ISSN 2321–340X
Journal of Aquatic Biology & Fisheries | Vol. 7 | 2019 | pp. 127 - 133

APPRAISAL ON THE MICROBIAL POLLUTION STATUS OF SASTHAMKOTTA LAKE, KERALA, INDIA

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Abstract: Watershed regions of the Ramsar Lake, Sasthamkotta in Kerala, have been exploited for various purposes. Thedecline in environmental services of the lake in the recent past is due to deterioration of catchment area, and decline in water both in quantity and quality due to various anthropogenic interferences. This paper estimates the microbial contamination of water, sediment and benthic community of the lake. Bimonthly collections of surface and bottom water and sediment samples were made from six stations during January to December 2016. Total Bacterial Count, Total Coliforms and *Escherichia coli* count was enumerated using standard methods. Bacteria isolated from three representative stations were selected for species identification employing morphological, biochemical and VITEK Identification System.The analysis indicated that the benthic fauna harbours maximum bacterial load from water the column and the total coliform count in water exceeds the desirable potable limit. Comparison of total coliforms in water, sediment and benthos showed that total coliform counts are the maximum in benthos. *Escherichia coli* status indicate that it increases during monsoon and post monsoon.

Key words: *E.coli*, Total coliforms, Water quality, Sediment, Benthic fauna

INTRODUCTION

Surface water resources like fresh water lakes are the preferred sources of drinking water in developing countries like India (Peter and Sreedevi, 2013). However, in the era of economic growth, surface water quality gets deteriorated due to urbanization and industrialization. Water quality describes the chemical, physical, and biological characteristics of water, usually in respect to its suitability for a particular purpose (Curtis, 2001). Entry of pathogenic bacteria in to surface water sources from anthropogenic discharges has become a challenge to water quality (Pandey *et al*., 2014).

Kerala State's largest drinking water source, the Ramsar notified Sasthamcotta freshwater lake in Kollam district has registered considerable environmental deterioration (Periera, 2013). The catchment area of the lake is subjected to serious environmental degradation due to excessive biotic disturbances and unscientific soil and water management practices (Chackacherry and Jayakumar, 2011). Anthropogenic interferences like domestic sewage from the thickly populated places in Kollam city can create bacteriological contamination of the fresh water system making it unsuitable for human consumption. The fecal bacteria can settle down in sediment and accumulate in benthic environment. Though some studies (George and Koshy, 2008), Peter and Sreedevi (2013) and Divya Raj and Morphin Kani (2018) have been initiated on deterioration of water quality of the lake including bacterial contamination, no study has been undertaken to estimate the bacteriological contamination in water, sediment and benthic environment together.

This paper records the microbial contamination of water, sediment and benthic organisms of Sasthamkotta Lake, which serves drinking water to Kollam city and nearby areas in Kerala state.

MATERIALS AND METHODS

Sathamkotta lake, located between 9°00'-9°05' N latitude and 76°35'-76°40' E longitude, is in Kollam district of Kerala state (Fig. 1). Its catchment area spreads in Sasthamkotta, West Kallada and Mynagapallygrama panchayaths. Bimonthly collections of both surface and bottom water and sediment samples were made from six stations during

January 2016 to December 2016. Microbiological analysis for Total Bacterial Count (TBC), Total Coliforms (TC) and *E.coli* count is carried out in six stations. Three representative stations viz., Station I in the northern end, station IV in the mid portion and station VI in the southern end, covering the entire area were selected for seasonal bacterial species identification. Enumeration of TBC, TC and *E.coli* in water samples is carried out using pour plate and MPN method (APHA, 2012), TBC in sediment and benthic fauna is estimated as per total plate count method (IS, 2002), and TC as per pour plate method and *E.coli* as per spread plate method (APHA, 2012). Benthos samples sieved from sediments collected using Ekman dredge and manually sorted are washed with sterile water and homogenized in pestle and mortar. One gram each of the homogenized sample is serial diluted up to 10^{-5} with sterile distilled water for enumeration of bacteria (APHA, 2012). Qualitative analysis of benthic organisms is done following Tonapi (1980). The benthic fauna identified are *Phaenopsectra* sp., *Sumatendipes tobaterdecimus*, *Procladius* sp., *Chaoborus asiaticus*, *Ecnomus puntung* and *Pristina leidyi*.

Bacterial strains isolated are initially analyzed by characteristic colony morphology and gram reaction (APHA, 2012). To confirm the bacterial species identity based on phenotypic traits, VITEK Cards through VITEK 2 Compact System is employed (Ligozzi *et al*., 2002). VITEK 2 System provides an automated computer based method of species identification as per the measurement of light attenuation associated with each biochemical reaction.

RESULTS AND DISCUSSION

Monthly and seasonal ranges of various bacteriological parameters are summarized in Table 1 to 6. Total Bacterial Count (TBC) in surface water ranged between 0.12×10^3 CFU/ml during October and 27×10^3 CFU/ml during June. In bottom water TBC varied between 0.22×10^2 CFU/ml during October and 27×10^2 CFU/ml during June. TBC in both surface and bottom water was the minimum during post monsoon and the maximum during monsoon. Monthly variation in TC in surface water ranged between 20 MPN/100ml during February and 720 MPN/100ml during June. TC in bottom water was

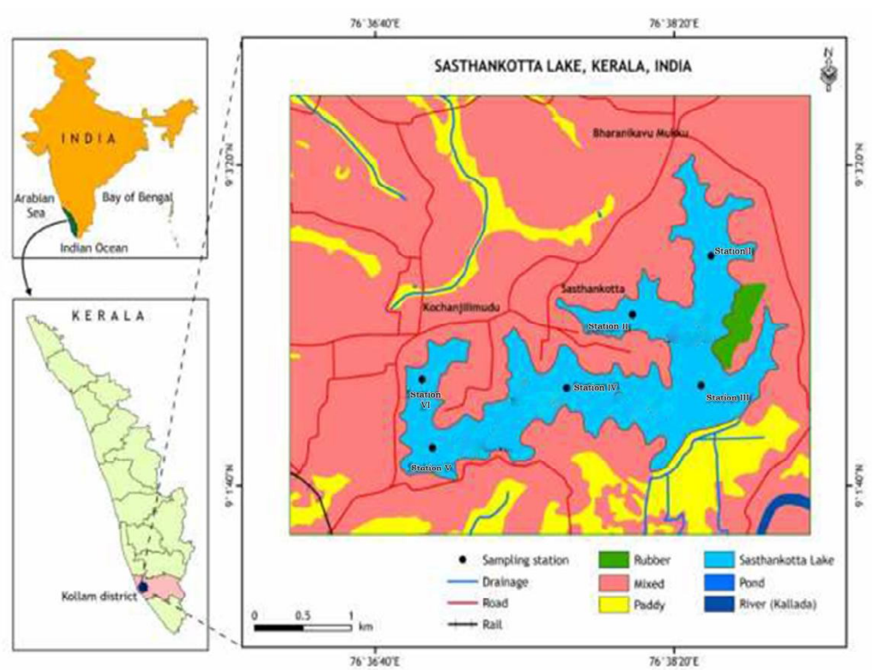

Fig. 1. Map of Sasthamkotta Lake, Kerala showing sampling stations

between 6 MPN/100 ml during August and 270 MPN/100 ml during October. The minimum TC count was recorded during pre-monsoon and the maximum during monsoon in surface water and during pre-monsoon and during post monsoon in bottom water. *E.coli* recorded the highest value of 20 MPN/100 ml during February in surface water and 92 MPN/100 ml during February in bottom water. *E.coli* was maximum during pre-monsoon in both surface and bottom water.

Highest TBC during monsoon season indicated surface runoff to the lake during the rainy days. The high coliform count noticed from the surface water during monsoon season also supports the observation. The overlying water with copious nutrients may also be responsible for proliferation of bacteria. Since the presence of *E.coli* indicates faecal contamination, the water is not fit for domestic use unless proper purification treatment is made. The permissible limit of both TC and *E.coli* in drinking water is 0 MPN/100 ml (IS, 2012). The acceptable limit for bathing purpose is 500 MPN/100 ml for total coliforms and 100 MPN/100 ml for *E.coli* (Singh and Singh,1995). However, the values recorded in the study indicate that they are lesser lesser compared to those observed in previous studies in the Sasthamkotta lake (Girijakumari, 2007; Girijakumari and Abraham, 2007; Peter and Sreedevi, 2013).

During monthly sampling the TBC in sediment ranged between $0.47x10^3$ CFU/g during December and $24x10^3$ CFU/g during February. The lowest TBC was observed in post monsoon and the highest values during pre-monsoon (Table 1). The monthly variation of TC in sediment ranged between <10 CFU/g and 390 CFU/g. TC count was the maximum during pre-monsoon at station VI. Total coliforms were not detected during monsoon and postmonsoon seasons and recorded from some stations during pre-monsoon (Table 2). Coliform bacteria may be more in clayey environment which is enriched with high organic load (Seo *et al.*, 2019). The runoff waste and human waste from nearby residential areas might be responsible for the increased faecal contamination in freshwater. *E.coli* in sediment was not detected throughout the study period. There is limited dilution of overlying water during pre-monsoon which might be accountable for the high microbial load.

Bimonthly variation of TBC in benthic fauna ranged between $0.0028x10^5$ CFU/g during February and $25x10^5$ CFU/g during October. The minimum count was observed during pre-monsoon and the maximum count during monsoon. TC ranged from <10 CFU/g to $12x10^2$CFU/g and the seasonal variation was between <10 CFU/g (pre-monsoon) and $11x10^2$CFU/g (monsoon). *E.coli* was not detected throughout the study period. In general the total bacterial load except *E.coli* was higher in benthic organisms compared to water and sediment (Table 3). Microorganisms might have accumulated in benthic fauna which in turn reduce the bacterial count in water and sediment.

A total of 21 species of bacteria was identified from the water samples (Table 4-6), which included *Actinetobacter baumanii, Actinetobacter pitii, Chryseobacterium gleum, Pantoea* sp., *Kocuria varians, Sphingomonas paucimobilis, Bacillus cereus, Kocuria kristinae, Methylobacterium* sp., *Stenotrophomonas maltophilia, Bacillus pumilus, Burkholderia vietnamiensis, Chromobacterium violaceum, Ralstonia picketti, Staphlycoccus epidermis, Bacillus licheniforms, Aeromonas salmonicida, Pseudomonas luteola, Aeromonas sobria, Cupriavidus pauculus,* and *Granulicatella elegans*. Twelve species of bacteria found in sediment samples were *Actinetobacter baumanii, Bacillus cereus, Bacillus coagulans, Bacillus pumilus, Bacillus thuringiensis, Brevibacillus choshinensis, Pantoea* sp., *Staphylococcus lentus, Burkholderia vietnamiensis, Kocuria varians, Brevundimonas diminuta* and *Sphingomonas paucimobili*. Ten bacterial communities isolated from benthic organisms were *Actineotobacter baumanii, Chryseobacterium gleum, Stenotrophomonas maltophilia, Bacillus pumilus, Burkholderia vietnamiensis, Chromobacterium violaceum, Ralstonia picketti, Staphlycoccus epidermis, Aeromonas sobria* and *Staphlycoccus xylosus*.

The species analysis indicates that bacterial species observed in water were seen both in sediment and benthic fauna. This indicates that source of bacteria in benthos is the overlying water and the settled sediment. The entry of anthropogenic wastes from organic pollutants acts as the sources of total and fecal coliforms in the lake. Girijakumari *et al.* (2006) reported total coliforms in the range of 450-1200

Table 1. Range and seasonal fluctuation in the bacterial count of water samples of Sasthamkotta Lake during 2016

		Minimum (Month, Station)	Maximum (Month, Station)	Minimum (Season, Station)	Maximum (Season, Station)
TBC (CFU/ml)	SW	$0.12x10^3$ (Oct. VI)	$21x10^3$ (June III)	130 (Post monsoon VI)	$20x10^3$ (Monsoon III)
	BW	$0.22x10^2$ (Oct. IV)	$27x10^2$ (June V)	240 (Post monsoon IV)	$21x10^3$ (Monsoon V)
TC (MPN/100ml)	SW	20 (Feb V)	720 (June IV)	20.5 (Pre-monsoon V)	520 (Monsoon IV)
	BW	6 (Aug. III)	270 (Oct.I)	23 (Pre-monsoon III)	900 (Post monsoon I)
E.coli (MPN/100ml)	SW	<1.8 (June, Aug., Oct., Dec-I, III, IV, V, VI)	120 (Feb. V)	<1.8 (Monsoon IV, V, VI, Post monsoon I, III, IV, V, VI)	17 (Pre-monsoon V)
	BW	<1.8 (June, Aug., Oct., Dec-I, II, III, IV, V, VI)	92 (Feb. VI)	<1.8 (Monsoon IV, V, VI, Post monsoon I, II, III, IV, V, VI)	86 (Pre-monsoon VI)

SW- Surface Water; BW- Bottom Water

Table 2. Range and Seasonal fluctuation in bacterial count in sedimentsamplesof Sasthamkotta Lake during 2016

	Minimum (Month, Station)	Maximum (Month, Station)	Minimum (Season, Station)	Maximum (Season, Station)
TBC (CFU/g)	$0.47x10^3$ (Dec. IV)	$24x10^3$ (Feb. III, IV)	$0.5x10^3$ (Post monsoon IV)	$22.5x10^3$ (Pre-monsoon x all stations)
TC count (CFU/g)	<10 (June, Aug., Oct., Dec. I, II, III, IV, V, VI)	390 (April IV)	<10 (Pre-monsoon III, IV, V, VI, Monsoon and Post monsoon- all stations)	320 (Pre-monsoon IV)
E. coli count (CFU/g)	Absent	Absent	Absent	Absent

Table 3. Range of bacterial count in benthic organisms of Sasthamkotta Lake during 2016

	Minimum (Month, Station)	Maximum (Month, Station)	Minimum (Season, Station)	Maximum (Season, Station)
TBC (CFU/g)	$0.0028x10^5$ (Feb. IV)	$25x10^5$ (Oct. IV)	$0.003x10^5$ (Pre-monsoon III, IV)	$22x10^5$- (Post monsoon IV)
TC count (CFU/g)	<10 (Feb., Apr. III, IV)	$12x10^2$ (June VI)	<10 (Pre-monsoon III, IV)	$11x10^2$ (Monsoon VI)
E. coli count (CFU/g)	Absent	Absent	Absent	Absent

MPN/100ml in pre-monsoon, 500-1400 MPN/100ml in monsoon and 240-2500 MPN/100ml in post monsoon and Peter and Sreedevi (2013) reported TC in the range of 467-1100 MPN/100ml in pre-monsoon and 1100 MPN/100ml in monsoon and post monsoon, both are higher than the counts observed in the present study. The range of *E.coli* reported from the lake water is 30-120 MPN/100ml in pre-monsoon, 140-260 MPN/100ml in monsoon and 120-260 MPN/100ml post monsoon (Peter and Sreedevi, 2013). This showed that pollution in Sasthamkotta lake is gradually decreasing. The present study indicates that the total bacterial load (TBC) in water is lesser than that in the sediment and benthos. It shows that the bacteria in water get settled down in sediments and accumulated in benthos. In effect the possibility of waterborne disease from bacterial contamination is controlled by the benthic environment, which support the self-purification process in the lake.

CONCLUSIONS

The bacterial species isolated from the Sasthamkotta lake system is represented by 21 species. The lesser seasonal counts of total coliforms and *E.coli* observed in the present study than that reported by earlier workers indicates that microbial pollution in the system is gradually decreasing due to the intervention by the authorities. The study shows that benthic environment is a complex milieu, which plays a pivotal role in maintaining the total bacterial load of the lacustrine ecosystem.

Table 4. Bacterial species identified by VITEK Method from Sasthamkotta Lake, pre-monsoon 2016

Bacteria	Gram Reaction	STATION I				STATION IV				STATION VI			
		SW	BW	Sediment	Benthic Fauna	SW	BW	Sediment	Benthic Fauna	SW	BW	Sediment	Benthic Fauna
Actinetobacter baumannii	GN	+	+	+	+	+	+	+	+	+	+	+	+
Actinetobacter pitii	GN	-	-	-	-	-	-	-	-	+	-	-	-
Bacillus cereus	BCL	-	-	-	-	-	+	+	-	-	-	+	-
Bacillus coagulans	BCL	-	-	+	-	-	-	-	-	-	-	-	-
Bacillus pumilus	BCL	-	-	+	-	-	-	-	-	-	-	-	-
Bacillus spp	BCL	-	-	+	-	-	-	-	-	-	-	-	-
Brevibacillus choshinensis	BCL	-	-	-	-	-	-	+	-	-	-	-	-
Chryseobacterium gleum	GN	+	+	-	+	+	+	-	-	+	+	-	+
Kocuria kristinae	GP	-	+	-	-	-	-	-	-	-	-	-	-
Kocuria varians	GP	+	-	-	-	+	-	-	-	+	+	-	-
Methylobacterium spp	GN	-	+	-	-	-	-	-	-	-	-	-	-
Pantoea spp	GN	-	-	+	-	-	-	+	-	+	+	-	-
Sphingomonas paucimobilis	GN	-	-	-	-	+	-	-	-	-	-	-	-
Staohylococcus lentus	GP	-	-	+	-	-	-	-	-	-	-	-	-
Stenotrophomonas maltophilia	GN	-	+	-	-	-	-	-	-	-	-	-	+

GN- Gram Negative; GP- Gram Positive; BCL- *Bacillus* Identification Card ; '+' - Presence; '-'- Absent

Table 5. Bacterial species identified by VITEK Method at Sasthamkotta during monsoon, 2016

Bacteria	Gram Reaction	STATION I				STATION IV				STATION VI			
		SW	BW	Sediment	Benthic Fauna	SW	BW	Sediment	Benthic Fauna	SW	BW	Sediment	Benthic Fauna
Bacillus pumilus	BCL	+	-	+	+	-	+	+	+	+	+	+	+
Bacillus licheniforms	BCL	-	+	-	-	-	-	-	-	-	-	-	-
Burkholderia vietnamiensis	GN	+	+	+	+	+	+	+	-		+	+	+
Chromobacterium violaceum	GN	+	-	-	+	+	+	-	+	-	+	-	+
Chryseobacterium gleum	GN	-	-	-	-	+	-	-	-	-	-	-	-
Kocuria varians	GP	-	+	-	-	+	+	+	-	+	-	-	-
Ralstonia pickettii	GN	+	-	-	+	-	-	-	+	+	-	-	+
Staphylococcus epidermidis	GP	+	-	—	+	-	-	-	+	-	+	-	+

GN- Gram Negative; GP- Gram Positive; BCL- *Bacillus* Identification Card ; '+' - Presence; '-'- Absent

Table 6. Bacterial species identified by VITEK Method at Sasthamkotta during post monsoon, 2016

Bacteria	Gram Reaction	STATION I				STATION IV				STATION VI			
		SW	BW	Sediment	Benthic Fauna	SW	BW	Sediment	Benthic Fauna	SW	BW	Sediment	Benthic Fauna
Aeromonas salmonicida	GN	-	-	-	-	+	-	-	-	+		-	-
Aeromonas sobria	GN	+	-	-	+	+	+	-	-	+	+	-	+
Bacillus cereus	BCL	-	-	-	-	-	-	+	-	-	-	-	-
Brevundimonas diminuta	GN	-	-	-	-	-	-	+	-	-	-	+	-
Cupriavidus pauculus	GN	-	+	-	-	-	-	-	-	-	-	-	-
Granulicatella elegans	GP	-	+	-	-	-	-	-	-	-	-	-	-
Pseudomonas luteola	GN	-	-	-	-	+	-	-	-	-	-	-	-
Sphingomonas paucimobilis	GN	-	-	+	-	-	-	-	-	-	-	-	-
Staphylococcus xylosus	GP	-	-	-	+	-	-	-	+	-	-	-	+

GN- Gram Negative; GP- Gram Positive; BCL- *Bacillus* Identification Card ; '+' - Presence; '-'- Absent

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