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Physico-Chemical And Microbial Investigation of Wardha And Wainganga River of Chandrapur And Gadchiroli District Exhibiting Infections in *Clarias Batrachus*

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ABSTRACT

Quantitative and qualitative analyses of bacterial flora associated with fresh water containing microbial pathogens present on the skin, gills, and fins of polycultured healthy common carp Clarias batrachus. Physico-chemical and biological parameters of Wardha and Wainganga river water were investigated and it was found that, during winter season these two river water has low alkalinity and low chloride concentration. The temperature was also found to be a fluctuating parameter. During winter season maximum affected fishes were reported. Pathogenic bacteria and fungi were also reported during these months in these water bodies. In the present study Clarias batrachus showed its presence throughout the year except the summer months in Wardha and Wainganga river water confirming their involvement in the initial process of damage, similarly the presence of fungi Saprolegnia parasitica, and Aspergillus sp. isolates from Wardha and Wainganga riverwater.

Keywords: *Clarias batrachus, Saprolegnia parasitica* and *Aspergillus sp.*etc.

INTRODUCTION

The bacterial composition may change with age, individuals, nutritional status, environmental conditions, and the complexity of the fish. Like all animals, fish suffers from environmental, nutritional and infectious diseases. Organisms such as bacteria and fungi are capable of causing disease in cultured and wild populations of fish. Poor nutrition and water quality can also cause disease under stressful conditions. Intensive and super intensive fish culture practices involve high rates of stocking and supplementary feeding which has substantially enhanced the incidence of diseases in fishes in our country.

The studies in the last decade (Kar *et al.*, 1993; 1994; 1995; 1996; 1997; 1998; 1999, 2000) showed that species like *Channa striatus, C. punctatus, Clarias batrachus and Anabas testudineus* have been severely affected by bacterial pathogens and the outbreak has been occurring during the period from November to March. Low temperatures appear to influence the severity of infectious lesions.

Rodgers and Burke (1981) observed infectious prevalence in estuarine fish populations with seasonal aggregations of fish stressed by low or rapidly changing water temperatures and rapid or prolonged depressions of salinity. Lowered salinity due to rainfall events (Callinan *et al*., 1995; Virgona, 1992) and excess water discharges appear to play a role in the subsequent appearance of lesions in fish (Kane *et al*., 2000).

Several water bodies in Chandrapur and Gadchiroli district were found to contain fresh water fishes Clarias *batrachus and Channa morulias* in infectious form. The paper deals with water profile containing both physiochemical and microbiological parameters of two major rivers from which infected fishes had been brought to the market for sale.

The present study reports on the composition of bacterial and fungal flora associated with two above mentioned river water. Bacterial and fungal flora in fish and water environment can be different in various geographical areas. Thus, the studies described in this paper are needed.

MATERIALS AND METHODS

Monthly water samples were collected preferably during 7 to 9 am each time from the Wardha and Wainganga River which have more infectious fishes. The physic-chemical parameters such as, D.O., alkalinity, total hardness, calcium hardness, chloride, pH were recorded during 2012 and 2013 following methods given in APHA (1998). The same samples were also used for the analysis of heavy metals like, Zn, and Mg Atomic Absorption Spectrophotometer (Fishman and Downs, 1966). Isolation of bacteria and fungi from the water samples was carried out. Pure cultures of bacteria were obtained by streaking out on a sterile nutrient Agar plate and were incubated at temperature 37^{o}C for 24 hrs. Then the Pure cultures were maintained on agar slants for further identification by morphological and biochemical tests.

For all these tests standard microbiological tests were carried (Aneja, 1996; Sneath *et al.*, 2000; Staley *et al.*, 2001; Cappucino and Sherman, 2002). For fungi the suspensions were streaked out in a circular pattern on specific media like Potato Dextrose (PD) Agar, Corn meal Agar, Czapex dox Agar. Fungal growth was observed 48 hrs after incubation at temperature between 25-30 ^{0}C. Pure cultures of fungi were obtained by reinoculation on fresh required media and their slides were prepared for identification. The media required were sterilized by moist method of sterilization using an autoclave at 15 lbs pressure and 121 ^{0} C as per the requirement and used.

RESULTS AND DISCUSSION

To know bacteriological identification of infections in fishes, a year survey of local market was carried out and it was found that the bottom dwelling fishes, *Clarias batrachus* were mostly affected by bacterial pathogens and they were brought by the fishermen to the market from two Wardha and Wainganga river. Then it was decided to study the water parameters of these two rivers. The observations are given below. The water quality of Wardha and Wainganga river was studied as most of the diseases of fishes from these river were brought to the local market for sale. Table 7 and 8, gives the percentage of infected fishes (*C.batrachus*) brought to the laboratory during September to January months of 2011-12 and 2012-13.

The water parameters of Wardha and Wainganga river (2012 and 2013) are shown in Tables 1 and 2, it reveals that both water bodies had low alkalinity and hardness a characteristic of acidic low calcium soils. In the present investigation total 08 different bacterial isolates were obtained from the water bodies (Table 3 and 4). Water samples from the two river were examined monthly for any bacterial and fungal contents and the results are given in Tables, 3 to 6. Total 7 different species of bacteria were isolated from water samples from Wardha river which includes, *Escherichia coli, Pseudomonas sp.,Staphylococcus sp., Streptococcus sp., Bacillus sp., Lactobacilli sp.* and

Klebsiella sp. (Table 3) and 5 species of bacteria were isolated from water samples of Wainganga river which includes *Pseudomonas sp., Staphylococcus sp., Streptococcus sp., Lactobacillus sp.and, Bacillus sp.s* (Table 4). *E.coli* was predominantly present in the Wardha water throughout the year except in the month of October. *Klesiella sp*., were recorded in Wardha river water which were found to be absent in Wainganga river water.

In Wainganga River *Staphylococcus* were found to be dominating with their absence only during May and June. The different kinds of fungal isolates were obtained by culturing them on various media such as Potato Dextrose Agar, Corn Meal Agar, Glucose peptone Yeast Agar and Czapex Dox Agar and their pure cultures were obtained by sub culturing these isolates on various medias as per the requirement. In the present investigation total 4 different types of fungus were obtained from water bodies from where EUS infected fishes were obtained. The fungi which were isolated from Wardha river were *Saprolegnia parasitica, Aphanomyces invadans, Achyla sp.* and *Aspergillus sp*. Similar fungi were also isolated from Wainganga river water (Table 5 and 6). The infected fishes mostly with red spots were considered as affected.

Clarias batrachus were found to be microbial infected from Wardha and Wainganga river during 2011-12 and 2012-13 in winter season. The results are shown in Tables, 7 and 8. During 2011-2012 out of 69 collected from Wardha river, 43 were microbial affected and during 2012-2013, 53 out of 83 were found to be microbial affected amounting to 62.32% and 63.86% infection respectively. However, from Wainganga River 54.22% and 59.09% infected were collected during 2011-12 and 2012-13 respectively.

Table 1: Water parameters from Wardha River during 2012-2013.

Water Parameters	Rainy	Winter	Summer
Temperature (^oC)	28(27^oC–$29\ ^oC$)	23.5 (22^oC–25^oC)	39 (38^oC–40^oC)
Conductivity (μm hos/cm)	344 (336.0–352.0)	220 (216.0–224.0)	326 (316.0–330.0)
Total Dissolved solids (mg/L)	198 (192.0–204.0)	111.5 (107.0-116.0)	128.5 (125.0–132.0)
pH	9.0 (8.8–9.2)	7.5 (7.4–7.8)	7.8 (7.5–8.1)
Dissolved oxygen (mg/L)	6.3 (6.2–6.4)	9 (8.8–9.2)	-
Chloride (mg/L)	24.15 (22.18–26.12)	20.10 (18.80–21.40)	41.77 (39.40–44.14)
Free CO_2 (mg/L)	3.50(2.40-4.60)	4.50(3.40-5.60)	4.82 (4.8–5.2)
Total Alkalinity (mg/L)	194 (192.00–196.00)	76.51(74.88–78.15)	154.5 (150.88–158.12)
Total hardness (mg/L)	192.20(190.00–194.40)	144.3 (138.40-150.20)	217.49(214.88220.10)
Magnesium (mg/L)	15.34 (12.22-18.46)	10.6 (8.84-12.36)	7.40 (6.00-8.96)
Zinc (mg/L)	0.78 (0.052-0.102)	0.055 (0.024-0.086)	0.058(0.024-0.92)
Copper (mg/L)	0.084 (0.008–0.16)	0.005 (0.002–0.008)	0.077 (0.002–0.14)
Lead	0.0185 (0.003–0.034)	0.006 (0.004–0.008)	0.0035 (0.002–0.005)

Table 2: Water parameters from Wainganga River during 2012-2013.

Water Parameters	Rainy	Winter	Summer
Temperature (^oC)	27.00 (26^oC–28^oC)	21.00 (18^oC–$24\ ^oC$)	37.50 (35^oC–40^oC)
Conductivity ((μm hos/cm)	353.11 (348.0–362.0)	258.35 (248.0–296.0)	333.90 (320.0–343.0)
Total Dissolved solids (mg/L)	215.26 (210.0–218.0)	188.11 (160.0–200.0)	165.56 (146.0–190.0)
pH	8.00(7.5–8.5)	7.90 (7.6–8.1)	8.25(7.7–8.8)
Dissolve oxygen (mg/L)	7.00 (6.4–7.6)	8.10 (7.9–8.5)	6.45 (6.1–6.8)
Chloride (mg/L)	28.87 (27.60–30.15)	22.72 (19.05–26.40)	38.16 (35.86–40.15)

Free CO_2 (mg/L) – – –	7.42 (6.25-8.60)	-	-
Total Alkalinity (mg/L)	187.54(176.64–198.44)	124.57(120.62–128.52)	152.77(145.35160.20)
Total hardness (mg/L)	136.86(131.08–142.65)	129.97(125.60–134.35)	189.81(180.82198.80)
Magnesium (mg/L)	14.110 (13.160-24.124)	21.708 (19.456-23.961)	20.154(18.185-22.122)
Zinc (mg/L)	0.142 (0.022-0.280)	0.109 (0.015-0.204)	0.185 (0.105-0.266)
Copper (mg/L)	0.29 (0.19–0.39)	0.024 (0.008–0.04)	0.173 (0.006–0.34)
Lead (mg/L)	0.039 (0.004–0.074)	0.032 (0.009–0.056)	0.032 (0.004–0.06

Table 3: Monthly isolation of bacteria from Wardha river water during 2012-2013

Sr.No.	Name of Bacteria	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
		2011				2012								2013											
1	*Bacillus sp.*	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
2	*Escherichia coli*	-	-	+	+	-	-	+	+	-	-	-	-	+	-	+	+	-	-	+	+	-	-	+	+
3	*Lactobacillus sp.*	-	+	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-
4	*Pseudomonas sp.*	-	-	+	-	-	-	+	-	-	+	-	-	+	+	+	-	-	-	-	+	-	-	+	-
5	*Streptococcus sp.*	+	+	-	-	-	+	-	-	+	-	+	+	+	+	-	-	-	+	-	-	+	+	-	+
6	*Staphylococcus sp.*	+	-	+	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	-	-	-	-	+	+
7	*Klebsiella sp.*	+	+	-	-	+	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-	+	+	+

(S-Sep,O-Oct,N-Nov,D-Dec,J-Jan,F-Fer,M-Mar,A-Apr,M-May,J-Jun,J-Jul,A-Aug)

Table 4: Monthly isolation of bacteria from Wainganga river water during 2012-2013.

Sr.No	Name of Bacteria	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
		2011				2012											2013								
1	*Bacillus sp.*	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+
2	*Escherichia coli*	-	-	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-	+	-	-	+	+	-	-
3	*Lactobacillus sp.*	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
4	*Pseudomonas sp.*	-	-	+	+	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+
5	*Streptococcus sp.*	+	+	-	+	-	+	-	-	+	-	+	+	+	+	-	+	-	+	-	-	+	+	-	+
6	*Staphylococcus sp.*	+	-	+	+	-	-	+	-	-	-	+	+	-	+	+	+	+	+	-	-	-	-	+	+

(S-Sep,O-Oct,N-Nov,D-Dec,J-Jan,F-Fer,M-Mar,A-Apr,M-May,J-Jun,J-Jul,A-Aug)

Table 5: Monthly isolation of fungi from Wardha river water during 2012-2013.

Sr.No	Name of Fungi	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
		2011				2012											2013								
1	*Saprolegnia parasitica*	-	-	+	+	+	-	-	-	-	+	-	+	-	-	+	+	+	-	-	-	-	+	-	+
2	*Aphanomyces invadans*	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	-	+	-	-	+	+	+	-	-
3	*Achyla sp.*	+	+	+	-	+	-	-	+	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+
4	*Aspergillus sp.*	-	+	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	+	+	+

(S-Sep,O-Oct,N-Nov,D-Dec,J-Jan,F-Fer,M-Mar,A-Apr,M-May,J-Jun,J-Jul,A-Aug)

Table 6: Monthly isolation of fungi from Wardha river water during 2012-2013.

Sr.No.	Name of Fungi	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
		2011				2012												2012							
1	*Saprolegnia parasitica*	-	+	+	+	-	-	-	-	+	-	+	-	-	+	+	+	-	-	-	-	+	-	+	+
2	*Aphanomyces invadans*	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	-	+	-	-	+	+	+	-	-
3	*Achyla sp.*	+	+	+	-	+	-	-	+	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+
4	*Aspergillus sp.*	-	+	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+

(S-Sep,O-Oct,N-Nov,D-Dec,J-Jan,F-Fer,M-Mar,A-Apr,M-May,J-Jun,J-Jul,A-Aug)

Table 7: Percent occurrence of infected fishes (*Clarias batrachus)* from Wardha river.

Month/Year	***Clarias batrachus***		
2012	Normal	Affected	% Affected
September	16	08	
October	12	07	
November	18	13	
December	09	06	**62.32**
January	14	09	
TOTAL	**69**	**43**	
2013			
September	12	05	
October	18	10	
November	16	11	
December	17	13	**63.86**
January	20	14	
TOTAL	**83**	**53**	

Table 8: Percent occurrence of infected fish (*Clarias batrachus*) from Wainganga river.

Month/Year	***Clarias batrachus***		
2012	Normal	Affected	% Affected
September	18	06	
October	16	11	
November	12	08	
December	18	11	**54.22**
January	17	13	
TOTAL	**83**	**45**	
2013			
September	22	10	
October	18	12	
November	16	10	
December	14	08	**59.09**
January	18	12	
TOTAL	**88**	**52**	

Pathogenic bacteria and fungi were also reported during these months in the water of these water bodies (Tables: 3 to 6). The role of water temperature with occurrence of the disease is still not clear. Lowering of water temperature may lower the innate defence competence of the fish, thereby the fish to infect. Sharp fall in the hardness of water from the higher summer values due to dilution during rainy season seems to be another predisposing factor for triggering the disease outbreak. Rodgers and Burke (1981) proposed that the Red Spot Disease of Noosa river of Queensland was related to low or rapidly changing temperature and rapid or prolonged depressions of salinity induced by heavy rains.

Progression of the disease to ulcers is reported to occur after rainfall and high river flows which also caused rapid changes in various water quality parameters such as salinity, temperature, dissolved oxygen, pH and turbidity (Virgona, 1992). Mohan and Shankar (1994) considered dissolved oxygen of the water had close relationship with distribution, seasonal changes and zoosporic production of aquatic fungi. Dayal and Tandon (1963) studied the ecology of Phycomycetes in relation to chemical factors of water and reported that dissolved oxygen favoured the development of fungi and bacteria. The present results are in agreement with above findings as

there was high prevalence of fungi and bacteria in both the river during winter months when DO was maximum (Tables :1 and 2). pH of water from Wardha and Wainganga river was also in the alkaline range and was minimum during winter when compared with pH during other seasons. Thus, the present results indicate that the extent of heavy metal pollution may create a stressed condition for fishes and may be the predisposing factor for infected fishes. Similarly during colder weather immune system of fishes gets suppressed. Thus sudden lowering down of temperature might make fish vulnerable to bacteria Willoughby (1994) called this phenomenon as winter kill. The identification test results of bacterial isolates from infected fishes are in agreement with previous information (Rodgers and Burke, 1981; Chakrabarty and Dastidar, 1991).These bacteria are a part of the normal microbial flora of water (Lio-Po *et al*., 1998) and is a potential disease agent for fish (Cotran *et al.,* 1999). During the present study, these bacteria caused natural microbial infection of fish *Clarias batrachus* were reported from Wardha river during 2012 (Table 8). Clinical signs were also similar to previous findings (Yadav *et al.*1992, Qureshi and Mastan, 1999; Kumari and Akela, 2002; Prasad and Verma, 2003; 2004).

Thus in the present study bacterial isolates in river water confirms their involvement in the initial process of tissue damage, where as fungi relates to their direct mycotic involvement causing infections.

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