International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 6, Issue 4 - 2019

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.04.009

Screening and Identification of Pathogenic Microorganisms in Backwaters of Alappuzha District, Kerala State, India

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Abstract

Microbial pollution in aquatic environments is one of the crucial issues with regards to the cleanliness of water. Among them the pathogenic microorganisms forms a major threat to the living beings. The present study focus on the identification of pathogenic microorganisms present in water and sediment samples in backwaters of Alappuzha district. Prior to the analysis of pathogens, physical parameters and total microbial count were analysed for each samples. Several pathogens such as *Salmonella typhimurium, Salmonella enteritidis, Salmonella abony, Enterococococcus faecalis, Enterobacter aerogens, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae, Vibrio fluvialis, Salmonella typhi, Klebsiella pneumoniae, E. coli were* found in water and sediment samples, where the water samples contain more amount of pathogens than the sediment samples.

Keywords: backwaters, contamination, medium, pathogenic microorganism, water.

Introduction

Water has been of great importance to human beings and other organisms of the environment for sustenance of life and maintaining the balance of the nature, hence water is called as the “life blood of the earth” (Ram et al., 2009). Backwaters and most of the water resources are highly contaminated due to the disposal of organic waste, fertilizer residues and run offs from agricultural fields into water bodies. As a result of this water bodies have become a congenial breeding ground for water borne vectors like mosquitoes and pathogenic microorganisms (Chandran, 2014).

Microorganisms can exist naturally or can occur as a result of contamination from human or animal waste (Chandran et al., 2011). Occurrence of aquatic weeds is a major cause of contamination. Aquatic weeds are those unabated plants which grow and complete their life cycle in water and cause harm to aquatic environment directly and to related eco-environment relatively (Lancar and Krake, 2002). Distribution of bacteria in water depends on the changes in water temperature, salinity and physicochemical parameters. Temperature and pH are limiting factors for the

survival of bacteria in the environment (Whipple and Rohovec, 1994). The microbial contamination of water is often of faecal nature related to humans (water sewage overflow, non collective sewage system) domesticated animals or wild life.

The abundance of pathogen in water depends on factors such as the contamination level, pathogen persistence in water bodies, biological reservoirs including aquatic plants and sediments (Dechense et al., 2006). Sediments and submerged aquatic vegetation are important reservoirs of microorganisms (Badgley et al., 2010). Aquatic weeds are also responsible for lowering quantity as well as quality of water. These weeds cause taste and odour problems and also increases biological oxygen demand because of organic loading (Gopal and Sharma, 1979). Faecal contamination of water introduces a variety of pathogens into water ways, including bacteria, viruses, protozoa and parasitic worms. *E. coli*, *Salmonella* and *Vibrio* spp. forms the most important pathogen that spread through water. *E. coli* is the only member of the total coli form group that is found exclusively in faeces, other members of the group are found naturally in water, soil, and vegetation, as well as in faeces. *Salmonella typhi* is one of the major causes of food and water borne gastroenteritis in human (Tsen et al., 2000) and remains an important health problem worldwide.

Materials and Methods

Study area

The water samples were collected from back water of Muhamma (site 1), Valavanad (site 2) and Kokkothamangalam (site 3) regions of Alappuzha district, Kerala State, India, which contains the floating aquatic weed.

Sample collection

The surface water sample was collected in sterile screw capped bottles for bacteriological assessment and the sediment sample was collected by employing an alcohol rinsed and air-dried small Peterson's grab, which were aseptically transferred into new polyethylene bags using a sterile spatula. All samples were brought to the laboratory in portable icebox within 2 h.

Physical parameter analysis

Water quality parameters such as pH, salinity, Electrical conductivity (EC), Total Dissolved Solids (TDS), temperature, specific gravity were monitored. The surface water temperatures were measured using standard mercury filled centigrade thermometer, salinity and specific gravity were estimated with the help of a hand held Refractometer (ERMA INC Tokyo, Japan) and pH was measured using pH meter (Systronics pH system 361). EC and TDS were estimated using conductivity and TDS meter (Systronics 308).

Heterophillic microbial analysis of water and sediment samples

Heterotrophic microbial population was expressed as colony forming units for water and sediments (Cfu mL^{-1}).

Bacterial analysis

The serially diluted water and sediment samples were spread over the surface of Glucose Tryptone Agar (GTA) to enumerate the total bacterial colonies of the samples. The dilutions were plated in triplicates.

Fungal analysis

The serially diluted water and sediment samples were spread over the surface of potato dextrose agar to enumerate the total fungal colonies grown on the surface of the agar. The dilutions were plated in triplicates.

Analysis of actinomycetes

The serially diluted water samples and sediments were used to enumerate the number of actinomycete colonies grown on the Actinomycete isolation agar. The dilutions were plated in triplicates.

Isolation of pathogenic microorganisms

One milliliter of water sample and 1 g of sediment sample were added separately in 9 ml distilled water and were serially diluted. 0.1 mL of the serially diluted samples was inoculated on to GTA and specific agar (Brilliant Green, Mac Conkey, Thiosulphate Citrate Bilesalt Sucrose (TCBS) Agar, Bismuth sulphite (BS), Antibiotic Assay medium D, Eosine Methyline Blue (EMB) Agar to enumerate and to isolate the specific

bacterial pathogens. After inoculation the plates were incubated at 28 ± 2 ^{o}C for 24 to 48 h. After incubation the colony was enumerated. Heterotrophic bacterial population was expressed as colony forming units for water and sediments (Cfu mL^{-1}).

Results

Water quality analysed showed that all three sample were turbid, coloured and had odour. The physical parameters studied were given in the (Table 2) where site 2 exhibit high pH and conductivity, whereas site 1 exhibit high value of TDS while temperature was high in site 3. Heterophillic plate count (HPC) of the water samples were analysed for identification of total bacterial, fungal and actinomycetes present in the water sample where huge number of bacterial and actinomycetes colonies were identified in site 3 and fungal colonies were high in site 2 (Table 3). HPC of sediment samples were analysed to identify the total bacterial, fungal and actinomycetes where high number of bacterial colonies were present in site 3 while fungal colonies were high in site 2 and actinomycetes were high in site 1 (Table 4). Water samples spread on specific agar surface helps to identify different pathogenic organisms on the basis of colony morphology of each water samples (Tables 5, 6, 7) where pinkish white colonies on brilliant green agar were recognized as *S. typhimurium, S. enteritidis* and *S. abony* while yellowish green as *E. coli*. On Mac Conkey agar colourless to pale pink colonies were recognized as *Enterococococcus faecalis*, Pink to red as *Enterobacter aerogens* and Pink to red with bile precipitate as *E. coli*, while on TCBS agar Bluish green colonies were recognized as *V. parahaemolyticus, V. vulnificus* yellow colonies as *V. cholerae*, greenish yellow as *V. fluvialis, S. typhimurium, S. enteritidis, S. typhi* were recognized on bismuth sulphite agar with black colonies having metallic sheen. Antibiotic assay medium D was used to identify *K. pneumoniae* which represents white colonies and EMB agar represents *E. coli* in the respective plates with green metallic sheen. Similar organisms were present in sediment samples (Tables 8, 9, 10) but microbial load was lower as compared to the water samples.

Table 1: Characteristics of water samples.

Sl. No.	Sampling sites	Odour	Colour	Turbidity
1	Muhamma	Earthy	Brown	Turbid
2	Valavanad	Earthy	Yellow	Turbid
3	Kokkothamangalam	Stinky	Yellow	Turbid

Table 2: Physical parameters of water samples

Sl. No.	Sampling sites	pH	Salinity	Conductivity	TDS (µs/ ppm)	Temperature	Specific gravity
1	Muhamma	6.37	Nil	203 µs	116.0	27.4 ° C	0
2	Valavanad	8.87	Nil	82.16 µs/mm	90.5	29 ° C	0
3	Kokkothamangalam	6.5	Nil	3.806 µs/ppm	1.970	30.2 °C	0

Table 3: Heterophillic plate count of water samples

Sl. No.	Sampling sites	Number of colonies (Cfu/ml)		
		Bacteria	Fungi	Actinomycetes
1	Muhamma	40 X 10^3	5.33 X 10^3	6 X 10^3
2	Valavanad	11.6 X 10^3	11 X 10^3	12 X 10^3
3	Kokkothamangalam	45.33 X 10^3	5 X 10^3	17 X 10^3

Table 4: Heterophillic plate count of sediment samples

Sl. No.	Sampling sites	Number of colonies (Cfu/ml)		
		Bacteria	Fungi	Actinomycetes
1	Muhamma	32×10^3	5×10^3	8×10^3
2	Valavanad	9×10^3	8×10^3	5×10^3
3	Kokkothamangalam	38×10^3	5×10^3	8×10^3

Table 5: Isolation of bacterial pathogens in water sample collected from Muhamma

Sl. No.	Dilution factor	Name of agar medium	Presence / absence of organism	Colony Morphology	Organism recognized
1	10^{-2}	Brilliant Green	+	Pinkish white colonies	*Salmonella typhimurium* *Salmonella abony* *Salmonella enteritidis*
2	10^{-2}	Mac Conkey	+	Colourless to pale Pink colonies	*Enterocococcus faecalis*
3	10^{-2}	TCBS	+	Bluish green colonies	*Vibrio parahaemolyticus*
4	10^{-2}	Bismuth Sulphite	+	Black with metallic sheen	*Salmonella enteritidis*
5	10^{-2}	Antibiotic assay medium D	+	Pinkish white colonies	*Klebsiella pneumoniae*
6	10^{-2}	EMB	+	Green metallic sheen	*E. coli*

Table 6: Isolation of bacterial pathogens in water sample collected from Valavanad

Sl. No.	Dilution factor	Name of agar medium	Presence / absence of organism	Colony Morphology	Organism recognized
1	10^{-2}	Brilliant Green	+	Pinkish white	*Salmonella typhimurium* *Salmonella abony* *Salmonella enteritidis* *Salmonella enteritidis*
2	10^{-2}	Mac Conkey	+	Colourless to pale pink	*Enterocococcus faecalis*
3	10^{-2}	TCBS	+	Yellow	*Vibrio cholerae* *Vibrio fluvialis*
4	10^{-2}	Bismuth Sulphite	+	Black with metallic sheen	*Salmonella typhimurium* *Salmonella enteritidis* *Salmonella typhi*
5	10^{-2}	Antibiotic assay medium D	-	Nil	Nil
6	10^{-2}	EMB	-	Green Metallic sheen	*E. coli*

Table: 7 Isolation of bacterial pathogens in water sample collected from Kokkothamangalam

Sl. No.	Dilution factor	Name of agar medium	Presence / absence of organism	Colony Morphology	Organism recognized
1	10^{-2}	Brilliant Green	+	Pinkish white Yellowish Green	*Salmonella abony* *Salmonella enteritidis* *Salmonella typhimurium* *E. coli*
2	10^{-2}	Mac Conkey	+	Pink to red with bile precipitate Colourless to pale pink Pink to red	*E. coli* *Enterococcus faecalis* *Enterobacter aerogens*
3	10^{-2}	TCBS	+	Yellow Greenish Yellow Bluish Green	*Vibrio cholerae* *Vibrio fluvialis* *Vibrio parahaemolyticus* *Vibrio vulnificus*
4	10^{-2}	Bismuth Sulphite	+	Black with metallic sheen	*Salmonella typhimurium* *Salmonella enteritidis* *Salmonella typhi*
5	10^{-2}	Antibiotic assay medium D	+	White colonies	*Klebsiella pneumoniae*
6	10^{-2}	EMB	+	Green Metallic sheen	*E. coli*

Table: 8 Isolation of bacterial pathogens in sediment sample collected from site -1 (Muhamma)

Sl. No.	Dilution factor	Name of agar medium	Presence / absence of organism	Colony Morphology	Organism recognized
1	10^{-2}	Brilliant Green	+	Reddish Pink Pinkish white	*Salmonella typhi* *Salmonella abony* *Salmonella enteritidis* *Salmonella typhimurium*
2	10^{-2}	Mac Conkey	+	Colourless to pale pink	*Enterococcus faecalis*
3	10^{-2}	TCBS	+	Yellow Bluish Greeen Yellow	*Vibrio cholerae* *Vibrio fluvialis* *Vibrio parahaemolyticus*
4	10^{-2}	Bismuth Sulphite	+	Black with metallic sheen	*Salmonella typhimurium* *Salmonella enteritidis* *Salmonella typhi* *Salmonella abony*
5	10^{-2}	Antibiotic assay medium D	_	White colonies	*Klebsiella pneumoniae*
6	10^{-2}	EMB	+	Green Metallic Sheen	*E. coli*

Table: 9 Isolation of bacterial pathogens in sediment sample collected from site -2 (Valavanad**)**

Sl. No.	Dilution factor	Name of agar medium	Presence / absence of organism	Colony Morphology	Organism recognized
1	10^{-2}	Brilliant Green	+	Pinkish white	*Salmonella typhimurium* *Salmonella abony* *Salmonella enteritidis*
2	10^{-2}	Mac Conkey	+	Colourless to pale pink	*Enterococcus faecalis*
3	10^{-2}	TCBS	_	Nil	Nil
4	10^{-2}	Bismuth Sulphite	_	Nil	Nil
5	10^{-2}	Antibiotic assay medium D	+	White Colonies	*Klebsiella pneumoniae*
6	10^{-2}	EMB	+	Green Metallic Sheen	*E. coli*

Table 10: Isolation of bacterial pathogens in sediment sample collected from Kokkothamangalam

Sl. No.	Dilution factor	Name of agar medium	Presence / absence of organism	Colony Morphology	Organism recognized
1	10^{-2}	Brilliant Green	+	Pinkish white Reddish Pink	*Salmonella typhimurium* *Salmonella abony* *Salmonella enteritidis* *Salmonella typhi*
2	10^{-2}	Mac Conkey	+	Colour less to pale pink	*Enterococcus faecalis*
3	10^{-2}	TCBS	_	Nil	Nil
4	10^{-2}	Bismuth Sulphite	+	Black with metallic sheen	*Salmonella typhimurium* *Salmonella enteritidis* *Salmonella typhi*
5	10^{-2}	Antibiotic assay medium D	+	White colonies	*Klebsiella pneumoniae*
6	10^{-2}	EMB	+	Green Metallic Sheen	*E. coli*

Discussion

Water is essential for life. Dead leaf, woody debris, animal remains etc. constitute the main sources of organic matter in backwater environment. Microorganisms distributed in the marine and backwaters play an important role in the decomposition of organic matter and mineralization (Hollibaugh *et al*., 1980). Microorganisms, like bacteria, fungi etc. have major role in the biodegradation of this organic matter, so their survival in water is inevitable. The existing bacterial communities are likely to play very active role in the rapid *in situ* degradative process, especially the salinity, pH, play a key role in the biological process (Nair et al., 2011). Drastic changes in cytoplasmic pH harm microorganisms disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins, whereas microorganisms can change the pH of their own habit by producing acidic or basic metabolic waste products. The water samples from different sites exhibit optimum pH and temperature of different pathogens hence abundant growth was observed in the present study. An increase in the turbidity of water results in interference of penetration of light. This will damage aquatic life and also deteriorate the quality of surface water. High values of turbidity minimize the filter runs which cause the pathogenic organisms to be more hazardous to human life which is similar to the current work as the water samples were highly turbid. The heterotrophic bacterial distribution, diversity and activities are controlled by various hydro biological factors and nutrient levels present in the aquatic environment and have been well studied in marine environment (Azam *et al*., 1983; Ducklow and Hill, 1985). Distribution of bacteria depends on changes in water temperature, salinity and physicochemical parameters. Pathogenic organisms present in the water determine the level of contamination that leads to threat for aquatic life forms and for the human beings. The presence of aquatic floating weeds in water reduces the pathogens from being accumulated in sediments as they help the pathogens to float to other regions of water rather than settling, which is evident from the sediment samples analysed in the study.

Conflict of Interest Statement

Authors declare that they have no conflict of interest.

Acknowledgments

The authors sincerely thank Dr. V.V. Pyarelal, Director, K.V.M. College of Science and Technology, Cherthala, Kerala, India, for providing necessary facilities and support for conducting this research work.

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Quick Response Code	Subject: Microbiology
DOI:10.22192/ijarbs.2019.06.04.009	

How to cite this article:
T.S. Athira, Bhavana Vijayan, G. Gopika, Theresa George, Reshmi Sudhakaran, Neethu Franklin, R. Pratap Chandran . (2019). Screening and Identification of Pathogenic Microorganisms in Backwaters of Alappuzha District, Kerala State, India. Int. J. Adv. Res. Biol. Sci. 6(4): 62-69.
DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.04.009