

SEASONAL BACTERIOLOGICAL ANALYSIS OF GOLA RIVER WATER CONTAMINATED WITH PULP PAPER MILL WASTE IN UTTARANCHAL, INDIA

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Abstract. The seasonal physico-chemical and microbial quality of Gola river water has been analyzed after confluence of pulp paper mill waste. The study revealed that it has enhanced 20–30 times pollution load of BOD, COD, TDS, TSS, sulphate, chloride, sodium, nitrate, potassium, lignin and phenol after mixing of pulp paper mill waste with river water in all season. Further, it induced the bacterial growth by increasing most probable number value of *E. coli* was 1.57×10^4 , 1.6×10^4 , 1.37×10^4 and SPC count was 1.68×10^4 , 1.64×10^4 , 1.67×10^4 /100 ml during summer, monsoon, winter respectively. While the most probable number value in river water before mixing of pulp paper mill waste was 1.4×10^2 , 1.82×10^2 , 1.5×10^2 and SPC count was 2.8×10^3 , 2.89×10^3 , 2.78×10^3 /100 ml during summer, monsoon and winter respectively. This indicated from 88–114 fold increase in most probable number value of *E. coli* and 56.55–60.0 times increase in SPC count of river water after mixing of effluent in summer, monsoon and winter. Moreover, the most probable number value in effluent itself before mixing was 3.4×10^2 , 3.3×10^2 , 2.8×10^2 and SPC count was 6×10^4 , 6.5×10^4 , 6×10^4 /100 ml during summer, monsoon, winter, respectively. Furthermore, it was revealed that the seasonal variation also regulated the bacterial population dynamics as per the physico-chemical quality, in which *E. coli* was found highest at the rate of (5.9×10^4), *E. aerogenes* (5.3×10^4), *P. aeruginosa* (1.3×10^4), *S. aureus* (3.2×10^3), *K. pneumoniae* (2.6×10^4), *Enteritidis* (1.1×10^4) on monsoon season and *V. cholerae* (7.4×10^2), *V. vulnificus* (9.2×10^2)/100 ml in river water when contaminated with pulp paper mill waste in monsoon season. Thus, the monsoon season showed presence of FC and TC indicated the thermo-tolerant and disease causing group of bacterial population in effluent and its sequence was observed as monsoon>summer>winter. This indicated the growth of many pathogenic and non-pathogenic bacteria for health hazards with contamination of pulp paper waste in aquatic ecosystem within the vicinity of pulp paper mill industry.

Keywords: bacteriological analysis, coliforms, physico-chemical parameters, pulp paper effluent, river water, seasonal

1. Introduction

Rivers are the most important water resource. Unfortunately, river is being polluted by indiscriminate disposal of sewerage, industrial waste and plethora of human activities, which affects its physico-chemical characteristics and microbiological quality (Koshy and Nayar, 1999). Prevention of river pollution requires effective

monitoring of physico-chemical and microbiological parameters (Bonde, 1977; Ramteke *et al.*, 1994). Thus detection and enumeration of indicator organisms are of primary importance for the monitoring of sanitary and microbiological quality of water (Gunnison, 1999; Kataria *et al.*, 1997). The bacteriological examination of water has a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health. For assessment of water quality is not only the physico-chemical characteristics of river water but also obtain information on whether the river conform to prescribed standard of microbiological water quality (APHA, 1998).

Physico-chemical quality of water also regulated the microbial growth. The elevated turbidities are often associated with the possibility of microbiological contamination, as high turbidity makes it difficult to disinfect water properly (Van Loon, 1982; Quality of Domestic Water Supplies, 1998). High nitrate level also stimulates algal growth (Fried, 1991; WRC, 2000) and plays a role in eutrophication.

Coliforms are the major microbial indicator of monitoring water quality (Brenner *et al.*, 1993; Grant, 1997). Coliforms bacteria are a natural part of the microbiology of the intestinal tract of warm-blooded mammals including man can be found in their wastes.

Total coliform (TC) and fecal coliform (FC) counts are the most widely used bacteriological procedures for assessment of the quality of drinking and surface waters (Mcdaniels *et al.*, 1985).

The TC bacteria test is a primary indicator of potability, suitability for consumption of drinking water. It measures the concentration of TC bacteria associated with the possible presence of disease causing organisms (Craun, 1978). Instead, the EPA has designated TC bacteria as a standard to determine bacterial safety of water found in their wastes. The EPA maximum contaminant level (MCL) for coliform bacteria in drinking water is zero TC/ 100 ml of water (APHA, 1998).

FC are selected members of the coliform group of bacteria which are able to ferment lactose at 44.5 °C are fairly specific for the feces of warm-blooded animals and are commonly used as indicators of fecal pollution in waters such as wastewater effluents, rivers, marine environments, recreational waters, and raw sources of drinking water supplies (Geldreich, 1978).

However, there is no report is available on bacterial quality of water due to industrial waste contamination. The wastewater discharge by the pulp paper mill effluent constitutes the major bulk of organic pollution of river. The pulp paper mill is major industrial sector utilizing huge amount of water during manufacturing process of paper. The industry produces 400 tons of paper/day and discharge 40,414 m³/day wastewater, which ultimately disposed into a main river at foot hills. i.e. Gola river, which is major source of water in the stretch area of 200 km.

The effluent discharged from industry known as black liquor. This effluent contains, lignin derivatives, colour, chlorinated phenol. Fibre, which creates problems in disposable and is hazardous to plants, animal and human being (Chauhan and

Thakur, 2002). Moreover the effluent contain organic and inorganic residual nutrient, which provide ample opportunity to flourishing a variety of pathogenic micro-organisms. The extent of physico-chemical and bacteriological change due to mixing of pulp paper effluent in river water is not known. Therefore, the simultaneous physico-chemical and bacteriological analysis has been felt to determine the effect of pulp paper mill effluent on receiving river water. This analysis will provide new information on the consequence of pulp paper mill effluent to river water.

In this study assesses seasonal water quality of Gola river water contaminated with M/s Century pulp paper mill with the uses of a number of biotic and abiotic factors such as physico-chemical variables and bacteriological variables.

2. Materials and Methods

2.1. SAMPLING SITE

The sampling sites were selected at M/s Century pulp paper mill, Lalkuan, Nainital, Uttaranchal, India which is located (79°10' E longitude and 29°3' N latitude) at foot hills of Himalayas. Water samples were collected from three sites in three seasons (monsoon, winter and summer). The river water sample was identified as sample A. The second sample was collected from pulp paper mill effluent was designated as sample B. Third sample was taken from the down stream of Gola River (Figure 1) after confluence of pulp paper mill effluent, which was designated as sample C. The samples were taken into pre-sterilized bottles kept in iceboxes, which were further transported laboratory for seasonal analysis of physico-chemical and microbiological parameters.

2.2. PHYSICO-CHEMICAL ANALYSIS

The collected samples from different site and seasons were analyzed for pH, total dissolved solid (TDS), total suspended solid (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), phenols, nitrogen, lignin, sulphate and phosphate as per method (APHA, 1998). Further, different salts i.e. chloride, sodium, nitrate, potassium were analyzed by ion meter (Orion Model 960) using selective ion electrode.

2.3. BACTERIOLOGICAL ANALYSIS

Quantitative bacterial analyses were done by standard plate count (SPC) on plate count agar (PCA), specific media and chromogenic media as shown in (Table I) (APHA, 1998).

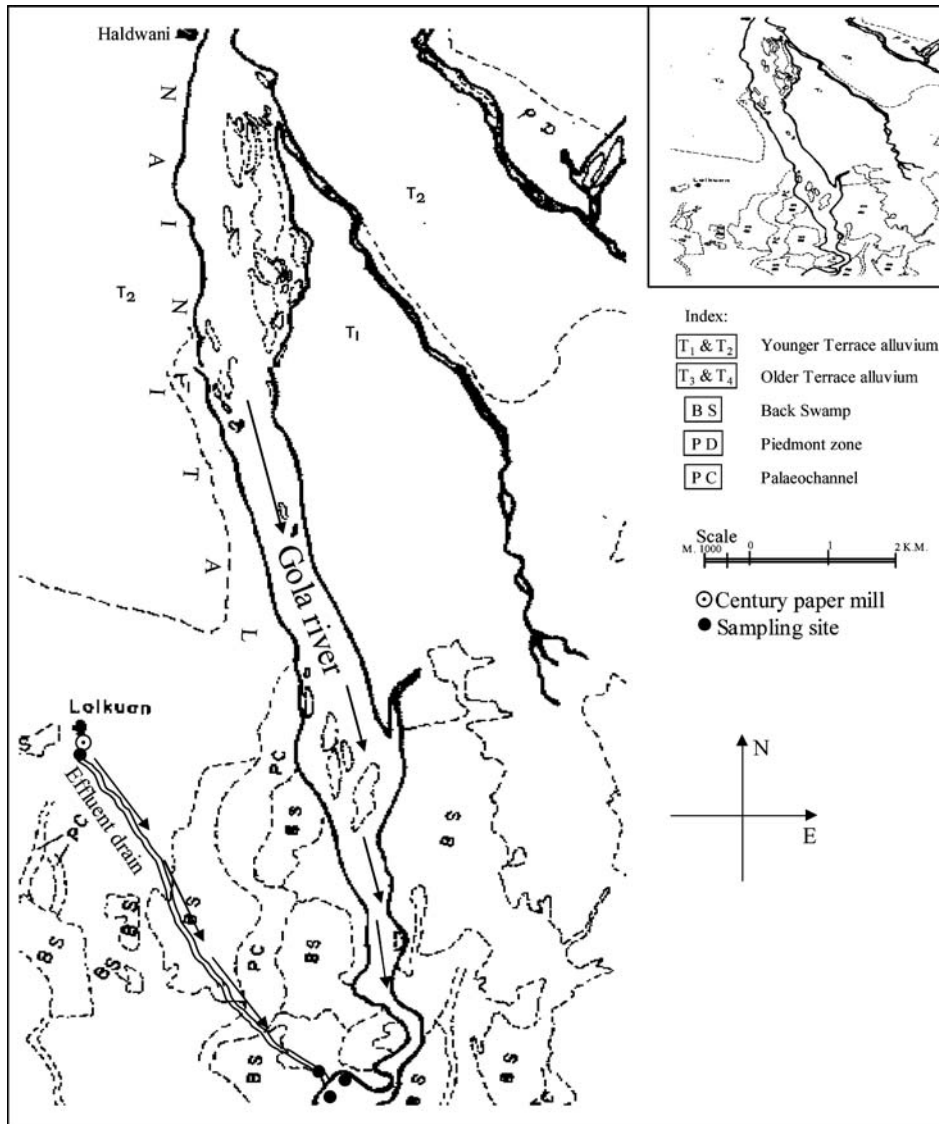


Figure 1. Geographical map of Lalkuan, Nainital showing location of century pulp paper mill and sampling site.

Qualitative analysis was carried by multiple tube fermentation technique (APHA, 1998) for members of the coliform group.

Coliforms were detected by presumptive inoculation into tubes of MacConkey broth and their incubation at $37 \pm 2^\circ\text{C}$ for 48 h. The positive tubes were sub-cultured into Levine's EMB and endo agar for confirmation. Subsequently, positive growth on plates were inoculated into Brilliant green Bile Broth (BGLB) and incubated at

TABLE I
Detail of specific and chromogenic culture media used for quantitative bacterial analysis

Serial no.	Media name	Specificity	Manufacturer/Trade
1.	TCBS, 0650-15 (specific media)	<i>Vibrio</i> species	DIFCO, UK
2.	BGLB, 64271 (specific media)	<i>Enterobacteriaceae</i>	Merck, Darmstadt, Germany
3.	EMB, M317 (specific media)	<i>E. coli</i>	HiMedia, Mumbai, India
4.	BCP-DCLS, M219 (specific media)	<i>Shigella</i> sp., <i>Salmonella</i> sp., <i>Arizona</i> sp.	HiMedia, Mumbai, India
5.	Rappaport vassiliadis medium, M880 (specific media)	<i>Salmonella</i>	HiMedia, Mumbai, India
6.	HiCrome ECC selective agar, M1294 (chromogenic media)	<i>E. coli</i> and other coliform	HiMedia, Mumbai, India
7.	HiCrome <i>E. coli</i> agar, M12951 chromogenic media	<i>E. coli</i>	HiMedia, Mumbai, India
8.	HiCrome coliform agar, M1300 chromogenic media	<i>E. coli</i> and TC	HiMedia, Mumbai, India
9.	HiCrome UTI agar, M1353 chromogenic media	Urinary tract infecting microorganisms	HiMedia, Mumbai, India

37 °C for 48 h for complete test. Gas production in BGLB was used for the detection of coliforms after 48 h incubation. Gram characters were also observed by Gram staining. MPN of coliforms were found in terms of index/100 ml by using standard tubes. All the results were compared with the standard MPN chart and the results were expressed as the total number of coliform/100 ml of the water. Positive results on EMB and Endo agar were tested for the biochemical properties in the BGLB broth finally *E. coli* were confirmed.

FC was analyzed by direct specific test method (APHA, 1998) for thermotolerant group of bacterial population taken a criterion for indicating coliform of fecal origin and simultaneously TC were determined in three steps by multiple tube fermentation technique (APHA, 1998)

2.4. STATISTICAL ANALYSIS

Standard deviation and two-way analysis of variance ANOVA test were used for correlating the data and elimination of variance is observed in results. Bacterial count was transformed prior to statistical treatment and results were analyzed by standard deviation and ANOVA test (Gomez and Gomez, 1984).

3. Results

The physico-chemical analysis carried out from different site during different seasons has been presented in (Table II). The data are shown the average of three samples per season. The data indicated that river water (sample A) was observed colorless and slight alkaline within prescribed limit of BOD, COD as per Indian standard in summer and winter season. According to the physico-chemical variables measured the chemical status of Gola River was bad at the monsoon season with high values of chloride, nitrate, TDS, BOD, COD. Pulp paper mill effluent showing beyond the permissible limit of phosphate, phenol, COD, BOD, chloride, sodium, nitrate. These high values were attributed the river water maximum in monsoon season as shown in Table II.

Microbial analyses were summarized in Table III, in which the MPN value of *E. coli* in river water (sample A) found highest among three samples. Sample C was found maximally polluted with fecal indicator bacteria in the monsoon season as shown in Table III. FC counts in sample B and C was also invariably low. A higher SPC was observed in pulp paper mill effluent (6.5×10^4) in monsoon season. TC counts were maximum in monsoon season among all samples as compared with pulp paper mill effluent displayed lower TC counts.

In Table IV, SPC counts on different specific and chromogenic medium among them in sample B showed highest SPC in monsoon season in EMB, BGLB, HiChrome ECC agar, HiChrome UTI agar, HiChrome coliform agar. In

TABLE II
Physico-chemical analysis of pulp paper mill effluent of different sites and seasons

Parameters	Sample A			Sample B			Sample C			ANOVA sample effect (F value)	Results season effect (F value)
	S	M	W	S	M	W	S	M	W		
pH	7.76	7.22	7.40	7.25	7.27	7.15	7.54	7.20	7.10	-	-
TDS	7.10	11.10	5.37	173.13	170.50	171.81	16.25	21.54	19.57	-	-
TSS	5.20	18.20	5.90	1170.37	1169.00	1173.00	119.93	146.16	130.30	21232.23*	0.89 [†]
Sulphate	3.33	3.40	3.00	0.26	0.28	0.29	0.04	0.03	0.03	1481.26*	1.19 [†]
Phosphate	3.00	3.80	2.33	131.49	133.07	129.40	19.22	19.51	13.73	13598.92**	9.90**
Phenol	6.00	6.50	3.00	1813.40	1914.52	1536.77	18.40	216.13	185.67	545.90**	2.90 [†]
COD	29.00	248.00	41.33	5049.73	5067.90	5036.40	510.41	633.72	556.59	33993.14*	13.30 [†]
BOD	13.33	123.33	19.33	2666.40	2603.67	2125.40	236.66	298.93	258.52	411.64*	2.83 [†]
Chloride	3.71	4.00	3.45	357.30	358.10	354.33	37.40	47.85	35.17	34661.92**	7.70*
Sodium	8.00	7.73	6.80	249.30	254.10	260.33	3.91	33.67	23.52	3437.45**	6.21 [†]
Nitrate	1.37	2.48	1.00	39.05	41.03	35.37	4.00	5.95	3.67	1249.65**	7.37**
Potassium	3.06	3.80	1.63	0.89	1.28	0.50	0.07	0.09	0.11	-	-
Lignin				264.87	273.73	236.33	13.44	24.50	11.39	-	-

(-) ANOVA not calculated, S: summer, M: monsoon, W: winter.

Average values are presented in mg/L.

*Indicates significant at $P < 0.05$, **Indicates significant at $P < 0.001$, [†]Indicates not significant.

TABLE III
Microbial quantity analysis of pulp paper mill effluent of different sites and seasons

Parameters	Sample A			Sample B			Sample C			ANOVA sample effect (F-value)	Results season effect (F-value)
	S	M	W	S	M	W	S	M	W		
MPN	1.4×10^2	1.82×10^2	1.5×10^2	3.4×10^2	3.3×10^2	2.8×10^2	1.57×10^4	1.68×10^4	1.37×10^4	2316.63**	6.03 [†]
FC	6.94×10^2	1.82×10^2	1.5×10^2	3.01×10^2	3.18×10^2	3.4×10^2	2.67	36.33	26	568.21 [†]	6.78 [†]
SPC	2.8×10^3	2.89×10^3	2.78×10^3	6×10^4	6.5×10^4	6×10^4	1.68×10^4	1.64×10^4	1.67×10^4	5327.60**	4.72 [†]
TC	24	39	36.67	26	35.67	30	21.67	30	22.67	27.14**	44.14**

* Indicates significant at $P < 0.05$, ** Indicates significant at $P < 0.001$, [†] Indicates not significant, (-) ANOVA not calculated.

TABLE IV
SPC count on different specific and chromogenic medium

Bacteria on specific medium	Sample A			Sample B			Sample C		
	S	M	W	S	M	W	S	M	W
EMB	30000 ± 0	30000 ± 0	30000 ± 0	23603.33 ± 4.2	33700 ± 2	23502 ± 2	8400 ± 0	8470 ± 53.70	8400 ± 2
TCBS	182 ± 3.0	188 ± 2.5	184 ± 2	208 ± 3	210 ± 3.5	210 ± 3.5	400 ± 2	402 ± 2	398 ± 2
BGLB	110 ± 2	120 ± 4.5	108 ± 3	1603.33 ± 4.16	2606.67 ± 6.11	1696 ± 4	208 ± 3.2	210 ± 2.5	208 ± 4
HiChrome ECC agar	1602 ± 2	16802 ± 2	1002 ± 2	20802 ± 2	30602 ± 2	15002 ± 2	2002 ± 2	6002 ± 2	1502 ± 2
HiChrome UTI agar	1140 ± 5	1260 ± 2.0	1120 ± 4.6	17200 ± 0	17200 ± 2	17196 ± 1.5	4000 ± 0	4135 ± 117.21	4202 ± 2
HiChrome Coliform agar	1405 ± 3	1440 ± 3.4	1410 ± 3.0	1605 ± 2.6	1600 ± 3.2	1604 ± 4	1502 ± 2	1602 ± 3.05	14808 ± 2

Values are in Mean ± SD; (-) Growth absent.

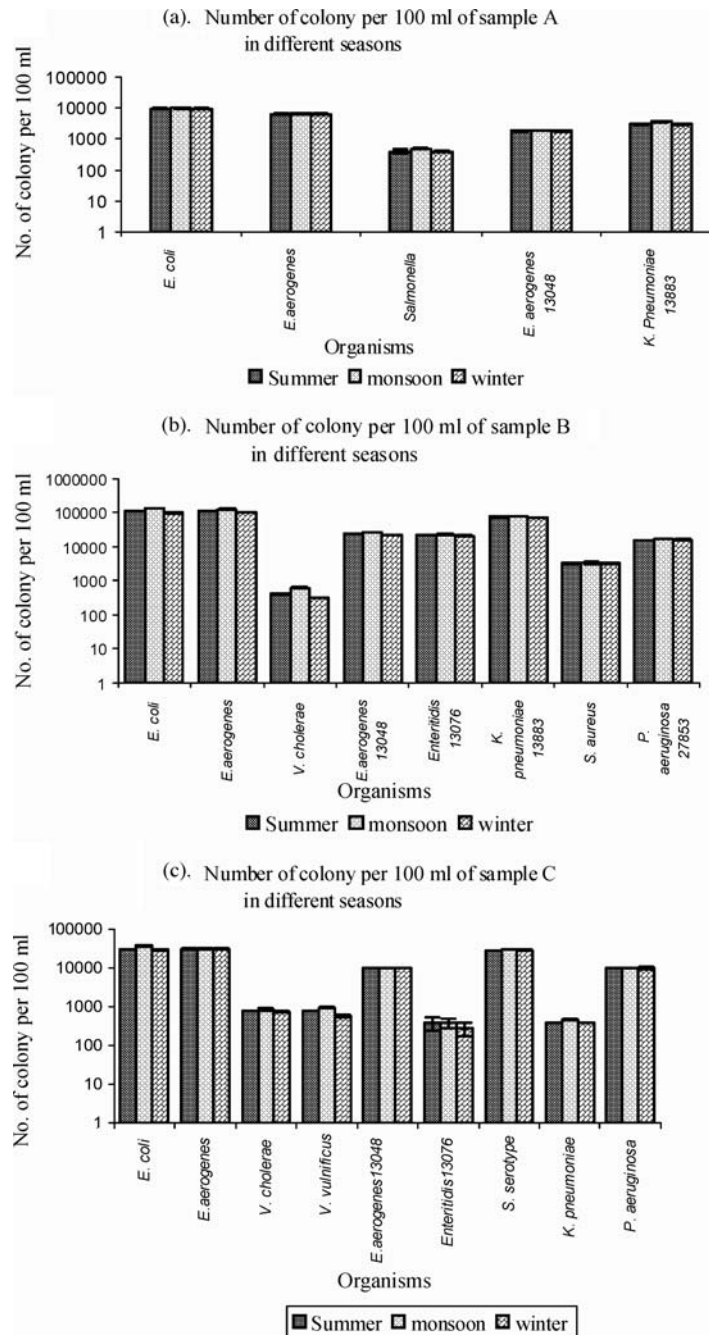


Figure 2. (a–c): Showing number of bacterial colony in different sample in different seasons.

Figures 2a–c shows the maximum number of colony of *E. coli* in summer season among three samples.

4. Discussion

The physico-chemical contents of pulp paper effluent contribute contamination to river water during the mixing therefore the physico-chemical properties of river water is altered as shown in Table II of results.

But, the slight variations were observed in river water in the monsoon season due to run off of organic matter into river from foothills and river basin. But the analysis of sample B (pulp paper effluent) showed high value of BOD, COD, lignin and phenols was due to soluble lignocellulosic material along with bleaching chemical of pulp manufacturing process (Jain *et al.*, 1996), which subsequently increased the pollutant parameter of physico-chemical quality of river water after mixing of all pollutants from the pulp paper effluent (Singh and Singh, 2003) as shown in sample C of (Table II) in all seasons.

However, the higher content of sodium, potassium, chloride and nitrate indicated rock leachate of hills are mixing into river water. The leaching properties of these ions have been also reported by (Mitra and Gupta, 1997).

Further, the effect of different seasons was also statistically analyzed within different parameters. But, the two-way ANOVA test revealed that there was not any significant variations were noted (ANOVA, $P < 0.05$) except chloride, which is probably added during rainy season from rock hills. However, the significant variations were observed between the different sample sites (ANOVA, $P < 0.05$ and $P < 0.001$) (Table II).

Average bacteriological enumeration showed that the MPN value was in order of sample A (river water) 1.82×10^2 < sample B (pulp paper mill effluent) 3.4×10^2 < sample C (river water after mixing of effluent) $1.6 \times 10^4/100$ ml (Table III). This indicated that due to high BOD and COD of effluent do not much support for bacterial growth but after mixing in river water support the bacterial growth due to addition of organic compounds in appropriate concentration.

Therefore, the significant variation was noted between different samples (ANOVA, $P < 0.001$). But, the insignificant variations were observed between different seasons (ANOVA, $P < 0.001$) as shown in Table III.

In the present study coliform bacteria also showed irregular pattern of their occurrence in different sample. The highest coliform was noted in monsoon season of sample A as shown in (Table III). This indicated the mixing of some domestic sewerage during the overflowing of run off water in monsoon time. Similar pattern was obtained in sample B (effluent) and sample C (effluent mixed water) also. The statistical analysis of seasonal change showed the significant data (ANOVA, $P < 0.001$). The irregular variations in the coliform bacteria due to seasonal change also corroborated the finding of (Legendre *et al.*, 1984; Barcina 1986 and Ramanibai,

1996). The existence of other members of the FC group (*Klebsiella*, *Enterobacter* and *Citrobacter*) has been reported for non-fecal origin (Alonso *et al.*, 1998). The higher FC has indicated the tolerant of high temperature as shown in Table III. This result coincides with observation of (Ravichandran and Ramanibai, 1988a).

The FC on most occasions in the sample A, B and C indicated that the coliforms are mainly of fecal origin. They showed a progressive increase from sample A to sample C (Table III). It appears there is decrease from the entry point towards the discharge flow of effluent due to mixing with pulp paper effluent, which make uneasy for existence of fecal origin (Holden, 1970). The significant coefficient of seasonal data and different sample showed significant variation (ANOVA, $P < 0.001$).

The SPC count for *E. coli*, *E. aerogenes* on EMB showed highest on sample A (River Water) in all seasons followed by effluent and effluent mixed river water as shown in (Table IV). While the *Vibrio* species were detected only in sample C. The common *Enterobacteriaceae* bacteria were observed in effluent only. The *E. coli* and coliform were prevalent in river water as well as effluent sample (Table IV) (Ramteke and Tewari, 2002; Ramteke, 1995; Ramteke *et al.*, 1994).

Statistical analysis also showed significant variation (ANOVA, $P < 0.001$). The FC were also analyzed for thermotolerant potency for *E. coli* throughout this study period among all site samples as shown in (Table III; Figures 2a–c). This might be due to mixing of sufficient pulp paper effluent in river, which directly increased the *E. coli* population. However, the insignificant correlation observed with TC. This may be due to the fact that the TC can also originate from non-fecal sources and the origin of pollution in the river pulp paper waste discharged the results are in accordance of earlier reports (Byamukama *et al.*, 2000).

In addition, the seasonal distribution pattern of different pathogenic and non-pathogenic bacteria in sample A, *E. coli* > *E. aerogenes* > *K. pneumoniae* > *E. aerogenes* strain 13048 > *Salmonella* (Figure 2a). While for sample B, *E. coli* > *E. aerogenes* > *K. pneumoniae* > *E. aerogenes* strain 13048 > *Enteritidis* strain 13076 > *P. aeruginosa* > *S. aureus* > *V. cholerae* (Figure 2b) and in sample C, order of distribution was *E. coli* > *E. aerogenes* > *E. aerogenes* strain 13048 > *S. serotypes* > *P. aeruginosa* > *V. cholerae* > *V. vulnificus* > *K. pneumoniae* > *Enteritidis* (Figure 2c).

The overall physico-chemical and bacteriological characteristics observed for river water quality is alarming. The bacteriological parameters analyzed revealed that the river water and pulp paper effluent is highly affected with high bacterial population at any point of river. The microbial quality of water also revealed abundant growth of pathogenic bacterial population.

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