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Study of Seasonal Variation in Mula-Mutha River of Pune City with respect to Total and Pathogenic Microbial Load

Kadhum Sabah Naser Alshekhmoham[1], Fadhil Kayoon Naser[1], Qasim Muhammad Abdulhussein Altaher[1], Sanjay Kale[1], Hemal Makani[2], Asha Salunke[2]

[1]Department of Environmental Science, Savitribai Phule Pune University, Ganeshkhind Road, Pune – 411007, Maharashtra, India
[2]BioEra Life Sciences Pvt. Ltd., BioEra Park, S. No. 125, Mumbai-Bangalore Highway, Tathawade, Pune – 411033, Maharashtra, India

Abstract: **The growth of Pune city, India has been accompanied by severe pollution of the Mula- Mutha River. In spite of Government regulations, lot of such waste is dumped around the cities and metropolis and deposited in natural water resources. In Pune, Mula-Mutha rivers are contaminated with such waste materials. This pollution has most immediate effect on human health, through water borne diseases. In present study, seasonal variations in the physicochemical characteristics as well as microflora of the Mula-Mutha river system were analyzed. The water samples were screened for presence of pathogens like Salmonella, Shigella, Pseudomonas, Escherichia coli, Streptococcus and Staphylococcus and their antibiotic resistance profiles were determined. For this purpose water samples were collected from 5 different sites on the banks of rivers Mula, Mutha and Mula-Mutha confluence in three seasons i.e. Monsoon, Winter and Summer. It was found that in Summer the pathogenic load was highest and all the isolated pathogens exhibited multiple drug resistance.**

Keywords: **Antibiotic Resistance, *Escherichia coli*, Mula, Mutha, *Pseudomonas*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus.***

I. INTRODUCTION

Pune became one of the winners of India's first smart city challenge and has grabbed attention of many. The city is ever developing with the new upcoming projects, proposals and changes those are made to transform the city into a better place to live in. The city is a cultural capital of Maharashtra and at the same time it is developing into a business center. It is recognized as one of the 9 cities worldwide with citation "Hosts IT and automotive companies". The city has its own historic value and it is situated on the banks of river Mutha (Nalawade, 2008).

Mutha river flows through Pune city and meets Mula river. After the confluence continues to flow to eastern side as Mula-Mutha river, it meets Bhima river. The river water is collected into dams Panshet and Khadakwasala which provides water for drinking and irrigation purpose throughout the year for the city. In recent years due to industrial development and improper disposal of waste materials into the river, the river has reached peak levels of pollution and the water can no longer be used for drinking or irrigation purposes. The pollution levels of river are an alarming signal of the health threat level to the population residing next to river.

There are reports highlighting the fact of pollution level of Mula-Mutha river. Mula-Mutha river was reported to have COD and BOD levels of much above the permissible limits (Pali et al., 2015). There are also reports on chemical analysis of river water and analysis of concentrations of the Chlorides, Phosphates, Nitrates levels in river water throughout the city. This indicated that the major cause of pollution in Mutha river is the population density and the discharge of domestic sewage from point and non-point sources. The industrial effluents also contribute to the increased levels of pollutants (D.G. Kanase, S.D. Jadhav, R.W. Jawale, M.S. Kadam, Bharati Vidyapeeth Deemed University, Yashwantrao Mohite College, Pune, personal communication).

Even though there are many reports that talk about the levels of chemical pollutants in Mula-Mutha river, very few reports are published focusing on biological contaminants present in river water. One of the study reports, presence of coliforms, the indicatives of pathogens, abundantly in river water. The estimated most probable number of coliforms was found to be 230000 per 100 ml of water (Kazmi et al., 2013).

The present study was carried out to estimate the chemical pollutants as well as to evaluate the total and pathogenic microbial load at different locations of Mula-Mutha river in all three season. The pathogens studied include *Pseudomonas*, *Salmonella*, *Shigella*, *Streptococcus*, *Staphylococcus* and *Escherichia coli*. These pathogens were selected on the basis of their known environmental ubiquity and potent pathogenesis. Also, many of these pathogens have been reported to have developed antibiotic resistance. Contamination of river water with multiple drug resistant pathogens may lead to outbreaks of severe diseases which are more difficult and costlier to treat. To check the presence of such multiple drug resistant microbes, the isolated pathogens were subjected to antibiotic susceptibility test.

II. MATERIALS AND METHOD

SAMPLE COLLECTION:

To analyze the pollution levels in Mula-Mutha river, five water samples were collected from various sites spanning the Mula-Mutha rivers and the confluence of these rivers. The sites for sample collection were same throughout the analysis. Sample collection was repeated in all three seasons from same sites to record the seasonal variations in microbial flora of the rivers.

PHYSICO-CHEMICAL ANALYSIS OF WATER SAMPLES:

Physical characteristics like colour, temperature, odour and turbidity of all the collected water samples. These were noted using standard procedures for all the samples. Chemical properties like pH, COD and BOD which were analyzed using standard procedures for all the samples (Directorate of irrigation research, 2009).

MICROBIAL ANALYSIS OF WATER SAMPLES:

Water samples were diluted using saline and were plated on sterile nutrient agar plates to evaluate the total viable count. Total number of coliforms was detected by carrying out MPN analysis for all water samples.

SCREENING FOR PRESENCE OF PATHOGENIC MICROORGANISMS

Each water sample was serially diluted to obtain dilution upto 10^{-9}. 100µl of 10^{-3}, 10^{-6}, 10^{-9} dilutions of each water sample were spread on SS Agar plate for isolation of *Salmonella* and *Shigella*, on Cetrimide Agar plate for isolation of *Pseudomonas*, on McConkey Agar plate for isolation of *Escherichia coli*, on Streptococcus selection agar plate for isolation of *Streptococcus* and on Mannitol Salt Agar plate for isolation of *Staphylococcus.* These plates were incubated at 37°C for 24 hours. After the incubation plates were observed for bacterial growth.

ANTIBIOTIC SENSITIVITY TEST:

The isolates obtained from selective agar plate were spread on a fresh selective agar plate at high density (by swabbing the overnight grown culture) and discs impregnated with various antibiotics of standard concentrations were placed on these plates. The antibiotics used were Penicillin (P) (10 µg/ml), Ampicillin (A) (20 µg/ml), Tetracycline (T) (30 µg/ml), Streptomycin (S) (10 µg/ml), Erythromycin (E) (15 µg/ml) and Gentamycin (G) (10 µg/ml). The plates were then incubated overnight at 37°C for 24 hours and were observed upon incubation. The results were by comparing the zone of inhibition values with the standard Kirby-Bauer chart values.

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III. RESULTS AND DISCUSSION

PHYSICO-CHEMICAL ANALYSIS:

Five sample sites were selected across the River Mula, Mutha and Mula-Mutha confluence. All the actual sample site photographs with GPS location are given in Fig. 1, Fig. 2, Fig. 3, Fig. 4 and Fig. 5. During Physical analysis (Table 1) it was observed that the water sample collected from Vitthalwadi and Lakadi Bridge had pungent or H_2S like smell in all three seasons and the water turbidity and temperature increased in summer season. The River water sample collected from Baner and Aundh were Green in colour, may be due to presence of algae. The turbidity and temperature increased in summer. The Sangamwadi water sample showed green colouration and increase in temperature in summer, but the sample remained transparent in all the seasons.

Chemical analysis Table 2 shows the pH of all the samples were alkaline in monsoon and summer season but had lowered down to neutral side in winter season. Water sample from Sangamwadi did not show any change in pH throughout all three seasons. The COD values decreased from monsoon to winter to summer for samples collected from Vitthal wadi, Lakadi Bridge and Baner. For water samples collected from Aundh and Sangamwadi the COD values were highest in winter season followed by monsoon and were least in case of summer. BOD values for samples collected from Vitthal wadi and Lakadi Bridge were maximum during winter and were minimum in summer. For sample collected from Baner the BOD values kept on increasing from monsoon to winter to summer. For Aundh water sample the BOD value was minimum in winter and was maximum in summer. Water sample collected from Sangamwadi showed continuous fall in BOD values from monsoon to winter to summer.

River water samples from Mula-Mutha were Physico-chemically analyzed (Pali et al., 2015) and it was found out that the agricultural run-off in a large proportion, disposal of waste, burning of fossil fuels, discharge of domestic wastes, hospitals and industrial effluents from small and large scale industries which are located at the bank of the rivers are polluting the river and making the waters unfit for consumption or use.

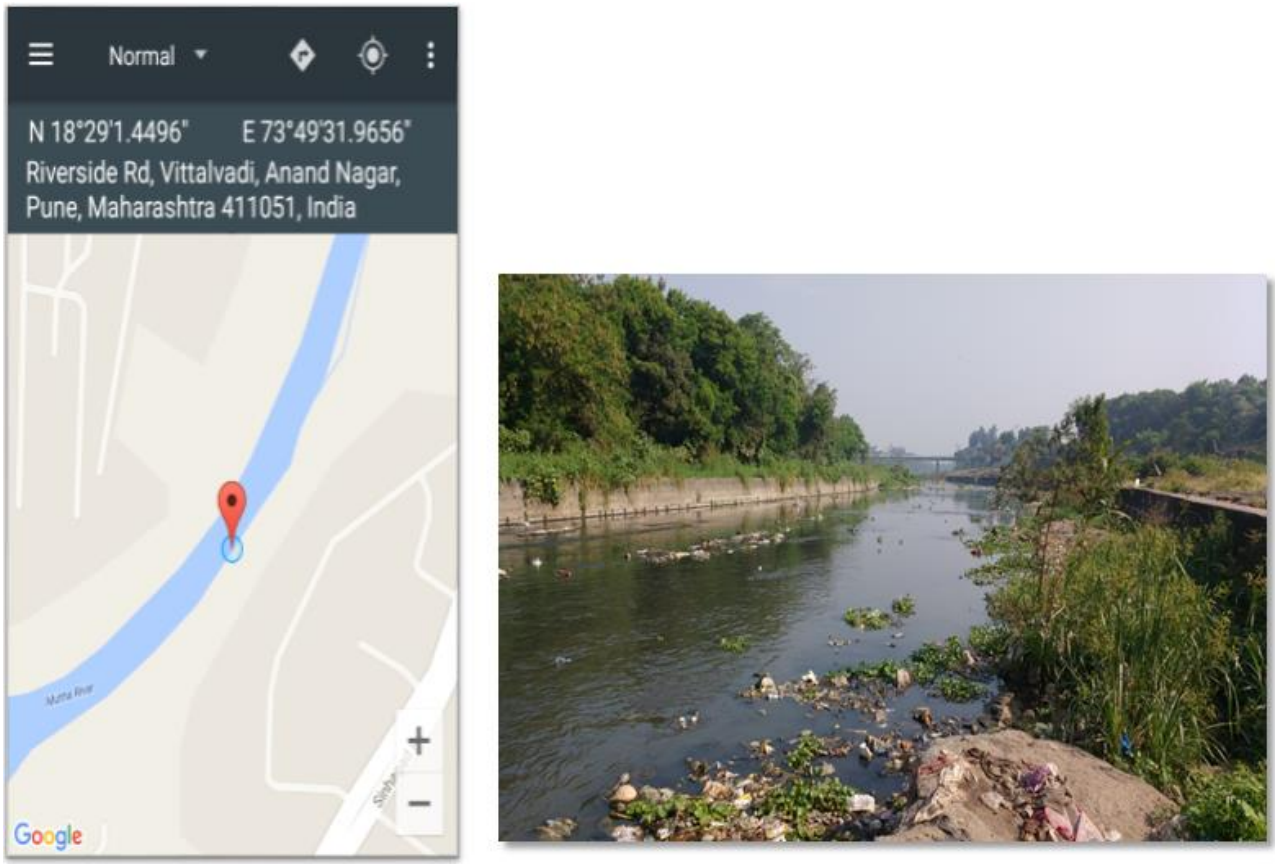

Fig. 1: Water Sample collection site Vitthal wadi (Mutha River)

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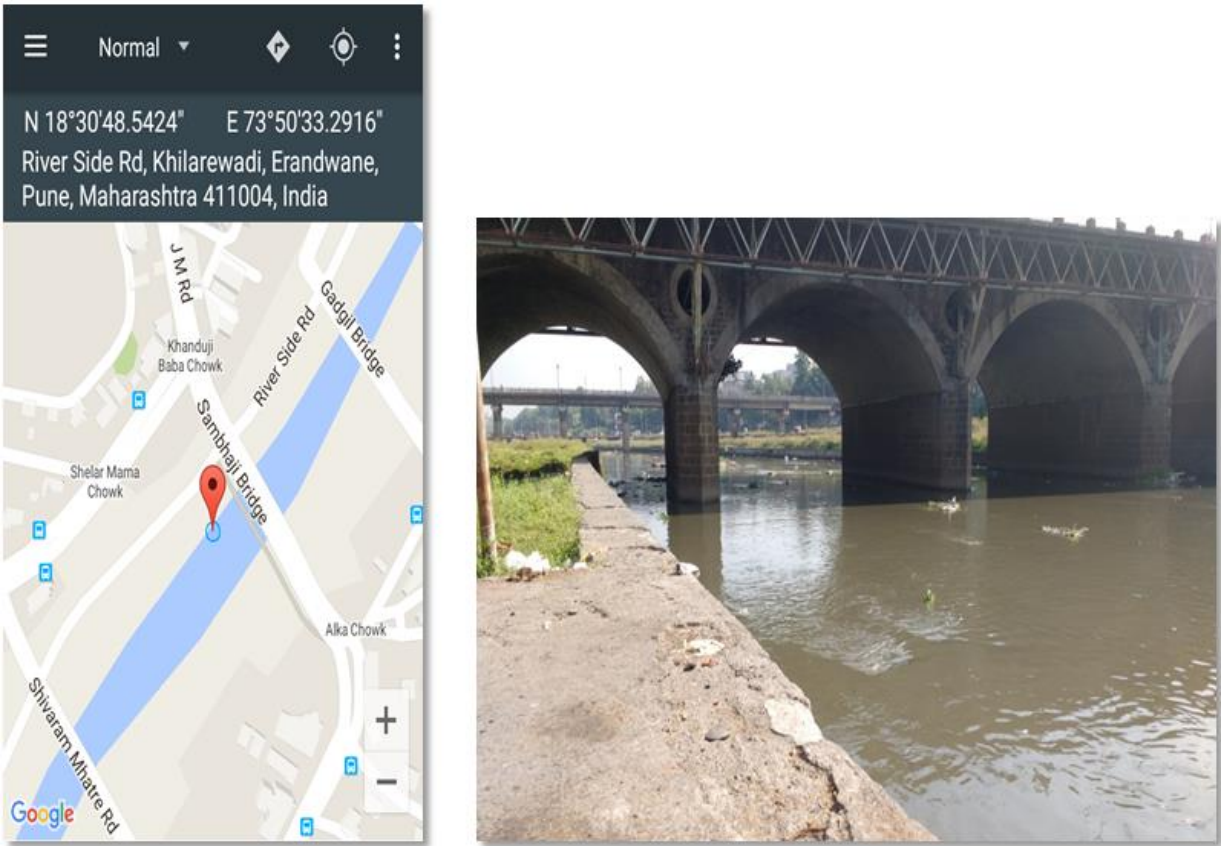

Fig. 2: Water Sample collection site Lakadi Bridge (Mutha River)

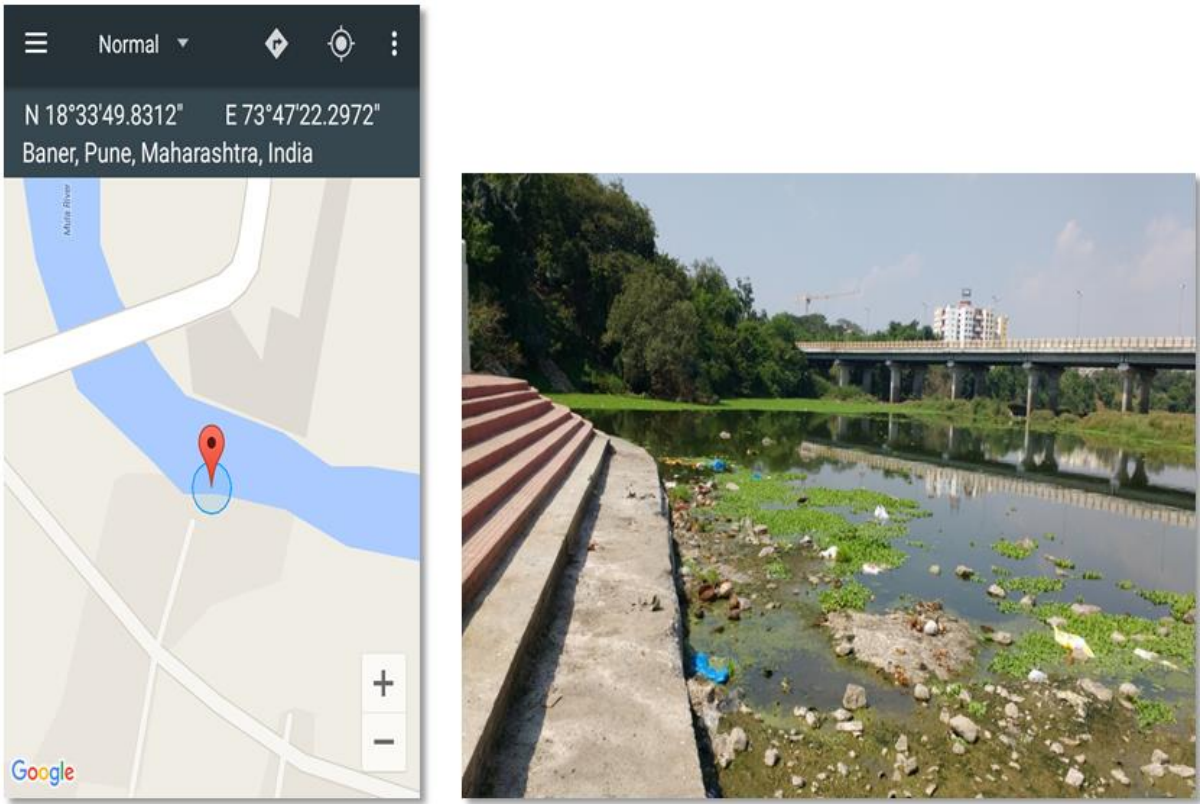

Fig. 3: Water Sample collection site Baner (Mula River)

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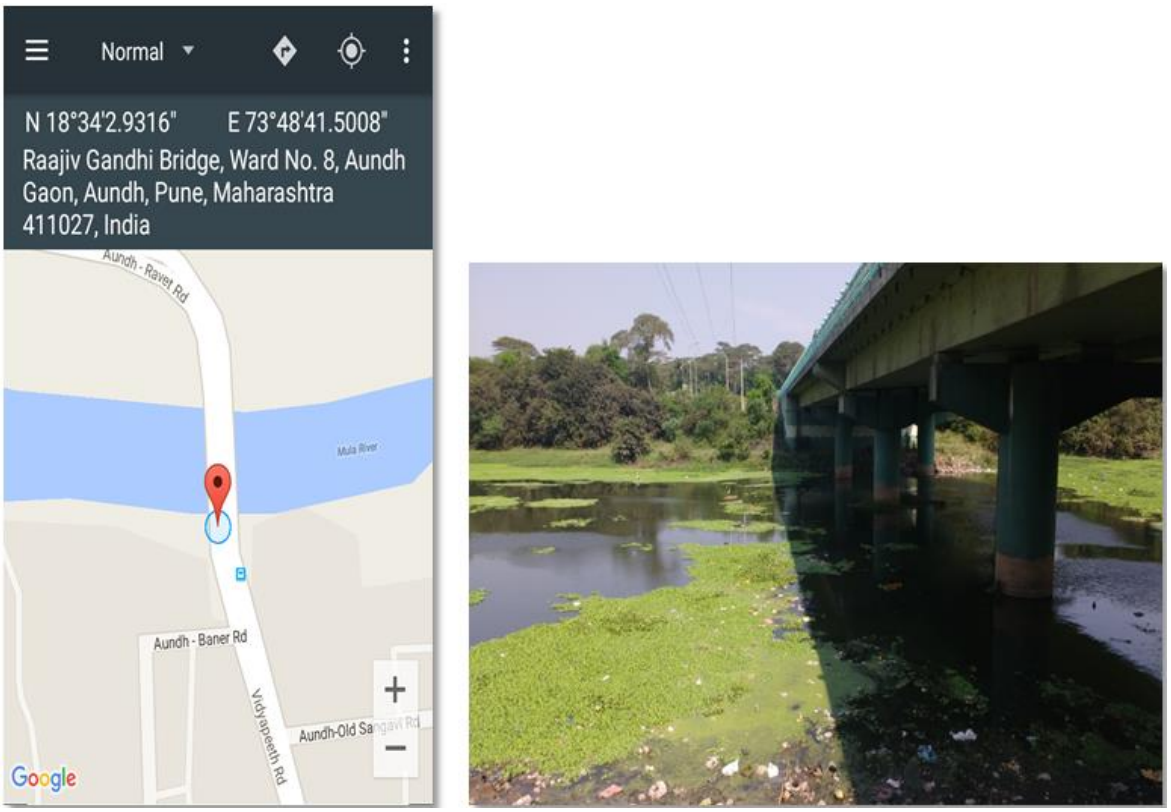

Fig. 4: Water Sample collection site Aundh (Mula River)

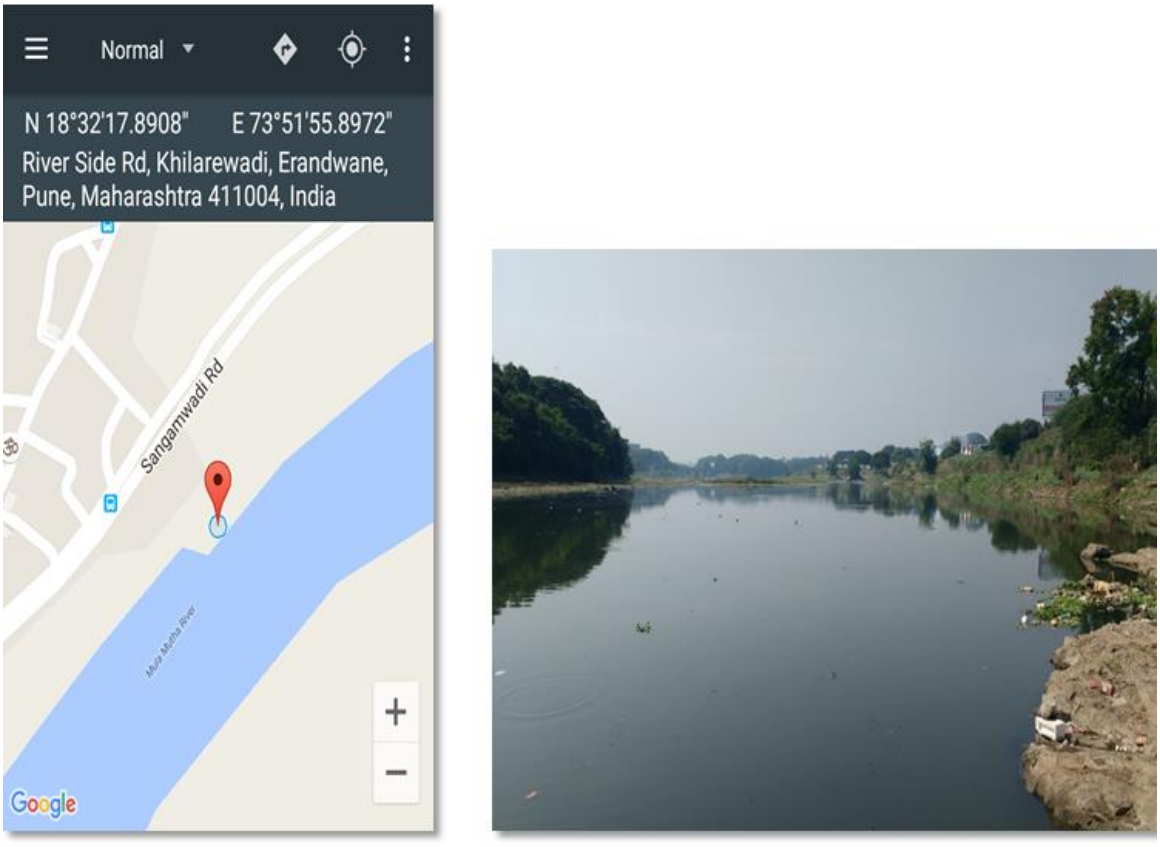

Fig. 5: Water Sample collection site Sangamwadi (Mula-Mutha)

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Table 1: Physical analysis of water samples

Samples	Physical parameter	Seasons		
		Rainy	Winter	Summer
Vitthalwadi	Colour	Colorless	Grey	Grey
Baner		Greenish	Greenish Yellow	Black
Aundh		Greenish	Black	Blackish
Lakadi bridge		Blackish	Blackish	Grayish
Sangamwadi		Greenish	Black	Blackish
Vitthalwadi	Temperature	25°C	21°C	25°C
Baner		28°C	22°C	27°C
Aundh		28°C	24°C	29°C
Lakadi bridge		27°C	23°C	27°C
Sangamwadi		28°C	24°C	29°C
Vitthalwadi	Odour	Pungent	Offensive, H_2S like	Offensive, H_2S like
Baner		None	Petrichor	Offensive, H_2S like
Aundh		None	Stale	Pungent, H_2S like
Lakadi bridge		Offensive, H_2S like	Offensive, H_2S like	Pungent
Sangamwadi		None	Stale	Pungent, H_2S like
Vitthalwadi	Turbidity	Transparent	Turbid	Turbid
Baner		Translucent	Turbid	Turbid
Aundh		Translucent	Highly Turbid	Turbid
Lakadi bridge		Turbid	Turbid	Turbid
Sangamwadi		Translucent	Highly Turbid	Turbid

Table 2: Chemical analysis of water samples

Water samples	pH			COD mg/L			BOD mg/L		
	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer
Vitthal wadi	9	7	9	48	45	39	30	42	28
Lakadi Bridge	8	8	8.5	40	42	32	30	41	26
Baner	9	9	9	52	50	43	42	48	52
Aundh	9	7	9	62	64	53	46	40	49
Sangamwadi	8	8	8	47	58	42	40	39	31

MICROBIAL ANALYSIS:

Microbial analysis of water samples generated information about the total microbial count and total coliform count which comments directly on potability of water. The microbial count and coliform count for water samples collected from Lakadi Bridge, Baner and Sangamwadi were highest in winter and were lowest in monsoon. This can be explained by the increased water levels and dilution of the microbial load due to rain water inlet. The Lakadi Bridge water sample showed highest microbial load in monsoon and lowest in summer whereas the water sample collected from Aundh had maximum microbial load in summer and lowest in monsoon. This can be correlated to the decreased water levels in summer and

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hence concentration of the microbial cells in river water. High levels of coliforms were found in all the samples in all three seasons suggesting that the water sample from any of these sites is not fit for drinking purpose.

Vitthal wadi water sample showed highest number of *Shigella*, *Salmonella*, *Pseudomonas*, *Escherichia coli* and *Staphylococcus* in winter season and they were lowest in number in summer season. It showed maximum number of *Streptococcus* in summer season water sample (Fig. 6).

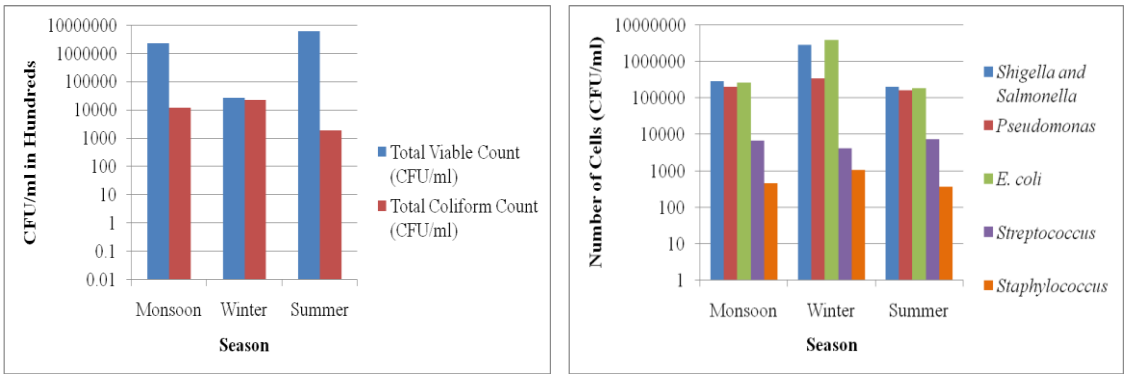

Fig. 6: Total bacterial and pathogenic microbial load in Vitthal wadi water sample

Lakadi bridge water sample showed highest number of *Shigella*, *Salmonella* and *Pseudomonas* in Monsoon season sample. Highest number of *Escherichia coli* was observed in winter sample whereas highest number of *Streptococcus* and *Staphylococcus* were observed in summer season water sample (Fig. 7).

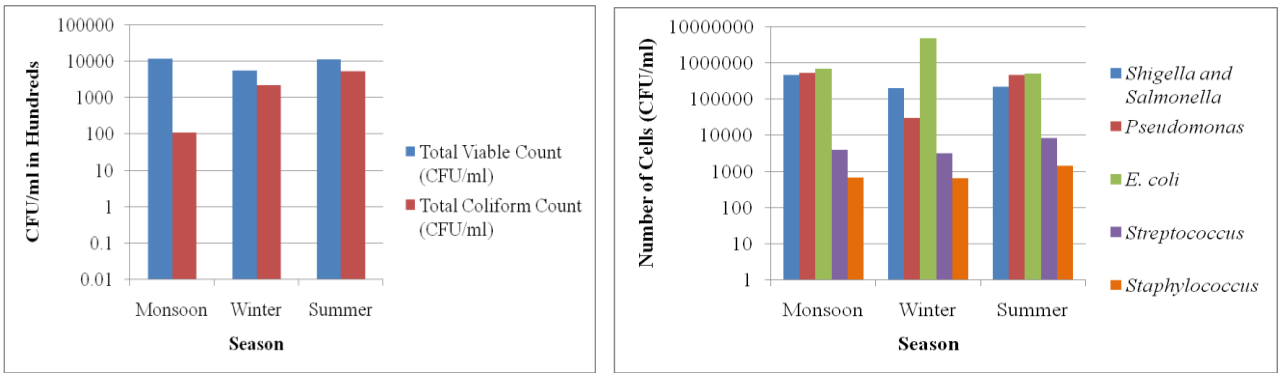

Fig. 7: Total bacterial and pathogenic microbial load in Lakadi Bridge water sample

Baner water sample showed highest number of *Shigella*, *Salmonella*, *Pseudomonas*, *Escherichia coli* and *Streptococcus* during winter season. Highest number of *Staphylococcus* was observed in summer season water sample whereas all other pathogenic bacteria were present in lowest numbers in the same sample (Fig. 8).

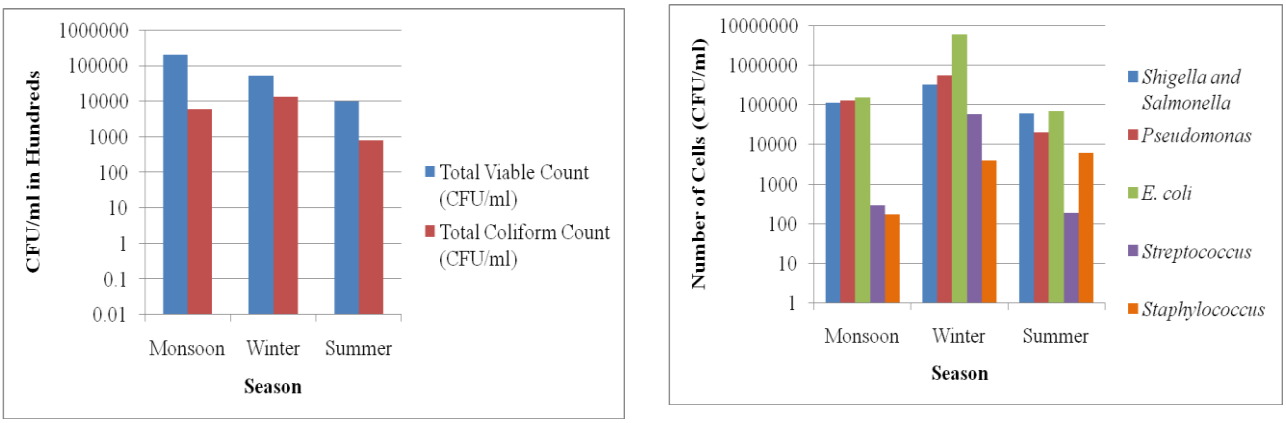

Fig. 8: Total bacterial and pathogenic microbial load in Baner water sample

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Aundh water sample showed presence of maximum number of *Shigella*, *Salmonella*, *Pseudomonas*, and *Escherichia coli* in winter water sample whereas minimum number of these organisms were observed in summer water sample. Summer season water sample showed maximum number of *Streptococcus* and *Staphylococcus* (Fig. 9).

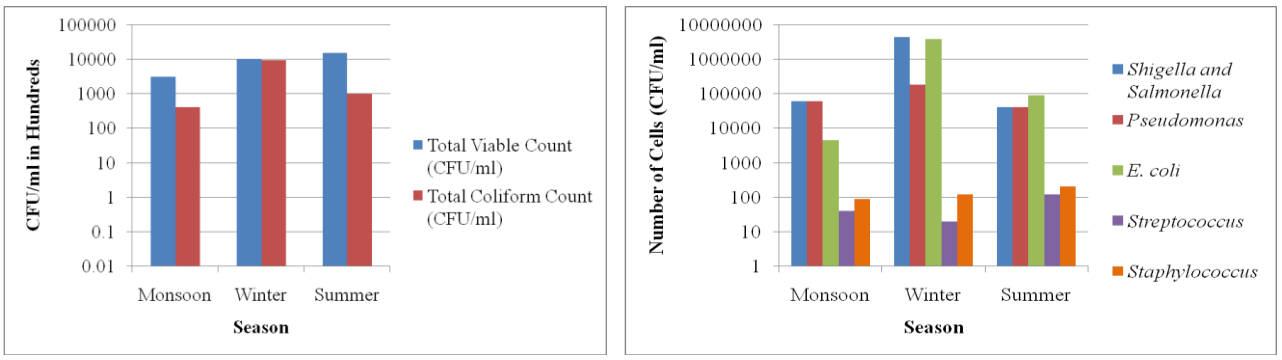

Fig. 9: Total bacterial and pathogenic microbial load in Aundh water sample

Sangamwadi water sample was found to inhabit maximum number of *Shigella Salmonella*, *Escherichia coli*, *Streptococcus* and *Staphylococcus* during winter season. Maximum number of *Pseudomonas* was observed in summer season (Fig. 10).

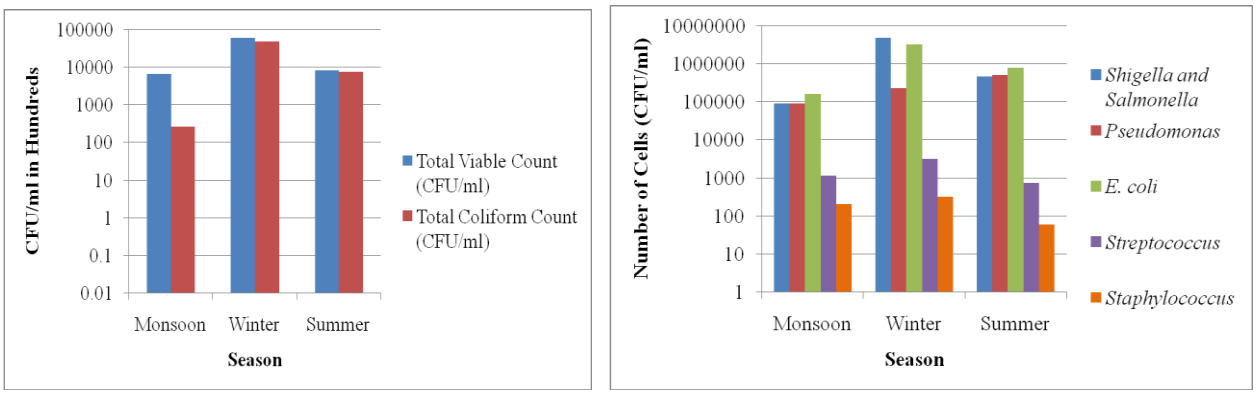

Fig. 10: Total bacterial and pathogenic microbial load in Sangamwadi water sample

ANTIBIOTIC SENSITIVITY TEST:

Table 3 shows the antibiotic resistance patterns of *Salmonella* isolates from Monsoon, Winter and Summer season respectively. It was observed that *Salmonella* isolate from Monsoon sample was sensitive to Tetracyclin and Gentamicin; it was intermediately resistant to Streptomycin and Erythromycin whereas it was resistant to Penicillin and Ampicillin. Isolate from winter sample was sensitive to Gentamicin; intermediately resistant to Tetracyclin and Streptomycin and it was completely resistant to Ampicillin and Penicillin. The isolate from summer season water sample was resistant to all the antibiotics mentioned above. Similar findings about *Salmonella* antibiotic resistance was reported (Santos et al., 2001) while studying *Salmonella* serotypes in humans, typhoid fever and enteritis, those can be modeled using *Salmonella enterica* serotype Typhimurium infections in mice and calves, respectively. The article reviews murine typhoid and bovine enteritis and discusses strengths, limitations and distinctive features of these animal models.

Table 3: Antibiotic sensitivity assay of *Salmonella* isolated from Monsoon, winter, and summer samples

Antibiotics (µg/ml)	Monsoon sample		Winter sample		Summer sample	
	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference
Penicillin (10)	8	R	8	R	8	R
Ampicillin (2)	8	R	8	R	8	R
Tetracycline (30)	19	S	15	I	8	R
Streptomycin (10)	17	I	15	I	8	R
Erythromycin (15)	16	I	10	R	8	R
Gentamicin (10)	16	S	16	S	8	R

Table 4 shows resistance pattern of *Shigella* in all three seasons. Isolate from Monsoon sample was found to be sensitive to Tetracyclin, Streptomycin and Gentamicin; it was intermediately resistant to Erythromycin whereas it was Resistant to Penicillin and Ampicillin. Isolate from winter sample was sensitive to Gentamicin; intermediately resistant to Tetracyclin and it was completely resistant to Ampicillin, Penicillin, Streptomycin and Erythromycin. The isolate from Summer season water sample was resistant to all the antibiotics mentioned above except Gentamicin. An earlier report of Shigella antibiotic resistance (Toshihiko and Sasakawa, 2001) explains the antibiotic resistance pattern of *Shigella* and their inter-cellular movements through cytoplasm to infect the adjacent epithelial cells.

Table 4: Antibiotic sensitivity assay of *Shigella* isolated from Monsoon, winter, and summer samples

Antibiotics (µg/ml)	Monsoon sample		Winter sample		Summer sample	
	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference
Penicillin (10)	8	R	8	R	8	R
Ampicillin (2)	10	R	8	R	8	R
Tetracycline (30)	20	S	17	I	8	R
Streptomycin (10)	25	S	8	R	8	R
Erythromycin (15)	15	I	13	R	8	R
Gentamicin (10)	20	S	15	S	16	S

Table 5 shows antibiotic resistance pattern of *Pseudomonas* isolates from all three seasons. The isolate from Monsoon sample was found to be sensitive to Tetracyclin, Erythromycin and Gentamicin; it was intermediately resistant to Streptomycin whereas it was Resistant to Penicillin and Ampicillin. Isolate from winter sample was sensitive to Gentamicin; intermediately resistant to Erythromycin and was completely resistant to Ampicillin, Penicillin, Tetracyclin and Streptomycin. The isolate from summer season water sample was resistant to all the antibiotics mentioned above. *Pseudomonas* antibiotic resistance was also reported in earlier work (Mena and Gerba, 2009) and it was found that *Pseudomonas* are highly versatile and can adapt to a wide range of habitats, and can even grow in distilled water. The review focuses majorly on the possible pathways and mechanisms that *Pseudomonas* adapts to overcome extreme environments such as high dosage of antibiotics.

Table 5: Antibiotic sensitivity assay of *Pseudomonas* isolated from Monsoon, winter, and summer samples

Antibiotics (µg/ml)	Monsoon sample		Winter sample		Summer sample	
	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference
Penicillin (10)	8	R	8	R	8	R
Ampicillin (2)	8	R	8	R	8	R
Tetracycline (30)	31	S	14	R	8	R
Streptomycin (10)	17	I	9	R	8	R
Erythromycin (15)	24	S	19	I	8	R
Gentamicin (10)	20	S	21	S	8	R

Table 6 shows *Escherichia coli* isolates from all three seasons and their resistance patterns. It was found that monsoon sample was sensitive to Tetracyclin, Streptomycin and Gentamicin whereas it was Resistant to Penicillin, Ampicillin and Erythromycin. Isolate from winter and summer sample were completely resistant to all the antibiotics mentioned above. Antibiotic resistant *Escherichia coli* was earlier reported (Salyers et al., 2004), exploring a more sinister side of intestinal bacteria; their role as traffickers in antibiotic resistance genes. Evidence is accumulating to support the hypothesis that intestinal bacteria not only exchange resistance genes amongst themselves but might also interact with bacteria that are passing through the colon, causing these bacteria to acquire and transmit antibiotic resistance genes.

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Table 6: Antibiotic sensitivity assay of *Escherichia coli* isolated from Monsoon, winter, and summer samples

Antibiotics (µg/ml)	Monsoon sample		Winter sample		Summer sample	
	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference
Penicillin (10)	8	R	8	R	8	R
Ampicillin (2)	8	R	8	R	8	R
Tetracycline (30)	28	S	8	R	8	R
Streptomycin (10)	28	S	8	R	8	R
Erythromycin (15)	13	R	8	R	8	R
Gentamicin (10)	28	S	11	R	8	R

Antibiotic resistance pattern of *Streptococcus* was observed Table 7 shows isolate from monsoon sample was found to be sensitive to all the antibiotics mentioned here except Streptomycin. Isolate from winter sample was sensitive to Penicillin, Tetracyclin and Gentamicin; it was intermediately resistant to Ampicillin and Erythromycin and was completely resistant to Streptomycin. The isolate from summer season water sample was resistant to all the antibiotics mentioned above. *Streptococcal* antibiotic resistance and Heavy colonization of the genital tract with group B *Streptococcus* was reported (Schuchat, 1998) and increased risk that a woman will deliver a preterm low-birth weight infant. Early-onset infections (occurring at < 7 days of age) are associated with much lower fatality than when they were first described, and their incidence is finally decreasing as the use of preventive antibiotics during childbirth increases among women at risk.

Table 7: Antibiotic sensitivity assay of *Streptococcus* isolated from Monsoon, winter, and summer samples

Antibiotics (µg/ml)	Monsoon sample		Winter sample		Summer sample	
	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference
Penicillin (10)	30	S	20	S	8	R
Ampicillin (2)	23	S	13	I	8	R
Tetracycline (30)	40	S	28	S	8	R
Streptomycin (10)	18	I	14	R	8	R
Erythromycin (15)	23	S	17	I	8	R
Gentamicin (10)	23	S	19	S	8	R

Table 8 illustrates the antibiotic profile of *Staphylococcus* isolates from all three seasons. The isolate from monsoon sample was found to be sensitive to Penicillin and Tetracyclin; it was intermediately resistant to Erythromycin whereas it was Resistant to Ampicillin, Streptomycin and Gentamicin. Isolate from winter sample was sensitive to Tetracyclin and Gentamicin and was completely resistant to Ampicillin, Penicillin and Streptomycin. The isolate from summer season water sample was resistant to all the antibiotics mentioned above except Tetracyclin. A recent study (Corey, 2009) explains in detail about the new antibiotics with proven efficacy against both susceptible and resistant strains of *Staphylococcus* particularly attractive for empirical therapy. The antimicrobial agents those are currently available for use in the treatment of both methicillin-susceptible and methicillin-resistant *S. aureus* bacteraemia and the scientific evidence that forms a basis for the use of these agents.

Table 8: Antibiotic sensitivity assay of *Staphylococcus* isolated from Monsoon, winter, and summer samples

Antibiotics (µg/ml)	Monsoon sample		Winter sample		Summer sample	
	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference	Diameter of zone of inhibition (in mm)	Inference
Penicillin (10)	32	S	20	R	13	R
Ampicillin (2)	27	R	16	R	11	R
Tetracycline (30)	33	S	25	S	16	I
Streptomycin (10)	8	R	11	R	8	R
Erythromycin (15)	22	I	28	S	8	R
Gentamicin (10)	10	R	18	S	8	R

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IV. CONCLUSION

Our study focused on analysis of Mula-Mutha river water and its pollution level. The Chemical and Biological pollutants have been analyzed and it was found that the biological pollutants pose a higher risk than the chemical pollutants. Major outcome of the research throws light on the microbial contamination and status of River water. The antibiotic sensitivity tests yielded antibiotic resistance profiles of bacteria, present in river water samples which can help in developing antibiotic therapies. Further analysis is needed to locate the sources of contamination. Proper preventive and corrective majors should be employed to reduce the contamination and microbial load of river water. A separate study should be carried out to understand the microbial ecosystem and interdependency of the river. An immediate Plan of action should be proposed to clean up Mula-Mutha river to reduce pathogenic microbial load. Awareness about the threat levels of river and regular surveys by local municipal bodies can keep a check on the contamination levels of river water.

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