mg/L), nitrate (40.00±0.00 to 51.00±1.00 mg/L) and ammonia (0.15±0.09 to 0.30±0.00 mg/L). The result showed that the maximum growth of fungal species in all fishes in the winter season may be due to low temperatures, neutral pH, high nutrient status (high nitrate), and large amounts of oxidizable organic matter (high total dissolved solids). Growth in rainy seasons may be due to the availability of high rainfall and surface run-off along with the nutrients and whereas a decrease in fungal species during summer may be due to high water temperatures and low nutrient status. The main challenge laid in identifying the main physico-chemical parameter which is confined to the aquatic ecosystems as the maximum level may become toxic to fishes and change their enzyme activities, oxidative stress response, and respiratory activities. Analytical measurement of water improves the production and survivability of fish.

Keywords: Indian major carps, Bhadra reservoir project, Physico-chemical parameters, spawns.

INTRODUCTION

Nutrition is one of the most vital factors and it contributes up to 50% of fish production (Djissou *et al.*, 2016). Therefore, fish breeding has been found necessary to increase fish production to make fish protein available to the population. Indian major carp (IMC's) such as *Catla catla, Labeo rohita,* and *Cirrhinus mrigala*, and minor carp i.e. *Cyprinus carpio* contribute about 87% of the total freshwater aquaculture production in India. Due to their fast-growing nature and taste, carps enjoy a prime position in the Indian aquaculture system of polyculture (Bais, 2018).

Water is the habitat of the fish. All living organisms have tolerable limits of water quality parameters in which they perform optimally (Bhatnagar and Devi 2013). Fish carp though attain maturity in confined or stagnant (pond) water, do not breed there, and need inducement for spawning (Bais, 2018). Physiological processes in fish are often carried out in a harsh aquatic environment, and fish face conditions and challenges that do not exist for terrestrial animals. The maintenance of internal homeostatic equilibrium is essential for the normal functioning of the animal and in case of any disturbance, the fish will try to establish a new equilibrium (Ahmed *et al.*, 2011). The optimal level of water quality parameter helps to increase the growth and production of the fish. A slight change or difference in the desired level of water quality parameters can result in fish being stressed and at risk of diseases. Mainly, most water molds live freely in the water, they can become associated with host organisms as mostly parasites and predators when the condition of the environment alters, or when the host is subjected to various stresses and allow infection (Kiziewicz and

Nalepa 2008). Fungal diseases are the second most serious cause of loss in aquaculture next to bacterial diseases. In India, fishermen are facing severe problems with fungal diseases (Rao, 2017). Fungi can attack fish of all ages. Almost every freshwater fish is exposed to at least one species of fungus during its lifetime (Ramaiah, 2006).

The great reduction of carp due to flooding, over manuring, exotic species invasion, and improper management of the qualitative property of water for the fish pond. Good quality production of fish and fish seeds mainly rely on water quality parameters. Water quality is estimated by physicochemical parameters which directly or indirectly affect the biotic component and aquatic component[s] (Sheikh *et al.,* 2017). Hence, it is necessary to know the tolerable level of water quality parameters of fish.

The present systematic study aimed to provide data on the physicochemical parameter of water in cement fish ponds for the Bhadra reservoir project fish farm at Shivamogga, Karnataka.

MATERIALS AND METHODS

Sampling site: The study area, Bhadra Reservoir Project fish seed production unit is located at 13°42'49"N and 75°38'27"E, Shivamogga district of Karnataka, India and it is one of the largest (40.80 hectares) and oldest fish seed production centers established during 1965-66 having 269 fish ponds and constructed through left bank canal system of Bhadra reservoir dam flooded with Bhadra river (situated near Lakkavalli village of Chikamagalur district, Karnataka).

Sampling. The various sampling ponds are located in the Bhadra reservoir project fish seed production unit shown in Fig. 1. Water samples were collected at monthly intervals for the winter (November, 2018-February, 2019), summer (March, 2019-May-2019), and rainy (June-2019-October, 2019) seasons. The samples were collected between 7 am to 9 am (Fig. 2). Each sample meant for physicochemical analysis was collected using a 5-liter polythene bottle with a screw cap which was thoroughly washed with detergent, then washed with dilute hydrochloric acid (1: 5 ratio), and rinsed twice with distilled water. At the sampling site, the polythene bottle was rinsed with the target water sample before it was filled and properly labeled. All the samples were stored in the laboratory after stabilizing them with 1ml concentrated hydrochloric acid in each container to avoid microbial action on their concentration (Eboagu *et al*., 2019).

Physicochemical analysis. The physicochemical properties such as temperature, pH, and total dissolved solids of freshwater were analyzed at the sampling site and the watercolor was estimated directly by observing the fish pond. Other parameters such as dissolved oxygen, biochemical oxygen demand, carbon dioxide, chloride, alkalinity, hardness, nitrate, and ammonia were analyzed in the laboratory by following the standard method (Eboagu *et al*., 2019) and correlated with standard values of WHO. The method of the analysis of the Physico-chemical parameter of water is described below.

Estimation of temperature. The temperature of each sample was measured in situ at the sampling site using a mercury thermometer. Both air and water temperature value were recorded (Ahmed *et al*., 2000).

Estimation of pH. The pH was determined in situ at the sampling site using a pH meter.

Estimation of watercolor. The color of the water was determined by directly observing fish pond water.

Estimation of Total Dissolved Solids (TDS). The total dissolved solids were measured in situ at the sampling site using a digital TDS meter.

Estimation of dissolved oxygen (DO). The dissolved oxygen of the sample was estimated by using Winkler's method of titration. 250ml stoppered reagent bottle was filled with fish pond water and 2cm^3 of $MnSO_4$ and 2 cm^3 alkaline iodide reagents were added at the side of the stoppered bottle. The bottle was stoppered with care to exclude any air bubbles and homogenized by inverting the bottle until a brown precipitate was formed. The stopper was removed and 2 cm^3 of concentrated sulphuric acid was added to the side of the bottle. The mixture was stoppered and mixed gently by inverting the solution until the solution turned to golden yellow color. From this, 50 ml of the solution was taken into a 250 ml conical flask and two drops of the freshly prepared starch solution were added. The solution turns to bluish-black color. It was titrated with 0.025 M of sodium thiosulphate until the solution turned colorless (Eboagu *et al*., 2019). Dissolved oxygen (DO) was calculated by using the following formula;

$$\text{Dissolved oxygen (mg/L)} = \frac{V \times M \times \text{molar mass of } O_2 \times 100}{V_1}$$

Where V = volume of sodium thiosulphate; M = molarity of sodium thiosulphate; the molar mass of O_2=32 g; V1=volume of sample used.

Estimation of biochemical oxygen demand (BOD). This is a continuation of the dissolved oxygen estimation. 250ml stoppered reagent bottle was filled with the fish pond water sample and immediately analyzed for dissolved oxygen (DO1). Reagent bottles were corked tight and incubated for 5 days at 20 °C in dark conditions. On the 5th day, the amount of dissolved oxygen remaining (DO5) in the incubated sample was estimated using the Winkler method (Eboagu *et al*., 2019). Biochemical oxygen demand (BOD) was calculated as follows:

Biological oxygen demand (mg/L) = DO1 – DO5

Estimation of chloride. 50ml of the water sample was taken in a conical flask and two drops of potassium chromate (K_2CrO_4) indicator was added. Then, the mixture was titrated with silver nitrate solution until the color turns to a light red (precipitation) end-point (Eboagu *et al*., 2019). The amount of chloride was calculated by using the following formula;

$$\text{Amount of Chloride (mg/L)} = \frac{V \times M \times E \times 1000}{V_1}$$

Where V = volume of silver nitrate; M = molarity of silver nitrate; E=equivalent weight of chloride; V_1=volume of sample used.

Estimation of alkalinity. 50ml of the water sample was taken in a 250 ml conical flask and two drops of phenolphthalein indicator were added. There was no color change showing that carbonate was not present. Three drops of methyl orange indicator were then added and titrated with 0.01 M HCl until the solution turned to a pale pink end-point (Eboagu *et al.*, 2019). Total alkalinity was calculated by the following formula;

$$\text{Alkalinity (mg/L)} = \frac{(V \times M \times 100,000)}{V_1}$$

Where V=volume of acid used in titration; M=molarity of HCl; V_1=volume of sample used.

Estimation of hardness. 50ml of the water sample was taken into a conical flask. Then, two drops of Eriochrome Black-T indicator and 2cm^3 of ammonia buffer solution were added. The solution was titrated with 0.01 M EDTA solution. The color changed from wine red to blue-black at the end-point. Total hardness was calculated by using the following formula;

$$\text{Hardness (mg/L)} = \frac{V \times M \times S \times 1000 \times 2.5}{V_1}$$

Where V=volume of EDTA used in titration; M=equivalent weight of Ca; S=strength of EDTA; V1=volume of sample used; 2.5= $\frac{\text{Molecular mass of } CaCO_3}{\text{Atomic mass of Ca}}$

Estimation of nitrate. 1.7 cm^3 of concentrated nitric acid was diluted with 100 cm^3 of distilled water (stock solution). 0.1, 0.2, 0.3, 0.4, and 0.5 cm^3 of the stock nitrate solution were pipetted into a 100 cm^3 volumetric flask to give 1, 2, 3, 4, and 5 ppm, respectively. These formed the working standards. 10 cm^3 of each of the standards was transferred to a set of 100-cm^3 volumetric flasks. To these standards, 2 cm^3 of 30% NaCl solution and 20 cm^3 of concentrated sulfuric acid were added. The mixture was then swirled. 0.2 g brucine reagent was added to all the volumetric flasks which produced a light yellow color. 10cm^3 of each of the samples and a blank were treated with the same reagents as the standards. The absorbance of the standards and the samples were read with the spectrophotometer at 410 nm. The readings of the standard were plotted against their concentration to obtain the calibration curve (Eboagu *et al.*, 2019). The nitrate concentrations were calculated from the calibration curve.

$$\text{Nitrate (mg/L)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard} \times 1000}{\text{Absorbance of standard} \times \text{volume of sample used}}$$

Estimation of ammonia. The ammonia was estimated by using an API freshwater test kit.

Isolation of Fungi. The aquatic fungi were isolated by taking a small portion of infected fish and washed thoroughly with distilled water. These tissues were inoculated over plates containing Potato Dextrose Agar (PDA) media and Sabourd's Dextrose Agar (SDA) media. Streptomycin (50 mg/l) was added to the media to avoid bacterial contamination. After inoculation, the plates were incubated at an ambient temperature (Aneja, 2007; Touhali, 2018).

Identification and Characterization of Fungi. Fungi were identified macroscopically by observing colony morphology and texture and prepared slides using lactophenol cotton blue stain, observed through the light microscope, and compared with authentic manuals (Barnett, 1975).

Frequency of fungal species

Seasonal variation and the percentage of fungal species were analyzed. The incidence of frequency of fungal species in percentage was calculated by using the following formula;

$$\text{Percentage of fungus} = \frac{\text{Total no. of infected fishes} \times 10}{\text{Total no. of observed fishes}}$$

Statistical analysis. All the statistical analyses were performed by ezANOVA (version 0.98) software.

RESULT AND DISCUSSION

Monitoring the Physico-chemical parameters is very important for studying the influence of parameters on the distribution of various fungal species. Water quality is influenced by geological, hydrological, climatic, and anthropogenic factors (Khatri and Tyagi 2015). The Physico-chemical parameter of the water of Bhadra Reservoir Project fish ponds in three seasons (winter, summer, and rainy) was recorded in Table 1-3 respectively.

Temperature is a physical property of water that expresses coldness and hotness in the body of living organisms. Fishes are cold-blooded animals and carp are living in warm water. The temperature is affected by season, Phytoplankton, and Zooplankton, and it directly affects fish productivity. The fluctuation in temperature has an impact on the metabolism, growth, development, and production of carp fishes. The optimum temperature for carp culture is between 24°C and 30°C (Bhatnagar and Devi 2013). In the present study, water temperature ranges from 1(Bhatnagar and Devi, 2013).75±1.57 to 28.45±3.29 (°C) and air temperature from 20.60±0.36 to 31.83±0.58 (°C). Maximum temperature received during summer (Table 2) and minimum in the winter season (Table 1). The variation of water and air temperature is shown in Fig. 1 and 2. The obtained result for temperature was supported by many workers (Nenavath and Kiran 2016; Mehta and Kumari, 2022).

pH is defined as the negative logarithm of hydrogen ion concentration (Bhatnagar and Devi 2013). The average pH of fish blood is 7.4; hence the optimum value of pH in fish pond water is neutral. It has been reported that the pH between 6 and 9 was appropriate for increased fish production (Agbaire *et al.*, 2015). The pH of freshwater ponds can fluctuate considerably both daily and seasonally, these fluctuations are due to photosynthesis and respiration by plants and animals (Dinesh *et al.*, 2017). In the present study, the pH ranges from 7.23±0.37 to 8.09±0.33 (Table 1-3). The current result showed a high pH value (8.09±0.33) during the summer season due to over manuring by the fisherman for the growth of phytoplankton and zooplankton assist the food and shelter for fish in the concrete pond (Table 2). The low value of pH was observed in the rainy season (pH 7.23±0.37) due to the dilution of the rainwater (Table 3). A similar kind of report of the pH was compared with many researchers (Dinesh *et al*., 2017; Mukherjee *et al*., 2022).

Watercolor is an important parameter for carp culture and it represents the pureness of water. The quality of an object or substance for light reflected by the object is usually determined visually by the measurement of saturation and brightness of the reflected light. National Agriculture Extension and Research in 1996 predicted that light green or greenish water is suitable for aquaculture. The abundance of phytoplankton and zooplankton is responsible for the determination of the color of an aquatic body and the Green, bluish-green/ brown greenish color of water indicates a good plankton population hence, well for fish health (Bhatnagar and Devi 2013). In the present estimation, all the carps pond were light greenish color hence the pond of water is suitable for carp culture. The color of pond-3 during summer was light brown color, which may be due to delay in water recycling or long-term storage of water and decreasing water level (Table 2). A similar result of the greenish color fish pond was noticed in Thanjavur, Tamil Nadu (Dinesh *et al*., 2017).

Total dissolved solids (TDS) are present naturally in water and contain organic molecules and minerals that provide nutrients and they also serve as sources of contaminants such as organic pollutants and toxic compounds. Sources of TDS include coal mines, agriculture (which includes aquaculture), and residual road runoffs (Hlordzi *et al*., 2020). Dissolved organic compounds, in the form of humic acids, derived from decaying matter may also contribute to TDS. The wastes from the inhabitants of the pond enhance the total dissolved solids of water of the pond (Elayaraj and Selvaraju 2014). TDS might be due to accumulation of the anthropogenic activity which troubles the quality of water and the growth of diatoms (Senthilkumar and Sivakumar 2008). In the present study, the TDS ranges from 182.67±2.08 to 291.00±1.00 mg/L (Table 1-3). In the present study, the maximum TDS recorded in winter seasons may be due to anthropogenic activity and high organic matter (Table 1). Elayaraj and Selvaraj(2014) estimated TDS (210-380 mg/L) in thachan pond Chidambaram taluk of TamilNadu.

Dissolved oxygen (DO) is the measurement of the amount of oxygen dissolved in the water. The principal source of oxygen in water is atmospheric air and photosynthetic plankton. Obtaining a sufficient source of oxygen is a greater problem for aquatic organisms due to the low solubility of oxygen in water (Bhatnagar and Devi 2013). The solubility of oxygen in water mainly depends on atmospheric pressure, water temperature, and rate of photosynthesis. DO is an important parameter to predict the status or condition of aquatic animals. In the present study, the DO ranges from 4.45±0.44 to 8.74±0.23 mg/L. In the present study, the maximum DO recorded during the winter season may be due to low temperature (Table 1) and the minimum during rainy due to high atmospheric pressure (Table 3). Haque (2016); Sharma *et al.*,(2017) recorded similar kinds of results.

Biological oxygen demand (BOD) refers to the amount of dissolved oxygen required by microorganisms to break down organic material present in a given water sample at a certain temperature over a specific period. The excess entry of cattle and domestic sewage from the nonpoint sources and similar increase in phosphate in village ponds may be attributed to the high organic load in these ponds thus causing a higher level of BOD (Bhatnagar and Devi 2013). In the present study, the BOD ranges from 1.62±0.01 to 3.22±0.03 mg/L and the variation of BOD in three seasons is shown in Table 1-3. Obtained BOD values of all the seasons are present in the permissible limit.

Carbon dioxide is a colorless, odorless gas and extremely dissolves in water to form carbonic acid. The CO_2 in the fish pond is highly influenced by atmospheric air, photosynthetic aquatic plants, respiration of aquatic animals, and decomposition of organic matter by microbes present in the pond. Carbon dioxide is subjected to wide fluctuation because of its capacity to combine with Ca, Mg, and other elements(Saha *et al*., 2017). The present study reveals CO_2 ranges from 1.07±0.06 to 8.60±0.35 mg/L (Table 1-3) and the value obtained study was in the acceptable range. Minimum CO_2 was recorded during the winter season in all fish ponds whereas, in the summer season, it is maximum may be due to increased temperature and increased amount of organic matter (Table 2). Mishra (2023) recorded similar kinds of results.

Chloride is a highly toxic gas. But Chloride ions are nontoxic inorganic anions of all the natural aquatic water bodies. A high concentration of chloride is considered to be an indicator of higher pollution due to higher organic waste of animal origin(Sahni and Yadav, 2017). In the present study, obtained the permissible limit of chloride level ranges from 15.57±0.09 to 30.27±0.23 mg/L (Table 1-3). Whereas a higher amount of chloride analyzed during summer (30.27±0.23) in pond-4 (Table 2) may be due to high temperature and the observed value of chloride was present in the permissible value. The same kind of findings (15.3 to 39.8 mg/L) were noticed in the Thanjavur district of Tamil Nadu (Dinesh *et al*., 2017).

Alkalinity is a measure of the water's ability to neutralize acidity. It also measures the sum of the concentration of bases in pond water like bicarbonates, carbonates, and hydroxides in water. The ideal range of total alkalinity varies from 25-100 mg/L. The average alkalinity during the study fluctuates in the range from 20.67±1.15 to 46.33±0.58 mg/L in all three seasons (Table 1-3). The alkalinity can be increased by adding calcium carbonate and by fertilizing ponds(Mishra, 2023). The lowest alkalinity (20.67±1.15 mg/L) was observed in the pond-4 in the rainy season due to the normal level of rain recorded during the study period (July to October 2019) and the highest value (46.33±0.58 mg/L) in the pond-3 during summer due to evaporation and decomposing organic matter (Bhatnagar and Devi 2013). There was higher alkalinity

(315mg/L) recorded during the summer season around Nadia district, West Bengal (Saha *et al*., 2017).

Hardness is the measure of metal ions such as calcium and magnesium present in a given water sample. Based on the degree of hardness, the water can be divided into soft (0-75 mg/L), moderately hard (75-150 mg/L), hard (150-300 mg/L), and very hard (above 300 mg/L). The optimum level of hardness ranges from 75 to 150mg/L is good for fish culture, lesser than 20mg/L and above 300mg/L lethal, to fish, as it increases alkalinity, due to lack of nutrients(Bhatnagar and Devi, 2013). Calcium and magnesium are essential for bone and scale formation in fish. Very low levels (3.84 to 5.80 mg/L) of total hardness were noticed in the concrete fish pond of the Delta state of Nigeria (Agbaire *et al*., 2015). In the present work, 47.07±0.12 to193.00±1.73 mg/L of total hardness were analyzed (Table 1-3). A similar result was found by Nenavath and Kiran (2016), from the water of a freshwater tank in Chikamaglore district of Karnataka.

Nitrate content is an excellent parameter to judge organic pollution and it represents the highest oxidized form of nitrogen (Elayaraj and Selvaraju 2014). The end products of digestion of feed in fish, mainly fecal waste are released into the rearing water, these excretory products contain high levels of nitrogen(Devaraj *et al*., 2013). Nitrogenous species such as ammonia, and nitrates are usually the main water pollutants associated with aquaculture in that they are toxic to aquatic life and aquatic ecosystems. Hence, they have drawn the attention of many researchers. Accumulation of nitrogen leads to stress in fish susceptible to infections, thus, threatening fish health and the environment (Hlordzi *et al*., 2020). In the present study, the nitrate ranges from 40.00±0.00 to 51.00±1.00 mg/L (Table 1-3). The maximum nitrate value obtained in the winter season could be attributed to the high amount of end products of digestion of feed. A maximum nitrate value was also obtained by Kiran (2010) in the same study area.

Ammonia is the by-product of protein metabolism excreted by fish and decomposition of organic matter such as wasted food, feces, dead plankton, sewage, etc., and can exist in two forms i.e. unionized form of ammonia (NH_3), which is extremely toxic and the ionized form (NH_4 +) is not toxic and both the forms are grouped as "total ammonia" (Bhatnagar and Devi, 2013). In the present study, the ammonia ranges from 0.15±0.09 to 0.30±0.00 (Table 1-3).

Fungal communities. Frequently, six different (*Saprolegnia* spp., *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Rhizopus* spp., and *Mucor* spp.,) combinations of fungal genera were observed in three seasons (winter, summer, and rainy) in Bhadra reservoir project seed production unit, Shivamogga, Karnataka. The percentage and variation of six different fungi in all four species of carp fishes in winter (Table 4 and Fig. 3), summer (Table 5 and Fig. 4), and the rainy season (Table 6 and Fig. 5) were noted.

The maximum percentage of fungus was recorded during the winter season in all the carp fishes (Table 1 and Fig. 3). Particularly, *Saprolegnia* spp. showed maximum growth in winter in all the fish ponds. It starts declining in the summer season and again showed an increase in its growth during the rainy season. Manoharachary and Ramaroa (1981) reported the highest number of fungi in winter months and whereas lowest in summer, and also observed that the saprolegniaceae family was the dominant group in a freshwater fish pond of Andra Pradesh, India. However, in tropical and sub-tropical countries like India, maximum numbers of fungi have been observed either in monsoon or winter (Dayal and Tandon 1962; Srivastava, 1967) and the least during summer.

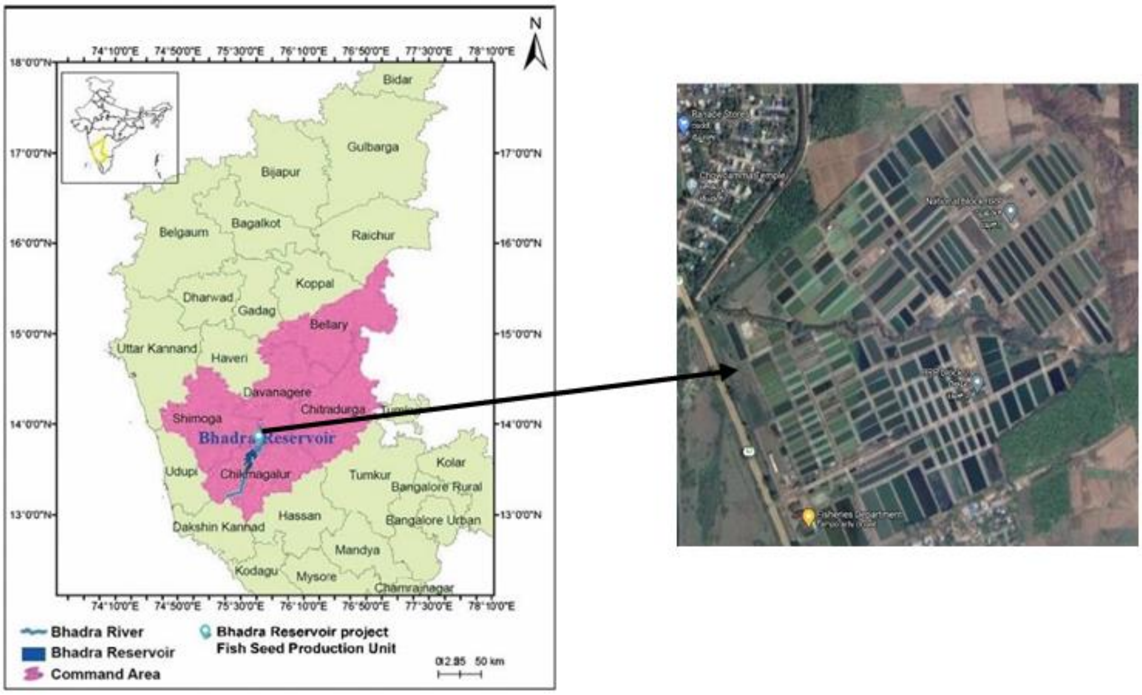

Fig. 1. Location map showing the study area.

Fig. 2. Collection of water samples from the pond in the study area.

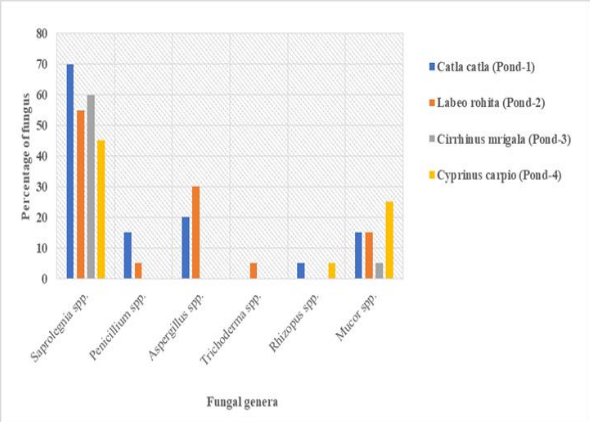

Fig. 3. Percentage of six different fungi in four fish species in the winter season.

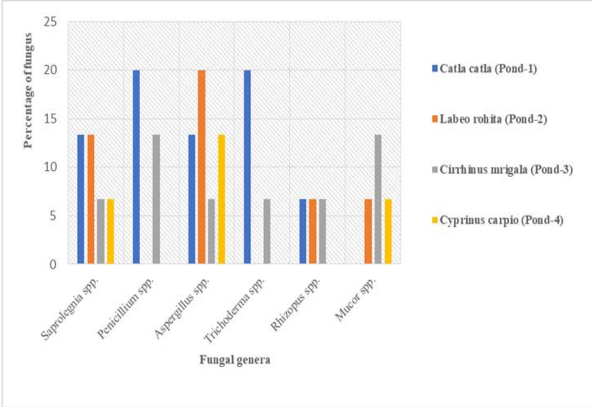

Fig. 4. Percentage of six different fungi in four fish species in the summer season.

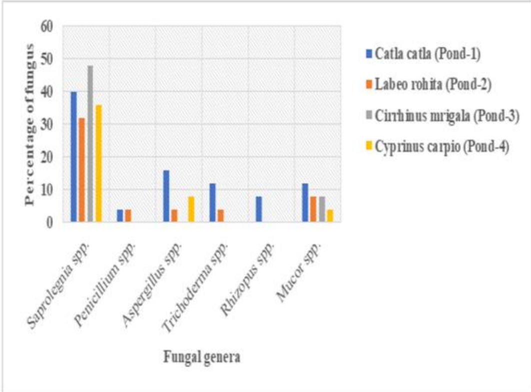

Fig. 5. Percentage of six different fungi in four fish species in the rainy season.

Table 1: Analytical value of a physico-chemical parameter in the winter season.

Parameter	Pond-1	Variance	Pond-2	Variance	Pond-3	Variance	Pond-4	Variance	Range	Acceptable range	Desirable range	Stress range	WHO
Water temperature (ºC)	19.33± 1.87	3.48	18.75± 1.57	2.48	18.83±1.69	2.84	19.30±2.24	5.04	18.75-19.33	15-35	20-30	<12, >35	28-35
Air temperature (ºC)	20.60± 0.36	0.13	20.60± 0.36	0.13	20.60±0.36	0.13	20.60±0.36	0.13	-	-	-	-	-
pH	7.54± 0.10	0.01	7.52± 0.08	0.28	7.48± 0.17	0.03	7.41± 0.16	0.03	7.41-7.54	7-9.5	6.5-9	<4, >11	6.5-8.5
watercolor	Light green	-	Light green	-	Light green	-	Light green	-	Light green	Pale to light green	Light green to light brown	Clearwater Dark green and brown	-
Total dissolved solids (mg/L)	272.67±2.08	4.33	252.67± 2.08	4.33	291.00±1.00	1.00	188.67±3.21	0.58	252.67-191	-	-	-	-
Dissolved oxygen (mg/L)	8.74± 0.23	0.05	8.29± 0.50	0.25	8.31± 0.27	0.07	8.58± 0.50	0.25	8.29-8.74	3-5	5	<5, >8	5
Biological oxygen demand (mg/L)	3.03± 0.06	0.01	3.22± 0.03	0.01	2.81± 0.01	0.01	3.07± 0.12	0.01	2.81-3.22	3-6	1-2	>10	10
Carbon dioxide (mg/L)	1.10± 0.00	0	1.17± 0.06	0.01	1.17± 0.06	0.01	1.07± 0.06	0.01	1.07-1.17	0-10	5-8	>12	-
Chloride (mg/L)	24.14± 2.46	6.05	20.47± 0.58	0.33	20.53± 0.46	0.21	21.13± 0.29	0.08	20.47-24.14	> 5	50-100	-	250
Alkalinity (mg/L)	31.67± 0.58	0.33	25.33± 1.15	1.33	24.00± 0.00	0.00	31.33± 1.15	1.33	24-31.67	50-200	75-100	<20, >300	75
Total Hardness (mg/L)	48.35± 0.09	0.09	47.07± 0.12	0.01	52.33± 0.58	0.33	52.00± 2.00	4.00	47.07-52.33	<20	75-150	<20, >300	
Nitrate (mg/L)	46.00± 3.46	12.00	44.00± 1.73	3.00	51.00± 1.00	1.00	50.67± 1.15	1.33	44-51	0-100	0.1-4.5	>100, <0.01	45
Ammonia (mg/L)	0.25± 0.00	0.00	0.27± 0.03	0.01	0.28± 0.03	0.01	0.25± 0.00	0.00	0.25-0.28	0-0.05	0-<0.025	>0.3	-

Table 2: Analytical value of the Physico-chemical parameter in the summer season.

Parameter	Pond-1	Variance	Pond-2	Variance	Pond-3	Variance	Pond-4	Variance	Range	Acceptable range	Desirable range	Stress range	WHO
Water temperature	28.11±2.99	8.92	28.22±2.80	7.82	28.45±3.29	10.81	28.00±2.90	8.43	28.00-28.45	15-35	20-30	<12, >35	28-35
Air temperature	31.83±0.58	0.33	31.83±0.58	0.33	31.83±0.58	0.33	31.83±0.58	0.33	31.83	-	-	-	-
pH	7.93±0.14	0.02	8.09±0.33	0.11	7.94±0.28	0.14	7.41±0.16	0.05	7.41-8.09	7-9.5	6.5-9	<4, >11	6.5-8.5
watercolor	Light green	-	Light green	-	Light brown	-	Light green	-	Light green-Light brown	Pale to light green	Light green to light brown	Clearwater Dark green and brown	-
Total dissolved solids	225.33±0.58	0.33	224.67±0.58	0.33	227.67±0.58	0.33	231±1.00	1.00	224.67-231	-	-	-	-
Dissolved oxygen	5.63±0.11	3.20	5.50±0.26	0.07	5.33±0.23	0.05	5.47±0.23	0.05	5.33-5.63	3-5	5	<5, >8	5
Biological oxygen demand	2.17±0.06	0.01	2.13±0.12	0.01	2.00±0.00	0.00	2.07±0.06	0.01	2.00-2.17	3-6	1-2	>10	10
Carbon dioxide	8.60±0.35	0.12	4.33±0.12	0.01	8.60±0.35	0.12	8.07±0.12	0.01	4.33-8.60	0-10	5-8	>12	-
Chloride	25.55±0.02	0.01	25.54±0.03	0.01	28.33±0.12	0.01	30.27±0.23	0.05	25.54-30.27	> 5	50-100	-	250
Alkalinity	43.33±1.15	1.33	41.67±0.58	0.33	46.33±0.58	0.33	45.33±0.58	0.33	41.67-46.33	50-200	75-100	<20, >300	75
Hardness	193±1.73	3.00	181.33±1.15	1.33	141.33±1.15	1.33	161.67±0.58	0.33	141.33-193	<20	75-150	<20, >300	-
Nitrate	40.00±0.00	0.00	40.00±0.00	0.00	41.00±0.00	0.00	40.00±0.00	0.00	40-41	0-100	0.1-4.5	>100, <0.01	45
Ammonia	0.25±0.03	0.01	0.25±0.03	0.01	0.25±0.00	0.00	0.25±0.00	0.00	0.25	0-0.05	0-<0.025	>0.3	-

Table 3: Analytical value of the Physico-chemical parameter in the rainy season.

Parameter	Pond-1	Variance	Pond-2	Variance	Pond-3	Variance	Pond-4	Variance	Range	Acceptable range	Desirable range	Stress range	WHO
Water temperature	23.27±3.22	10.35	23.27±3.12	9.75	22.80±3.56	12.64	23.07±2.99	8.92	22.80-23.07	15-35	20-30	<12, >35	28-35
Air temperature	24.87±0.58	0.33	24.87±0.58	0.33	24.87±0.58	0.33	24.87±0.58	0.33	24.87	-	-	-	-
pH	7.31±0.42	0.18	7.31±0.46	0.22	7.23±0.37	0.14	7.24±0.48	0.23	7.23-7.31	7-9.5	6.5-9	<4, >11	6.5-8.5
watercolor	Light green	-	Light green	-	Light brown	-	Light green	-	Light green	Pale to light green	Light green to light brown	Clearwater, Dark green and brown	-
Total dissolved solids	193.67±2.31	5.33	185.00±5.00	25.00	184.00±1.73	3.00	182.67±2.08	4.33	182.67-193.67	-	-	-	-
Dissolved oxygen	4.74±0.23	0.05	4.45±0.44	0.19	4.87±0.01	0.01	4.60±0.23	0.13	4.45-4.87	3-5	5	<5, >8	5
Biological oxygen demand	1.62±±0.01	0.01	1.83±0.02	0.01	1.67±0.03	0.01	1.82±0.01	0.01	1.62-1.83	3-6	1-2	>10	10
Carbon dioxide	2.20±0.00	0.00	2.10±0.10	0.01	4.33±0.12	0.01	4.13±0.23	0.05	2.10-4.33	0-10	5-8	>12	-
Chloride	17.51±0.82	0.67	17.99±0.82	0.67	15.57±0.09	0.01	19.85±0.3	0.11	15.57-19.85	> 5	50-100	-	250
Alkalinity	24.33±0.58	0.33	27.67±0.58	0.33	25.33±0.58	0.33	20.67±1.15	1.33	20.67-27.67	50-200	75-100	<20, >300	75
Hardness	51.67±0.58	0.33	56.00±0.00	0.00	51.67±0.58	0.33	54.00±1.73	3.00	51.67-56.00	<20	75-150	<20, >300	-
Nitrate	40.67±1.15	1.33	41.33±1.15	1.33	42.67±1.15	1.33	43.00±1.73	3.00	40.67-43.00	0-100	0.1-4.5	>100, <0.01	45
Ammonia	0.3±0.00	0.00	0.15±0.09	0.01	0.27±0.03	0.01	0.27±0.03	0.01	0.15-0.3	0-0.05	0-<0.025	>0.3	-

Table 4: Percentage of different fungi in different fishes in the winter season.

Fungal species	Percentage of different fungi in different fish species			
	Catla catla(Pond-1)	*Labeo rohita* (Pond-2)	*Cirrhinus mrigala* (Pond-3)	*Cyprinus carpio* (Pond-4)
Saprolegnia spp.	70	55	60	45
Penicillium spp.	15	5	0	0
Aspergillus spp.	20	30	0	0
Trichoderma spp.	0	5	0	0
Rhizopus spp.	5	0	0	5
*Mucor*s pp.	15	15	5	25

Table 5: Percentage of different fungi in different fishes in the summer season.

Fungal species	Percentage of different fungi in four different fish species			
	***Catla catla* (Pond-1)**	***Labeo rohita* (Pond-2)**	***Cirrhinus mrigala* (Pond-3)**	***Cyprinus carpio* (Pond-4)**
Saprolegnia spp.	13.34	13.34	6.67	6.67
Penicillium spp.	20	0	13.34	0
Aspergillus spp.	13.34	20	6.67	13.34
Trichoderma spp.	20	0	6.67	0
Rhizopuss pp.	6.67	6.67	6.67	0
Mucor spp.	0	6.67	13.34	6.67

Table 6: Percentage of different fungi in different fishes in the rainy season.

Fungal species	Percentage of different fungi in four different fish species			
	***Catla catla* (Pond-1)**	***Labeo rohita* (Pond-2)**	***Cirrhinus mrigala* (Pond-3)**	***Cyprinus carpio* (Pond-4)**
Saprolegnia spp.	40	32	48	36
Penicillium spp.	4	4	0	0
Aspergillus spp.	16	4	0	8
Trichoderma spp.	12	4	0	0
Rhizopus spp.	8	0	0	0
Mucor spp.	12	8	8	4

The winter peaks may be due to the presence of more oxygen, low temperatures, high nutrient status, and large amounts of oxidizable organic matter. The monsoon peaks may be due to the availability of high rainfall and surface run-off along with the nutrients and whereas a decrease in fungal species during summer may be due to high water temperatures and low nutrient status (Manoharachary and Ramarao 1981).

CONCLUSIONS

The present study described the seasonal variations of Physico-chemical parameters and their influences on the growth of fungal species. The obtained Physico-chemical parameter was correlated with the limit of the World Health Organisation (WHO) standard. By the result of analytical research, it can be concluded that manuring the pond, continuous supply of nutrients, and the normal level of rain (floods) often create abrupt changes in water quality parameters such as temperature, pH, total dissolved solids, dissolved oxygen, nitrate, and ammonia causing infectious disease (saprolegniasis) in fishes ultimately leading to an economic loss of carp production. The periodical measurement of physico-chemical parameters of water helps in correcting the abnormalities in each fish pond by training the farmers and aquaculture extension workers to adopt new techniques to respond effectively to reduce the risk of flood. So that ponds can be used to increase the production and survivability of fish.

FUTURE SCOPE

Maximum growth of *Saprolegnia* spp. during the winter season, identified as a fish pathogen in the present research work may be used further to develop a bio-controlling agent against devasting fish pathogen i.e. *Saprolegnia* spp.

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Conflict of Interest. None.

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