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STUDIES ON THE PHYSICOCHEMICAL PARAMETERS AND THE MICROBIAL INVESTIGATION OF THE FRESHWATER EXOTIC CARP *Catla Catla* IN LOWER ANAICUT RESERVOIR, TAMIL NADU, INDIA

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ABSTRACT

In this study, we examined the physico-chemical parameters and microbial population in soil sediment, water and gill region of *Catla catla* from Lower Annicut Reservoir were examined. Based on their growth characteristics on specific culture media, the following human bacterial pathogen such as *Aerococcus* Spp, *Bacillus* Spp, *E. coli*, *Klebsiella* Spp, *Staphylococcus aureus*, *Proteus* Spp, *Pseudomonas* Spp and fungal pathogens like *Aspergillus flavus, Aspergillus tamari, Aspergillus nidulans, Aspergillus fumigatus, Mucor and Penicillium* were isolated. The medium of the fishes and the sediments of their habitat were further more collected and examined for pathogens. However, the five species of bacteria and four species of fungus were found in gill region. This research highlights the quality of *Catla catla* in Lower Annicut Reservoir and to create awareness amid fish eating population.

INTRODUCTION

Aquaculture is an emerging industrial sector which requires continued research with scientific and technical developments, and innovation. The important physical and chemical parameters influencing the aquatic environment are temperature, rainfall, pH, salinity, dissolved oxygen and carbon dioxide and these parameters are the limiting factors for the survival of aquatic organisms [1]. In particular, fish is a rich source of animal protein and its culture is an efficient protein food production system from aquatic environment. Physicochemical and functional properties of fish protein play a fundamental role in the food industry and its end products [2]. Unfortunately, human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the exposed individual [3]. Microbial investigation for characteristics of potential pathogenic microorganisms for fish will allow the application of adequate measures to prevent and control the main diseases limiting the production of fishes [4]. Hence, the present study was focused on the physicochemical parameters as well as isolate and characterized the fungal and bacterial strains in soil, water and gill region of *C. catla* from Lower Annicut Reservoir.

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MATERIALS AND METHODS

Description of the Study Area

The data for the present study were collected from April 2012 to August 2012 in the freshwater of Lower

Annicut Reservoir, Thanjavur district, Tamilnadu, India. Fishes are feed with natural supplementary feed.

Analysis of Physicochemical Parameters in Water Samples

For the determination of physicochemical properties, water samples are collected for a period of four months (April 2012 to August 2012) from the lower anaicut. Temperature was measured with a mercury thermometer (0.5°C). Transparency was determined by dipping thermometer directly on the water about the minute and reading was recorded. The pH of the water sample was determined by using digital pH meter. The light penetration was determined with the help of Sacchi's disc. Dissolved oxygen samples were collected in colored bottles and analyzed by a standard Winkler's method [5] and then salinity was measured by Mohr's titration method as well as free carbon dioxide and nitrite were analyzed in the laboratory, APHA [6].

Biochemical tests

Indole test

The test requires sterilized (121ºC for 15 min) tryptophan broth. The culture was inoculated in cooled sterilize broth. After 24 hrs of incubation, 0.3 ml of Kovac's reagent was added into the tubes for observe the result.

Methyl Red and Voges-Proskauver's Test

The culture was inoculated into the tubes containing sterilized (121ºC, 15 min at 15 lbs) MR–VP broth; Tubes were incubated at 37ºC for 24 hrs; Add 0.5ml of MR reagent, 0.2ml of VP reagent A and B; Results were observed after adding reagent.

Citrate Utilization Test

Lightly inoculate a pure culture into a tube of sterilize (121ºC, 15 min at 15 lbs) Simmon'scitrate medium, using needle to stab, then streak the medium; Be careful not to carry over anynutrient material; incubated at 37ºC for 24 hrs; The results were taken after incubation.

Urease Test

In this test 20% urea solution added and added on sterilized (121ºC, 15 min at 15 lbs/Inch2) media and it was transferred into the slant tubes for slanting position solidification. The culture was inoculated into the tubes and incubated at 37ºC for 24 hrs. The results were observed after incubation.

Triple Sugar Ion Test

Inoculate pure culture by stabbing and streaking the triple sugar iron (TSI) slant tube; incubate at 37ºC for 24 hrs in a incubator. The reaction was recorded.

Catalase Test

A drop of culture broth was placed on clean microscope slide; two or three drops of hydrogen peroxide solution were added to culture broth on the slide for observe air bubble formation.

Oxidase Test

Oxidase disc coated with 1% N–N tetra methyl para phenylenediamine dihydrochloride was placed at the centre of the clean microscope slide. A drop of culture broth was placed over the surface of the oxidase disc for observe the colour change.

Microbial test

The bacterial and fungal species were isolated from the lower annaicut lake soil, water and gill region of *C.catla.*

Serial dilutions of the sample

The nutrient agar medium were prepared, sterilized and poured in sterile Petri plates and allowed to solidify. The 10gm of the sample was added to 90ml of the distilled water in a flask. It was shaked vigorously and 1ml was transferred in test tube containing 9ml of distilled water. The content was mixed well and 1ml was transferred from 10-1 dilution to the next dilutions up to 10-9 dilution. After solidifying, the nutrient agar plates with dilution 10-6 and 10-7 were taken. 0.1mlsample was poured in Petri plates using spread plate technique. The Nutrient agar plates with dilution 10-4, 10-5and 10-6 were taken 0.1ml sample was poured in Petri plates using spread using spread plate technique. The plates were incubated for bacteria at 37ºC for 24 hrs.

Streak plate method

In this method a sterilized loop of transfer needle was dipped into a streak plate method offers in the most practical method of obtaining discrete colonies and pure cultures. Suitable diluted suspension of organisms, which is then streaked on the surface of an already solidified agar plate to make a serious of parallel. The aim of this method is to check whether the organisms are growing i.e., growth derived from a single or spore.

Isolation of bacteria by gram staining

Gram staining was done by the method of Hans Christian's gram [7].

Sabouraud dextrose agar method

The Rose Bengal agar and Sabouraud dextrose agar medium were prepared and sterilized. The medium was poured in sterile Petri plates and allowed to solidify. For primary isolation of the fungus, 10gm of the sample was added to 90ml of the distilled water; shaked vigorously and 1ml was transferred 9ml of distilled water. The content was mixed well and 1ml was transferred from 10-1 dilution to the next dilutions up to 10-9 dilution. After

solidifying, the nutrient agar plates with dilution 10-2, 10-3 and 10-4 were taken. 0.1ml sample was poured in Petri plates using spread plate technique. The Sabouraud dextrose agar plates with dilution10-2, 10-3 and 10-4 were taken 0.1ml sample was poured in Petri plates using spread using spread plate technique. The plates were incubated at 37ºC for 72 hrs.

Lactophenol cotton blue method

One or two drops of lacto phenol cotton blue stain were placed on the clean glass slide. Tuft of fungus suspension was mixed with the stain. A clean cover slip was placed over the preparation without the formation of any air bubbles and observed fungus under the microscope.

RESULT AND DISCUSSION

Temperature

Recorded temperatures during the study period (March to September 2012). The minimum temperature (27°C) recorded in March and maximum water temperature (34° C) recorded in May 2012. It was observed during the present study indicates that the surface water temperature is lesser and closely associated with the air temperature, because temperature is one of the most common ecological factor. It has a universal influence and is frequently a limiting factor and distribution of animals and plants (Fig 1). Patra *et al.,* observed that seasonal variation in physicochemical parameters of Chilika Lake after spawning [8]. Physico-chemical parameters in relation to meterological and climatic conditions in fish pond were determined by Nargis *et al* [9].

Turbidity

Turbidity of the fish culture pond depends on the suspended material like clay, water plants,plankton bloom etc. The turbidity values varied from 21.0 to 28.0 cm. It was found to be minimum (21.0cm) June 2012 and maximum (28.0cm) April 2010 (Fig 2). The turbidity plays an important role in the productivity of the pond thereby controlling other physical factors. The level of turbidity depends upon the bottom of the pond and depth of the water. It has general effect of an aquatic biota. The penetration of light is affected by turbidity and therefore the temperature of water affected. In the pond transparency found between 23 and 28 cm. In the present study, transparency showed significant fluctuation. The variation in the turbidity may be due to the suspended particle nutrients and the abundance of phytoplankton.

Hydrogen Ion Concentration (pH)

The maximum level of pH 7.8 was recorded in May 2010 and minimum value of pH 7.0 was recorded in March 2010. The hydrogen ion concentration was always found to be neutral and slightly above 7.0. Hydrogen ion concentration (pH) is important hydrological parameters which influence the growth and metabolism of aquatic organism. The hydrogen ion concentration of the pond was found in the range 7.0 – 7.7 (Fig 3). The pH of the culture pond is maintained by applying lime and gypsum periodically. Significant variations in values were observed in the various locations. Generally, the parameters analyzed within the desirable and acceptable limits. Furthermore, a similar range was obtained by many researches [10].

Salinity

The salinity was variable throughout present study period it was recorded between 0.11 ppt and 0.16 ppt. Salinity to be lower 0.11 ppt in May 2012 and higher 0.16 ppt in July 2010 (Fig 4). The salinity of the culture pond is one of the abiotic factors having slight influence on growth of the freshwater fishes. The salinity of freshwater body should be below 0.5 ppt and it is more suitable for freshwater fish culture. The salinity did not show significant fluctuation during the study period. These results are identical to those reported by Igbinosa *et al* [11].

Dissolved Oxygen

The fluctuation in the concentration of dissolved oxygen content varied from 2.8 ml/1 to 6.1ml/1. The maximum value of 6.1 ml / l was observed in March 2010 and minimum value of 2.8 ml/l was observed in August 2012 (Fig 5). Oxygen distribution in water bodies is important as it is the direct need of many aquatic organisms and it favours the solubility and availability of many nutrients to the organisms and therefore increases the productivity of the aquatic ecosystem. Dissolved oxygen (DO) plays an important role in aquatic environment and is essential for growth of phytoplankton and fish productivity. The inhabitant organisms are affected greatly due the diurnal and seasonal variation in the dissolved oxygen of the ambient water [12]. However, in the pond dissolved oxygen content found between 2.807 and 8.983 ml/l. Fish cannot take (O_2) if carbon dioxide remains high in water. (O_2) transportation of blood decreases. As a result fish suffer from suffocation and die.

Free carbon dioxide

Free carbon dioxide is one of the important factors in aquatic habitat. It is highly soluble in water and is the main source of carbon path way in the nature which required for photosynthesis of all aquatic plants. In the present study, the free carbon dioxide level slightly fluctuates during the observation period (Fig 6). It varied from 0.002 to 0.7. The dissolved carbon-di-oxide is another important biological factor which is obtained in the pond water either through decomposition of organic material or respiratory exchange of aquatic plants and animals. It is essential for photosynthetic activity and retaining calcium in water.

Nitrite

In the present study, the level of nitrite content of the pond varied between 0.12 and 0.87 mg/l (Fig 7). Nitrite concentrations generally peaked during the autumn and winter months. This was attributed to increased decomposition of organic material in the pond and concurrent decreases in algal and bacterial populations which normally absorbed those nutrients. In late spring and early summer urban runoff into the pond from precipitation increased the concentrations of those nutrients. By the end of summer ammonia, nitrite, and nitrate concentrations had decreased. The concentration of orthophosphate remained high throughout most of the study indicating the eutrophic condition of this pond.

Bacteria

Escherichia coli (Gram +ve), *Klebsiella* (Gram –ve), and *Proteus* (Gram –ve) are exist in gill region of *C. catla* and in the water sample but absent in soil, while the bacterial species like *Bacillus subtilis* (Gram +ve), *Staphylococcus aureus* (Gram +ve) were absent in gill but they present in soil and water. However, the remaining species *Aerococcus* (Gram +ve), and *Pseudomonas* (Gram –ve) were present among these three source (Table 1). Human Infections caused by pathogens transmitted from fish are quite common [13]. Establishment and persistence of *Staphylococcus epidermidis* in Tilapia (*Oreochromis* spp.), *Sparus aurata* was investigated by Huang *et al* [14] and Kubilay and Uluköy [15]. Eissa *et al* [16] Characterized Pseudomonas Species in Qaroun and Wadi-El-Rayan Lakes, isolated from *Oreochromis niloticus*. Eze *et al* [17] have confirmed the occurrence of *E. coli* in frozen mackerel fish (*Scomber scombrus*) in a humid tropical environment.

Fungal Populations

In the present investigation, the analysis of fungal flora in soil sediments, water sample of culture pond and gill region were isolated from the diseased fish *C. catla*. The table clearly shows that the seven species of fungal flora in soil sediments and water sample of culture pond and gill region of diseased fish *C. catla* such as *Aspergillus flavus, Aspergillus tamari, Mucor* and *Penicillium* were recorded at the same time the *Aspergillus Nidulans* and *Aspergillus fumigates* were absent in gill region (Table 2). The present investigation has the similarity withthe following findings. Bukola *et al* [18] observed the mycofloral of smoke-dried fishes sold in Uyo, Eastern Nigeria.

Figure 1. The Temperature variation in Lower Annicut Reservoir

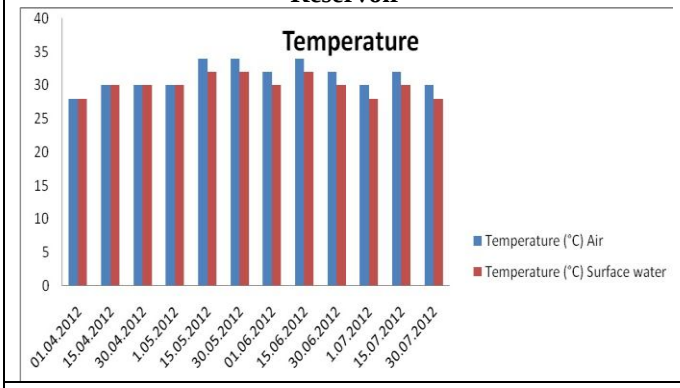

Figure 2. The turbidity variation in Lower Annicut Reservoir

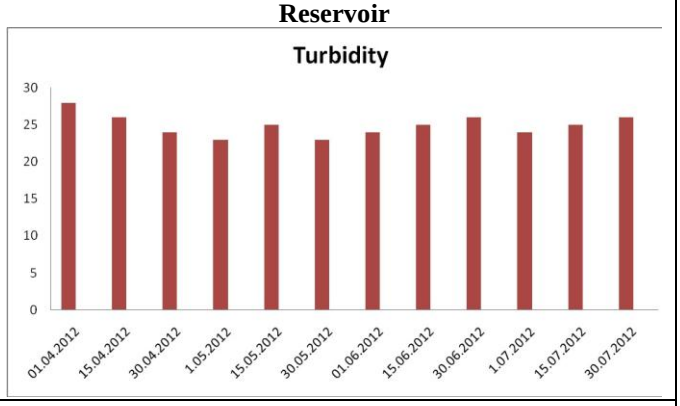

Figure 3. The pH variation in lower Annicut Reservoir

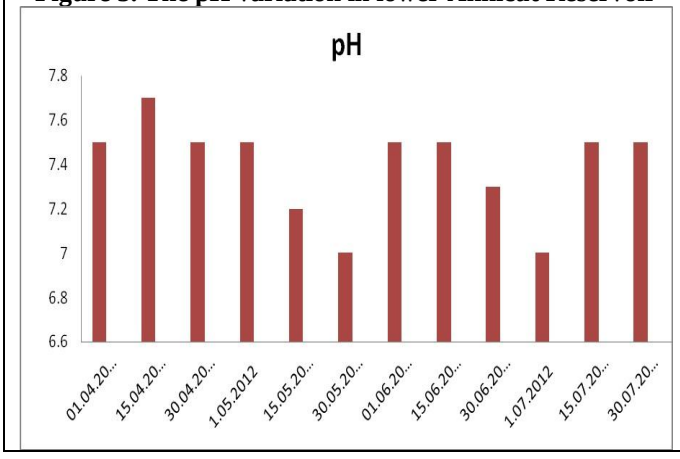

Figure 4. the salinity in Lower Annicut Reservoir

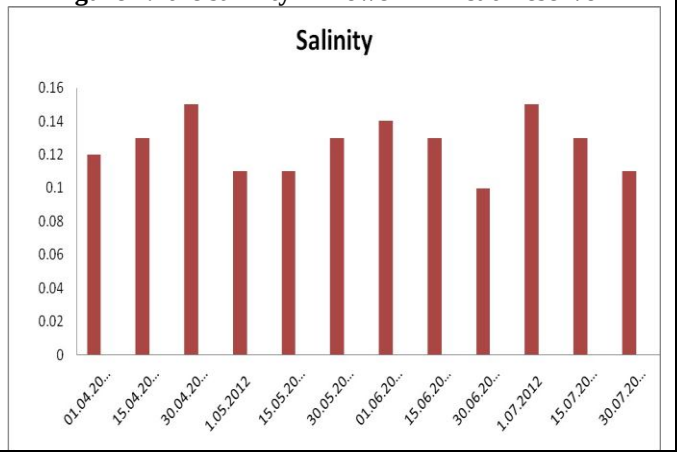

Figure 5. The Dissolved oxygen variation level in Lower Annicut Reservoir

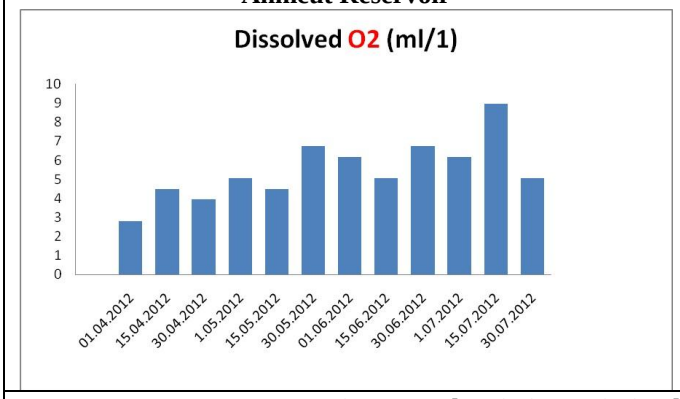

Figure 6. The CO_2 in Lower Annicut Reservoir

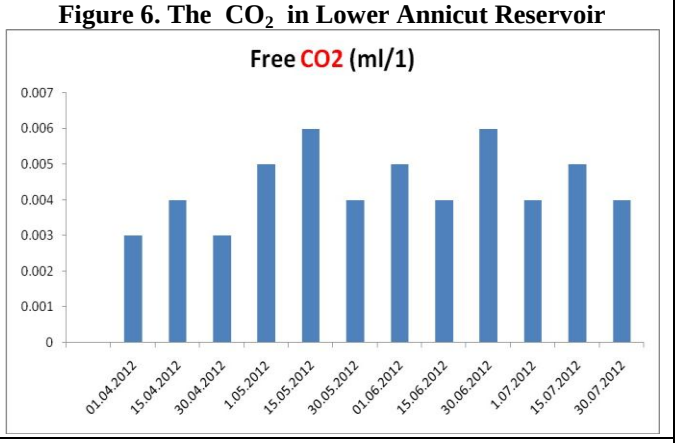

Figure 7. The nitrite variation level in Lower Annicut Reservoir

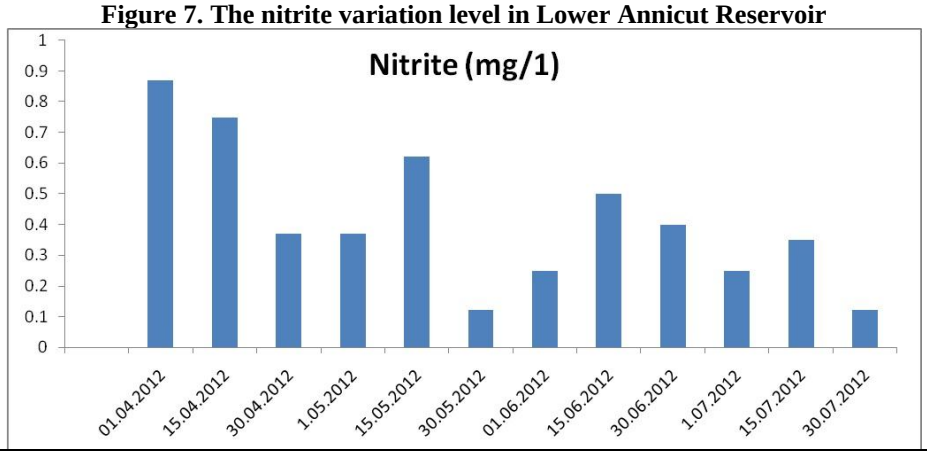

Table 1. The Analysis of Bacterial Population in Soil, Pond Water and Gill Region of Diseased fish *Catla catla*

Name of the Organisms	Soil	Lake Water	Gill
Escherichia coli	-	+	+
Bacillus subtilis	+	+	-
Klebsiella	-	+	+
Proteus	-	+	+
Staphylococcus aureus	+	+	-
Pseudomonas Species	+	+	+
Aeroccocus Species	+	+	+

(+) Denote Present, (-) Denote Absent

Table 2. The Analysis of Fungal Population in Soil, Pond Water and Gill Region of Diseased fish *Catla catla*

Name of the Organisms	Soil	Lake Water	Gill
Aspergillus flavus	+	+	+
Mucor	+	+	+
Penicillium	+	+	+
Aspergillus tamari	+	+	+
Aspergillus nidulans	+	+	-
Aspergillus Fumigates	+	+	-

(+) Denote Present, (-) Denote Absent

CONCLUSION

From this investigation, it has been concluded that the physicochemical characteristics are suitable for the growth of the fishes, but the fungal and bacterial species showed harmful effect on the culture organisms and creates awareness among fish eating people.

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