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An assessment of removal efficiency for the bacterial pathogens in Mysore urban water treatment system, Karnataka, India—A case study

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ABSTRACT

The present study mainly aims to determine whether the water treatment process is microbiologically feasible to reduce the microbial load and safe for consumption. The work was focused on four water treatment plants (WTP's) supplying drinking water to Mysore urban city (Karnataka, India). A total of 144 samples were collected and analyzed during three seasons (December 2011 to August 2013) for microbiological and physico-chemical parameters. Water samples were collected from different stages of treatment such as raw water, stage-1 (coagulation/flocculation/sedimentation), stage-2 (filtration), and finally water from four WTP's. In this study most probable number method to assess the microbiological water quality and heterotrophic plate count (HPC) was used to assess microbial load reduction in different stages of treatment. The result of the study indicates that treated water samples pH values were within the permissible range of World Health Organization (WHO). The reduction of turbidity for treated water samples were in the range of 0.6–1.3 NTU. The average residual chlorine level for treated water ranges from 0.5 to 0.8 mg/l. The results of HPC in raw water were moderately high during monsoon season compared to other seasons for all WTP's. The treatment plants showed that there were not much variation in raw water and stage-1 water (coagulation/flocculation/sedimentation). The significant level of reduction occurs in stage-2 (filtration). The final water was clear due to the application of chlorination process. For all seasons the MPN count of treated water was zero. The treated water values are within permissible limit recommended by WHO. In Mysore city, all the four treatment plants use the same method of treatment and same level of reduction, which occurred in terms of microbial load.

Keywords: Coliforms; MPN; Water treatment plants; *Escherichia coli*; *Salmonella*; *Shigella*; *Yersinia*

1. Introduction

The provision of safe drinking water is one of the major challenges for developing counties. Nowadays, one-sixth of the world population still does not have access to safe drinking water [1]. The purpose of all drinking water systems is to deliver safe and sufficient quantity of water, and to protect sources of water from contamination. Treating raw water and providing esthetically-acceptable drinking

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water to the consumers is important [2,3]. The safe water has also been implicated by physico-chemical qualities such as turbidity, temperature, pH, and hardness. These qualities are readily affected by variation in climatic conditions and impact on the survival of micro-organisms and efficiency of treatment process [4]. The contamination of natural water with fecally-polluted domestic, agriculture, and industrial waste may result in an increased risk of disease transmission to individuals those who use this water. Thus, human pathogenic micro-organisms that are transmitted by water include bacteria, protozoa, and viruses. Most of them usually grow in the human intestinal tract and thrown out through the fecal matter.

The most important pathogenic organisms transmitted by water were *Escherichia coli, Campylobacter, Shigella, Salmonella*, *Cryptosporidium, Giardia,* and enteric viruses. Usually gastroenteritis, which is caused by poor sanitation and by contaminated water, is part of those diseases in the developing countries [5]. According to World Health Organization (WHO), there were estimates of four billion cases of diarrhea, and 2.2 million deaths annually in these countries due to consumption of unsafe drinking water [6]. Ideally, drinking water should not contain any micro-organisms known to be pathogenic. Since the presence of these pathogens has been traditionally seen as an indicator for fecal contamination, tests are useful for monitoring the microbiological quality of water used for consumption. The microbiological quality of water is commonly measured by indicators such as heterotrophic plate count (HPC), total coliform count (TCC), fecal coliform count (FCC), and *E. coli*. The HPC is a useful method in judging the efficiency of various treatment processes in drinking water treatment plant [7,8].

In India, most of the drinking water treatment plants (WTP's) were using the conventional method of treatment systems. Generally the performance of the WTP's was based on the quality of treated water and to compare to the regulatory requirements. The performance of each stage-wise process in a WTP's is an important evaluation method, and to ensure the process whether it is successful or not [3]. In Mysore city, not much information is available on microbiological quality of water due to the lack of continuous monitoring. Hence, the present study is an attempt to assess the bacterial pathogens reduction in stage-wise treatment during different seasons, and also the ability of treatment process to remove specific pathogens has been directly measured.

2. Materials and methods

2.1. Study area

The study was carried out in Mysore, Karnataka, India, with an estimated population of about 8,87,446 people (Census India, 2011). Mysore is located at 12° 18′N 76° 39′E 12.30° N 76.65° E and has an average altitude of 770 m (2,526 feet). It is situated in the southern region of the state of Karnataka. The work was mainly focused in four WTP's supplying drinking water to Mysore city. They are Belagola, Hongally-II, Hongally-III, and Melapura [9].

2.2. Sampling and analysis technique

A total of 144 samples were collected and analyzed during three seasons of 2011–2013 (winter, summer, and monsoon) for microbiological and physico-chemical parameters. Water samples were collected in presterilized bottles from each stages of treatment such as raw water, stage-1 (coagulation/flocculation/sedimentation), stage-2 (filtration), and final water from each plant. The microbiological parameters were examined within 6 h of sample collection.

2.3. Microbiological analysis

The water samples were analyzed for the microbiological parameters such as HPC, TCC, FCC, and *E. coli* using standard methods [7,10,11]. The total plate count was done by HPC technique [7]. In this present study the most probable number (MPN) method was used to assess the microbiological quality of water, and the HPC was used to assess the microbial load reduction during the stages of treatment.

The total coliform count was performed by multiple tube fermentation technique using set of three tubes inoculated with 10 ml of lactose broth of different strength with samples of 10, 1, and 0.1 ml, respectively. A pH indicator bromocresol purple blue was added to lactose broth for the detection of acid. The samples were inoculated in the appropriate tubes and incubated at 35˚C for 48 h. The tubes were examined for gas, and acid production indicate positive presumptive test for coliform organisms [7]. The positive presumptive tubes were used for confirmed test. Positive presumptive tubes were transferred to a special media tube of brilliant green lactose bile broth (BGLB) and incubated at 35˚C for 48 h. The positive tubes are used to determine MPN.

The results were expressed as MPN per 100 ml of the sample. In completed test, the positive BGLB samples were streaked on EMB agar, MacConkey agar, Salmonella–Shigella agar, and xylose lysine deoxycholate (XLD) agar plates and incubated at 35˚C for 24 h. Bacterial pathogens isolated on respective media were identified on the basis of their morphological and biochemical properties.

For isolation of *Yersinia* spp., 20–30 ml of water samples was inoculated into 10-fold volume of phosphate-buffered saline containing sorbitol and bile salts. It was kept at 4˚C for three weeks. After this period a loopful of cold enriched sample was treated with 5% KOH in 5% sodium chloride for 30 s. Cold enriched and KOH-treated samples were plated on cefsulodin-irgasan-novobiocin (CIN) agar and MacConkey agar (Himedia, Mumbai). The plates were incubated at 25˚C–26˚C for 24–48 h. Colonies having features of characteristic bull's eye morphology with deep red centers and white to translucent periphery on CIN agar, and flat non-lactose fermenting (NLF) growth on MacConkey agar were selected [12]. These suspected isolates were subjected to detailed biochemical characterization [13].

2.4. Physico-chemical analysis

The physico-chemical characteristic includes pH, turbidity, and residual chlorine; these were measured according to the standard methods [14]. All the measurements were done in triplicate and this average were considered.

3. Results and discussion

The microbiological and physico-chemical parameters of water samples collected from Belagola and Melapura plants were shown in (Table 1). The water samples collected from Hongally II and Hongally III were shown in Table 2.

3.1. Physico-chemical parameters

pH: The average pH value of raw water, stage-1, stage-2, and treated water in all four treatment plants were within permissible limit recommended by CPCB, 2008 and WHO [15,16]. The hydrogen ion concentration in all water samples remained alkaline throughout the study period. The alkaline nature of pH in water shows the eutrophic and mesotrophic nature of water bodies [17,18]. The average pH of treated water for all plants showed maximum of 8.0 during summer and minimum of 7.4 during monsoon.

3.1.1. Reduction in turbidity

Water is considered to be of good quality when it contains turbidity values one or below. In this present study the raw water contains turbidity value ranges of 3.2–6.5 NTU. For stage-1 water samples 1.7–3.4 NTU and stage-2 water samples within the ranges of 1.1–2.3 NTU. The treated water samples from all four plants showed values in the range of 0.6–1.3 NTU. The stage-wise reductions of turbidity were shown in Fig. 1.

The raw water turbidity was slightly increased during monsoon season when compared to winter season. It may be due to the surface run-off water often during or shortly after heavy rainfall, and also contribute the presence of suspended colloidal particles such as clay, silt, finely divided organic matter, plankton, and other microscopic organisms. The turbidity of treated water was maintained low and under the WHO guideline value throughout the study period.

3.1.2. Residual chlorine

For all treated water samples residual chlorine level ranges from 0.5 to 0.8 mg/l. Most of the treated water samples slightly exceed the permissible limit recommended by WHO standards (Tables 1 and 2). In some instance proper chlorine dosage depends on many factors including chlorine demand, residual chlorine, contact time, pH, and temperature [4]. The disinfective agent's mainly using liquid chlorination is effective for bacterial pathogens, but some parasites are more resistant toward chlorination. Therefore, the absence of coliforms from treated water does not indicate the absence of protozoan pathogens like *Cryptosporidium* and *Giardia* [19].

Micro-organisms have a great diversity of habitat with different physico-chemical conditions during treatment [20]. Overall the pH, turbidity, and residual chlorine are important water quality parameters for

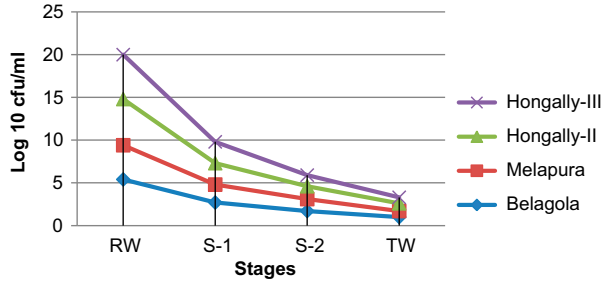

Fig. 1. Stage-wise reduction of turbidity.

Table 1
Microbiological and physico-chemical parameters of water samples collected from Belagola and Melapura WTP's in Mysore city during December 2011 to August 2013

Sample location	pH	Turbidity (NTU)	HPC cfu/ml	TCC MPN/ 100 ml	FCC MPN/ 100 ml	*E. coli*	*Salmonella* spp.	*Shigella* spp.	*Yersinia* spp.	Residual Cl (mg/L)
Belagola water treatment plant										
Raw water										
Winter	8.0	4.1	2.4×10^8	1,100	+	+	ND	ND	ND	
Summer	8.2	5.6	2.7×10^8	1,533	+	+	ND	ND	ND	
Monsoon	7.7	6.5	2.9×10^8	1,966	+	+	+	+	+	
Mean	7.9	5.4	2.6×10^8	1,533						
SD	0.2	1.2	–	–						
Stage-1 (coagulation/flocculation/sedimentation)										
Winter	7.9	2.1	1.8×10^8	1,100	+	+	ND	ND	ND	
Summer	8.1	2.7	1.7×10^8	1,100	+	+	ND	ND	ND	
Monsoon	7.6	3.4	2.4×10^8	1,100	+	+	+	+	ND	
Mean	7.8	2.7	1.9×10^8	1,100						
SD	0.2	0.6	–	–						
Stage-2 (filtration)										
Winter	7.8	1.3	4.7×10^5	210	ND	ND	ND	ND	ND	
Summer	7.9	1.5	7.0×10^5	240	ND	ND	ND	ND	ND	
Monsoon	7.5	2.3	1.2×10^6	460	ND	ND	ND	ND	ND	
Mean	7.7	1.7	7.9×10^5	303.3						
SD	0.2	0.5	–	–						
Treated water										
Winter	7.8	1.0	ND	0	ND	ND	ND	ND	ND	0.8
Summer	7.8	0.8	ND	0	ND	ND	ND	ND	ND	0.7
Monsoon	7.6	1.3	ND	0	ND	ND	ND	ND	ND	0.9
Mean	7.7	1.03	–	0						0.8
SD	0.1	0.2	–	–						0.1
Melapura water treatment plant										
Raw water										
Winter	8.1	3.2	1.5×10^8	1,100	+	+	+	ND	ND	
Summer	8.2	4.3	2.6×10^8	1,533	+	+	ND	ND	ND	
Monsoon	7.7	4.6	2.8×10^8	1,966	+	+	+	+	+	
Mean	8.0	4.03	2.3×10^8	1,533						
SD	0.2	0.7	–	–						
Stage-1 (coagulation/flocculation/sedimentation)										
Winter	7.9	1.7	6.1×10^7	460	ND	ND	ND	ND	ND	
Summer	8.1	2.2	9.8×10^7	460	ND	ND	ND	ND	ND	
Monsoon	7.6	2.4	1.3×10^8	1,100	+	+	ND	ND	ND	
Mean	7.9	2.1	9.6×10^7	673						
SD	0.2	0.3	–	–						
Stage-2 (filtration)										
Winter	7.8	1.2	4.2×10^5	210	ND	ND	ND	ND	ND	
Summer	8.0	1.5	7.9×10^5	240	ND	ND	ND	ND	ND	
Monsoon	7.5	1.7	1.0×10^6	460	ND	ND	ND	ND	ND	
Mean	7.7	1.4	7.3×10^5	303.3						
SD	0.2	0.2	–	–						

(*Continued*)

Table 1 (*Continued*)

Sample location	pH	Turbidity (NTU)	HPC cfu/ml	TCC MPN/ 100 ml	FCC MPN/ 100 ml	*E. coli*	*Salmonella* spp.	*Shigella* spp.	*Yersinia* spp.	Residual Cl (mg/L)
Treated water										
Winter	7.8	0.6	ND	0	ND	ND	ND	ND	ND	0.6
Summer	8.0	0.7	ND	0	ND	ND	ND	ND	ND	0.5
Monsoon	7.5	0.8	ND	0	ND	ND	ND	ND	ND	0.7
Mean	7.7	0.7	–	–						0.6
SD	0.2	0.1	–	–						0.1

Notes: Monthly average values are presented, MPN—most probable number, HPC—heterotrophic plate count (0.1 ml of sample), ND—not detected.

describing microbiological quality of drinking water. These parameters recommended as either directly influence microbiological quality or may influence disinfection efficiencies [21].

3.2. Microbiological parameters

3.2.1. Reduction of heterotrophic plate count (HPC)

The seasonal and stage-wise microbial load reduction in Belagola plant are shown in Fig. 2. The average HPC of raw water for winter, summer, and monsoon were 2.4×10^8, 2.7×10^8, 2.9×10^8 cfu/ml, respectively. In stage-1 (coagulation/flocculation/sedimentation) the count slightly decreased. The avarage HPC for winter is 1.8×10^8 cfu/ml, in summer 1.7×10^8 cfu/ml, and in monsoon is 2.4×10^8 cfu/ml. The results of the this study showed that not much variation in raw water and stage-1 treatment. In case of stage-2 (filtration) the average HPC for winter is 4.7×10^5 cfu/ml, in summer is 7.0×10^5 cfu/ml, and in monsoon is 1.2×10^6 cfu/ml; in this stage the significant level of reduction occurred. The final water remains clear for consumption.

In Melapura plant the average heterotropic plate count of raw water for winter, summer, and monsoon seasons were 1.5×10^8, 2.6×10^8, 2.8×10^8 cfu/ml, respectively. In stage-1(coagulation/flocculation/sedimentation) the count was slightly decreased. The avarage HPC for winter is 6.1×10^7 cfu/ml, in summer 9.8×10^7 cfu/ml, and in monsoon season 1.3×10^8 cfu/ml. It shows that moderate reduction that occured in this stage of treatment. The average HPC of stage-2 water samples were 2×10^5, 7.9×10^5, 1.0×10^6 cfu/ml, respectively. This shows the filtration process was moderatly effective to reduce the pathogen load in this stage. The final water remains clear due to the application of chlorination.

In Hongally-II and Hongally-III the raw water source were same. The average HPC of raw water for Hongally-II during winter is 1.5×10^8 cfu/ml, in summer is 2.2×10^8 cfu/ml, and monsoon is 2.7×10^8 cfu/ml. In stage-1 water sample were 1.2×10^8 cfu/ml and 1.4×10^8 cfu/ml. In case of Hongally-III the avarage heterotropic plate count for raw water were 1.4×10^8 cfu/ml in winter, 2.0×10^8 cfu/ml in summer, and 2.5×10^8 cfu/ml in monsoon. The stage-1 water samples from Hongally-II shows the avarage heterotropic plate count were 9.1×10^7, 9.4×10^7, and 1.8×10^8 cfu/ml. In case of Hongally-III the avarage heterotropic plate count for stage-1 water samples for winter is 1.8×10^8 cfu/ml, 1.5×10^8 cfu/ml in summer, and 1.7×10^8 cfu/ml in monsoon season. The stage-2 water it shows the avarage values 7.5×10^5 cfu/ml and 8.8×10^5 cfu/ml, respectively for all seasons. The final water was clear for consumption.

The results of HPC in raw water were moderately high during monsoon season in comparsion to other seasons for all WTP's. Most of the plants showed that there was not much variation in raw water and stage-1 water samples (coagulation/flocculation/sedimentation). The significant level of reduction occured in stage-2 (filtration) and the final water was clear due to the application of chlorination process (Fig. 3).

3.2.2. Total coliform count (TCC)

The total coliform count was measured in all stages of treatment during the study period.The average total coliform count in raw water samples from Belagola plant were 1,100 MPN/100 ml during winter, 1,533 MPN/100 ml during summer, and 1,966 MPN/100 ml during monsoon. In stage-1 water samples the average count of total coliform for all season were 1,100 MPN/100 ml. In stage-2 water for all season shows in between 210 MPN/100 ml and 460 MPN/100 ml. No coliforms were found in the treated water.

Melapura plant shows the similar pattern of reduction in total coliforms. During monsoon season the MPN count was increased. The average total coliform count in raw water of Melapura plant was 1,533 MPN/100 ml. In stage-1 the total coliform count

Table 2
Microbiological and physico-chemical parameters of samples collected from Hongally-II and Hongally-III WTP's in Mysore city during December 2011 to August 2013

Sample location	pH	Turbidity (NTU)	HPC cfu/ml	TCC MPN/ 100 ml	FCC MPN/ 100 ml	*E. coli*	*Salmonella* spp.	*Shigella* spp.	*Yersinia* spp.	Residual Cl (mg/L)
Hongally-II water treatment plant										
Raw water										
Winter	8.0	4.4	1.5×10^8	>2,400	+	+	ND	+	ND	
Summer	8.2	5.4	2.2×10^8	>2,400	+	+	+	ND	ND	
Monsoon	7.6	6.5	2.7×10^8	>2,400	+	+	+	+	ND	
Mean	7.9	5.4	2.1×10^8	>2,400						
SD	0.3	1.0	–	–						
Stage-1 (coagulation/flocculation/sedimentation)										
Winter	7.9	1.8	9.1×10^7	1,100	+	+	ND	ND	ND	
Summer	8.1	2.5	9.4×10^7	1,100	+	+	ND	ND	ND	
Monsoon	7.5	3.2	1.8×10^8	1,100	+	+	+	ND	ND	
Mean	7.8	2.5	1.2×10^8	1,100						
SD	0.3	0.7	–	–						
Stage-2 (filtration)										
Winter	7.8	1.2	4.1×10^5	240	ND	ND	ND	ND	ND	
Summer	8.0	1.5	8.4×10^5	240	ND	ND	ND	ND	ND	
Monsoon	7.4	1.8	1.0×10^6	460	+	+	+	ND	ND	
Mean	7.7	1.5	7.5×10^5	306						
SD	0.3	0.3	–	–						
Treated water										
Winter	7.8	0.8	ND	0	ND	ND	ND	ND	ND	0.8
Summer	7.9	0.9	ND	0	ND	ND	ND	ND	ND	0.7
Monsoon	7.4	1.0	ND	0	ND	ND	ND	ND	ND	0.6
Mean	7.7	0.9	–							0.7
SD	0.2	0.1	–							0.1
Hongally-III water treatment plant										
Raw water										
Winter	8.0	4.4	1.4×10^8	>2,400	+	+	ND	ND	ND	
Summer	8.2	5.2	2.0×10^8	>2,400	+	+	ND	ND	ND	
Monsoon	7.6	6.2	2.5×10^8	>2,400	+	+	+	+	ND	
Mean	7.9	5.2	1.9×10^8	2,400						
SD	0.3	0.9	–	–						
Stage-1 (coagulation/flocculation/sedimentation)										
Winter	7.9	2.1	1.1×10^8	1,100	+	ND	ND	ND	ND	
Summer	8.1	2.4	1.5×10^8	1,100	+	+	ND	ND	ND	
Monsoon	7.5	3.2	1.7×10^8	1,100	+	+	ND	ND	ND	
Mean	7.8	2.5	1.4×10^8	1,100						
SD	0.3	0.5	–	–						
Stage-2 (filtration)										
Winter	7.9	1.1	8.7×10^5	240	ND	ND	ND	ND	ND	
Summer	8.0	1.2	8.8×10^5	460	ND	ND	ND	ND	ND	
Monsoon	7.4	1.8	9.0×10^5	460	ND	ND	ND	ND	ND	
Mean	7.7	1.3	8.8×10^5	386						
SD	0.3	0.3	–	–						

(*Continued*)

Table 2 (*Continued*)

Sample location	pH	Turbidity (NTU)	HPC cfu/ml	TCC MPN/ 100 ml	FCC MPN/ 100 ml	*E. coli*	*Salmonella* spp.	*Shigella* spp.	*Yersinia* spp.	Residual Cl (mg/L)
Treated water										
Winter	7.9	0.6	ND	0	ND	ND	ND	ND	ND	0.8
Summer	7.9	0.7	ND	0	ND	ND	ND	ND	ND	0.7
Monsoon	7.5	0.8	ND	0	ND	ND	ND	ND	ND	0.6
Mean	7.7	0.7	–	–						0.7
SD	0.2	0.1	–	–						0.1

Notes: Monthly average values are presented, MPN—most probable number, HPC—heterotrophic plate count (0.1 ml of sample), ND—not detected.

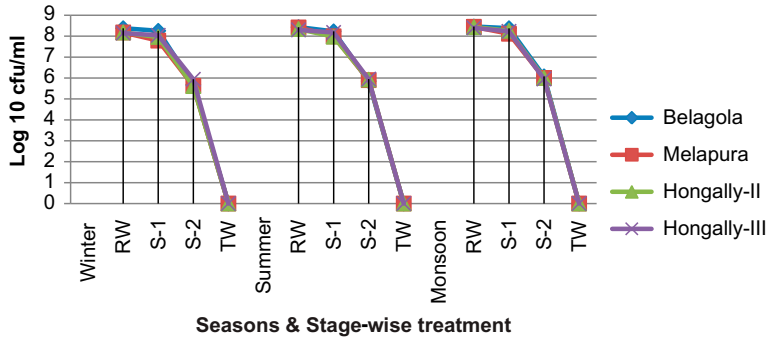

Fig. 2. Graph showing seasonal and stage-wise microbial load reduction in Mysore WTP's.

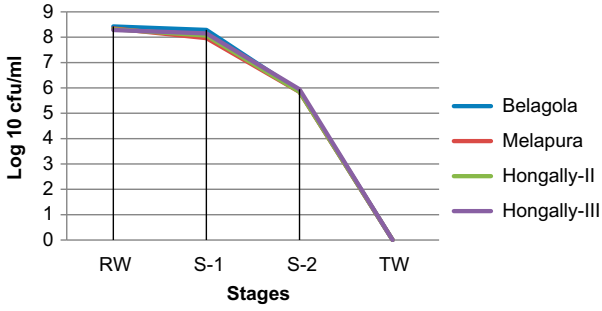

Fig. 3. Treatment perfomance based on reduction in HPC.

was 673 MPN/100 ml. In stage-2 water samples the count was 303 MPN/100 ml. The final water remained clear for consumption. In this study it shows that the perfomance of Melapura plant was much effective than the Belagola plant in terms of total coliform reduction.

In Hongally-II and Hongally-III the average total coliform count of raw water for all season was 2,400 MPN/100 ml. It shows that the raw water was moderatly polluted as the anthopogenic activities are going on in the nearby water intake points. In stage-1 water, the average coliform count was 1,100 MPN/ 100 ml for both plants. The stage-2 water, the average coliform count was 306 MPN/100 ml and 386 MPN/ 100 ml, respectively. For all seasons the MPN count of treated water was zero. The treated water values are within permissible limit recommended by WHO.

3.2.3. Fecal coliform count & E. coli

The average FCC of raw water for Belagola plant was 18 MPN/100 ml. In Melapura plant the average values for all seasons were 12 MPN/100 ml. In Hongally-II and HongallyIII the values were 24 MPN/ 100 ml.

The other pathogenic organisms such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Yersina* spp. were also detected during some seasons from raw water, stage-1, and stage-2 water. Overall *E. coli* spp. are the predominant bacterial flora in these water bodies.

4. Conclusion

This study is focused on each stage of treatment process based on microbiological and physico- chemical properties of water. In Mysore all the four

treatment plants using the same method of treatment and same level of reduction occurred in terms of microbial load. The treatment performance in WTP's of Mysore showed that there were not much variation in the quality of raw water and stage-1 water. It may be due to the operational failure in stage-1 (Clarifloculation). The significant variations occurred in stage-2 treatment processes. For all season the values of microbiological physico-chemical parameters are within the limit of water quality standards except for residual chlorine in some months. This may be because of the adjustment of the quality of finished water due to the substandard treatment of previous stages. In some plants we noticed the prolonged equipment errors, but the finished water quality will be maintained based on the excess chlorine dosage. During monsoon season the values are higher in comparison to the other seasons. The study also revealed the ability of treatment process to remove specific pathogens. The overall conclusion of this study is the operational status of treatment, using all four WTP's, which are moderately safe for controlling pathogenic organisms.

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