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Quantitative Analysis of Pollution Indicator and Pathogenic Bacteria in River Tawi, J&K (India)

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The present study aimed at the assessesment of water quality and to quantify microorganisms of health concerns of river Tawi - the main source of drinking water supplies for Jammu city and its outskirts. Seasonal changes in quantitative estimation of bacterial load (SPC & MPN) at four different stations of river Tawi showed a significant change. Various bacterial species viz. *Escherichia coli*, *Salmonella* sp. & *Pseudomonas* sp. were isolated from water as well as fish fauna of river Tawi, with the help of biochemical tests and further confirmation was done by molecular analysis (PCR). The correlation ($p<0.05$) of bacterial population with physico-chemical parameters was also enumerated and it was found to be positively correlated with temperature & alkalinity and negatively with pH, DO & FCO_2 .

Keywords: River Tawi, Bacterial load, Physico-chemical, Correlation, PCR.

River Tawi is the main source of drinking water supplies for Jammu city and its outskirts. As river is being polluted day by day (with sewage and other effluents), it is therefore necessary to know the degree of pollution load so as to assess its potability. For assessment of water quality, it is not only the physico-chemical characteristics but information whether river conforms to standard of microbiological water quality is more significant (APHA, 1998). Coliform count and *Escherichia coli* detection in particular, remains the major and most reliable tool in the assessment of the health risks posed by pathogens in water (Brenner *et al*. 1993, Grant 1997, Byamukama *et al*. 2000).

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Methodology

Sampling sites

During the study period (April 06 - March 07), four sampling stations were selected along the river Tawi (within the city limits of Jammu) viz., Station I (Har ki Pouri), Station II (Peer Kho), Station III (Gujjar Nagar) and Station IV (Bhagwati Nagar). Station I has negligible sewage entry where as there is a regular increase in sewage entry as we move from Station I to Station IV.

Physico-chemical analysis

Physico-chemical analysis viz., Temperature, pH, DO, FCO_2 & alkalinity was done according to the procedures recommended by American Public Health Association (APHA, 1998). Samples were collected monthly from all the stations.

Bacteriological analysis

Quantitative estimation of bacteria by Standard Plate Count (SPC/ml of facultative aerobic bacterial count) & Most Probable Number (MPN/100ml) count or coliform count methods.

Qualitative estimation of bacteria includes

(i) Isolation and growth of bacteria includes dilution of sample (either water or swabs

from different organs i.e. skin, gills & intestine of fishes) followed by streaking on nutrient agar plates again & again till the appearance of single colonies.

(ii) Identification on the basis of cultural characteristics, staining reactions and biochemical reactions (Bergey's manual 8th edition, 1974; Collins & Lyne, 1985 and Austin & Austin, 1987).

Molecular analysis

Specific PCR assay was performed using chromosomal DNA. The cultures were grown in 3-5 ml of LB broth (Hi media, India) at 37°C for 24 hrs. 1 ml of grown culture was pelleted down, supernatant drained out and the pellet suspended in 300 ml of mixture of STE buffer, SDS, BSA and ß-merceptoethanol. After vortexing, 250µl ice cold 7.5 M ammonium acetate was added, incubated on ice and centrifuged (after adding 500 µl chloroform) at 7000 rpm for 15 minutes. Aqueous phase again centrifuged after addition of chloroform, repeated thrice. Precipitation was effected by 7.5 M ammonium acetate and ethanol. Pellet was air dried & resuspended in 30µl of TE.

Two pairs of primers were designed for PCR assay: QVR184 (5'ACGCATTTTG CGTTTATTCC 3') & QVR185 (5'GGATT GCCTG GCTCATAAAC 3') for *Salmonella* flg B gene and QVR201 (5'GGCTTCTGTCAA CGCTGTTT3') & QVR202 (5'ACAGTTTTCGC GATCCAGAC 3') for *E. coli* uidA gene.

PCR was performed using extracted DNA. The reaction assay of 20 µl contained 10X PCR buffer, 200µM dNTP mixture, 10 pmoles of each primer, 1-10 ng of template DNA and 1U of Taq polymerase. The reactions were performed in Mastercycler (Eppendorf, Germany). PCR programme was set up as follows: Initial denaturation at 72°C for 3 min. followed by 30 cycles of denaturation (94°C for 15 sec.), annealing (60°C for 45 sec.) and extension (72°C for 15 sec.). Final extension was allowed at 72°C for 5 min.

A PCR reaction with all reaction components without DNA was run as a negative control to rule out any carry over contamination. The PCR products were run on 1% agarose gel and visualized under UV transilluminator. The primers used for *Salmonella* sp. yielded a specific PCR product of 752 bp and those for *E. coli* yielded a PCR product of 293 bp**.**

Statistical analysis

Bacterial load (SPC & MPN count) and Physicochemical parameters (temperature, pH, DO, FCO_2 & alkalinity) were statistically analysed with the help of Pearson correlation coefficient (calculated by Minitab software). Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Correlation between bacterial load and physico-chemical parameters

The data and correlation of bacterial load (SPC & MPN) & physico-chemical parameters presented in Tables 1, 2, 3 & 4 clearly revealed that the highest bacterial load (SPC as well as MPN) was recorded at sampling sites III & IV (high level of sewage contamination) as compared to I & II. Further, Station IV had significantly higher load than Station III. The present observations are in line with the studies of Austin & Austin (1985) and Agbogu *et al*. (2006), who also associated the high levels of bacterial load with the amount of sewage contamination.

Temperature showed positive correlation with SPC (r=0.60,0.39,0.55,0.70) and MPN (r=0.73,0.81,0.76,0.39) at all the sampling stations (I-IV). A significant correlation ($p<0.05$) was noticed between SPC and temperature at Station I & IV and insignificant ($p>0.05$) at Station II & III. MPN was also found to be significantly correlated ($p<0.05$) with temperature at Station I, II & III, where as at Station IV it showed insignificant ($p>0.05$) correlation with temperature. In tune with the present observations Sharma (1993), Gupta (1996) and Al-Harbi (2003) also held that the bacterial count of a water body bears a direct correlation with water temperature.

pH showed negative correlation (r = -0.54, -0.61, -0.30, -0.75) with SPC at all the stations (Station I–IV). Both the parameters recorded a significant correlation ($p<0.05$) at Station II & at Station IV and insignificant correlation ($p>0.05$) at Station I & III. The data further revealed a negative correlation (r= -0.81, -0.56, -0.74, -0.01) between pH and MPN at all the experimental stations (I-IV). MPN recorded significant relation ($p<0.05$) with pH at Station I, II & III where as at Station IV, it showed insignificant ($p>0.05$) correlation. Contrary to the

Table 1. Physico-chemical factors and bacterial load at Station I of River Tawi

Months	Temp. (ºC)	pH	DO (mg/l)	FCO_2 (mg/l)	Alkalinity (mg/l)	SPC(cfu/ml)	MPN/100ml
April'06	27.5	8.2	7.5	Nil	159.8	9.4×10^2	19
May	28.5	8.3	7.7	Nil	125.6	1.2×10^3	23
June	32.5	8.2	7.2	Nil	144.2	2.5×10^3	28
July	31.5	8.2	7.2	Nil	145.9	2.6×10^3	31
August	30.0	8.1	7.4	Nil	154.1	1.0×10^2	22
September	28.5	8.4	7.1	Nil	142.6	4.8×10^2	9
October	23.5	8.5	8.1	Nil	160.8	5.2×10^2	15
November	21.0	8.5	8.2	Nil	197.4	4.6×10^2	11
December	17.5	8.6	8.8	Nil	157.0	2.9×10^2	12
January'07	14.5	8.6	9.4	Nil	195.8	2.2×10^2	8
February	16.0	8.5	8.9	Nil	157.9	5.3×10^2	13
March	21.5	8.3	8.5	Nil	149.0	1.3×10^2	22

Relative significance(r):

(S = Significant)
($p<0.05$)
(NS = Not significant)
($p>0.05$)

Statistical Analysis

Temp.	Vs.	SPC(cfu/ml): r = 0.60(S)
	Vs.	MPN/100ml : r = 0.73(S)
pH	Vs.	SPC(cfu/ml): r = -0.5(NS)
	Vs	MPN/100ml : r = -0.81(S)
DO	Vs.	SPC(cfu/ml): r = -0.50(NS)
	Vs.	MPN/100ml : r = -0.59(S)
Alkalinity	Vs.	SPC(cfu/ml):r = -0.46(NS)
	Vs.	MPN/100ml : r =-0.59(S)

Table 2. Physico-chemical factors and bacterial load at Station II of River Tawi

Months	Temp. (ºC)	pH	DO (mg/l)	FCO_2 (mg/l)	Alkalinity (mg/l)	SPC(cfu/ml)	MPN/100ml
April'06	28.0	8.2	6.6	Nil	227.8	4.0×10^3	84
May	29.0	8.2	5.5	Nil	102.8	1.1×10^4	95
June	33.0	8.2	5.3	Nil	103.8	2.9×10^3	240
July	32.0	8.0	5.4	Nil	105.8	2.7×10^3	170
August	31.0	8.0	5.8	Nil	122.8	3.4×10^4	110
September	29.0	8.2	6.0	Nil	222.9	4.0×10^2	220
October	24.0	8.3	6.6	Nil	120.6	4.3×10^2	56
November	21.0	8.3	7.9	Nil	124.5	9.6×10^2	32
December	18.0	8.4	8.0	Nil	129.3	1.8×10^2	38
January'07	14.5	8.4	8.4	1.7	129.9	7.3×10^2	26
February	16.5	8.3	8.4	Nil	148.4	3.0×10^3	47
March	21.5	8.2	7.3	Nil	135.3	3.7×10^3	58

Relative significance(r):

(S = Significant)
($p<0.05$)
(NS = Not significant)
($p>0.05$)

Statistical Analysis

Temp.	Vs.	SPC(cfu/ml): r = 0.39(NS)
	Vs.	MPN/100ml : r = 0.81(S)
pH	Vs.	SPC(cfu/ml): r = -0.61(S)
	Vs.	MPN/100ml : r = -0.56(S)
DO	Vs	SPC(cfu/ml): r = -0.37(NS)
	Vs.	MPN/100ml : r = -0.79(S)
FCO_2	Vs.	SPC(cfu/ml): r = -0.15(NS)
	Vs.	MPN/100ml : r = -0.30(NS)
Alkalinity	Vs.	SPC(cfu/ml): r = -0.18(NS)
	Vs.	MPN/100ml : r =-0.12(S)

Table 3. Physico-chemical factors and bacterial load at Station III of River Tawi

Months	Temp. (ºC)	pH	DO (mg/l)	FCO_2 (mg/l)	Alkalinity (mg/l)	SPC(cfu/ml)	MPN/100ml
April'06	28.0	7.9	4.8	Nil	174.8	2.0×10^4	540
May	29.0	7.9	4.2	Nil	128.5	6.1×10^3	280
June	33.0	7.7	4.6	Nil	243.5	1.8×10^4	920
July	32.0	7.9	5.6	Nil	167.2	7.2×10^4	430
August	31.0	7.9	5.9	Nil	153.6	6.0×10^3	600
September	29.0	8.1	6.7	Nil	158.8	5.5×10^3	220
October	24.0	8.3	6.9	Nil	200.6	5.3×10^3	150
November	21.0	8.2	6.9	Nil	266.0	2.8×10^3	95
December	18.0	8.2	7.3	Nil	209.6	2.5×10^2	84
January'07	15.0	8.3	7.3	3.4	222.6	3.7×10^2	79
February	16.5	7.8	6.4	Nil	236.9	2.0×10^3	220
March	21.5	7.8	5.8	3.3	209.4	3.0×10^3	280

Relative significance(r):

(S = Significant)
(p<0.05)
(NS = Not significant)
(p>0.05)

Temp.	Vs.	SPC(cfu/ml): r = 0.54(NS)
	Vs.	MPN/100ml : r = 0.76(S)
pH	Vs.	SPC(cfu/ml): r = -0.29(NS)
	Vs.	MPN/100ml : r = -0.74(S)
DO	Vs.	SPC(cfu/ml): r =-0.34(NS)
	Vs.	MPN/100ml : r = -0.73(S)
FCO_2	Vs.	SPC(cfu/ml): r = -0.24(NS)
	Vs.	MPN/100ml : r = -0.26(NS)
Alkalinity	Vs.	SPC(cfu/ml): r = -0.26(NS)
	Vs.	MPN/100ml : r = -0.13(NS)

Statistical Analysis

Table 4. Physico-chemical factors and bacterial load at Station IV of River Tawi

Months	Temp. (ºC)	pH	DO (mg/l)	FCO_2 (mg/l)	Alkalinity (mg/l)	SPC(cfu/ml)	MPN/100ml
April'06	28.0	8.0	3.6	0.3	588.2	8.0×10^4	920
May	29.0	7.9	3.8	0.2	359.3	1.9×10^4	2400
June	33.0	7.3	3.2	Nil	324.8	9.2×10^4	600
July	32.0	7.0	4.2	Nil	360.8	8.4×10^4	220
August	31.0	7.7	4.5	5.5	276.5	2.9×10^4	240
September	29.0	8.1	5.1	1.9	302.6	3.8×10^3	350
October	24.0	8.0	5.3	4.3	246.7	2.7×10^3	240
November	21.0	8.1	5.3	4.3	212.3	3.5×10^3	95
December	18.0	8.1	4.8	5.0	227.7	2.2×10^3	64
January'07	15.0	8.2	3.0	5.8	467.7	1.8×10^3	58
February	16.5	7.8	5.8	5.3	398.2	2.1×10^3	120
March	22.0	7.8	4.4	5.9	512.9	2.3×10^4	540

Relative significance(r):

(S = Significant)(p<0.05)
(NS = Not significant) (p>0.05)

Temp.	Vs.	SPC(cfu/ml): r = 0.72(S)
	Vs.	MPN/100ml : r = 0.39(NS)
pH	Vs.	SPC(cfu/ml): r = -0.89(S)
	Vs.	MPN/100ml : r = -0.01(NS)
DO	Vs.	SPC(cfu/ml): r = -0.51(NS)
	Vs.	MPN/100ml : r = -0.36(NS)
FCO_2	Vs.	SPC(cfu/ml): r = -0.67(S)
	Vs.	MPN/100ml : r = -0.55(S)
Alkalinity	Vs.	SPC(cfu/ml): r = 0.16(NS)
	Vs.	MPN/100ml : r = 0.25(NS)

Statistical Analysis

present observations, Sharma (1993) and Gupta (1996) reported no marked correlation between seasonal pH value and bacterial count in pond water samples studied by them.

With Dissolved oxygen (DO) SPC, showed a negative correlation (r = -0.50, -0.37, -0.35, -0.56) in all the experimental stations. DO recorded significant ($p<0.05$) relationship with

Table 5. Bacterial pathogens isolated from different samples (Water & Fish).

	Total isolates	Identified bacterial isolates			
	(141)	*E. coli*	*Salmonella sp.*	*Pseudomonas sp.*	Unidentified
Water	97	33	19	11	34
Fish	44	21	6	1	16

Table 6. Number of bacterial species isolated from different stations.

Station code	*E. coli*	*Salmonella sp.*	*Pseudomonas sp.*	Unidentified
I	3	0	0	7
II	9	0	2	6
III	9	7	6	9
IV	12	12	3	12
Total	33	19	11	34

(I = Har ki Pouri, II = Peer Kho, III = Gujjar Nagar & IV = Bhagwati Nagar)

Table 7. Number of bacterial species isolated from different organs of fishes

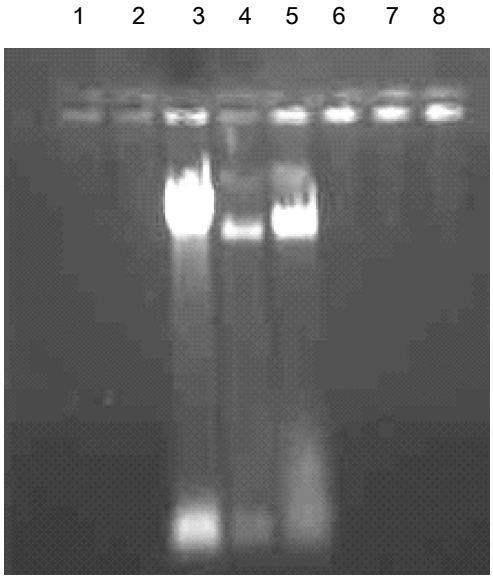
Fish sample	*E. coli*	*Salmonella sp.*	*Pseudomonas sp.*	Unidentified
Skin	9	3	1	5
Gills	7	3	0	7
Intestine	5	0	0	4
Total	21	6	1	16

Table 8. Biochemical characterists of isolated bacteria

Biochemical tests	*E. coli*	*Salmonella* sp.	*Pseudomonas* sp.
Gram Staining	-	-	-
Catalase test	+	+	+
Oxidase test	-	-	+
MR test	+	+	-
VP test	-	-	-
Nitrate test	+	+	+
Indole test	+	-	-
Citrate test	-	+	+
Urease test	-	-	-
H_2S test	-	+	-
TSI test	Sucrose / Lactosefermentation	Glucose fermentation	No carbohydrate fermentation
Growth on Mac-conkey	+	+	+
Gelatin hydrolysis	-	-	+
Lipid hydrolysis	-	-	+
Starch hydrolysis	-	-	-

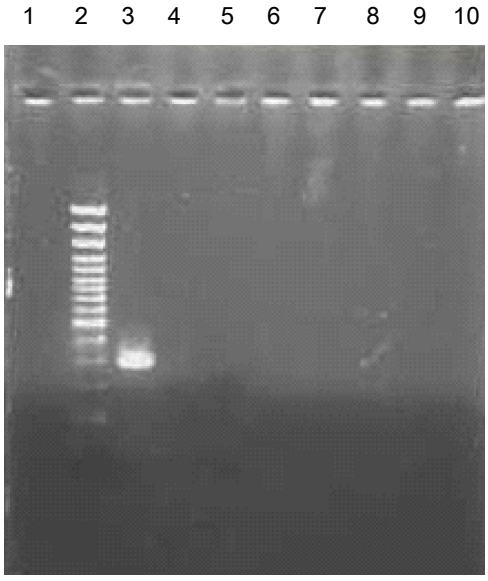
SPC at Station IV (highly polluted) and insignificant ($p>0.05$) at Station I, II & III. The results further suggest that there is a negative correlation between DO and MPN ($r = -0.59, -0.79, -0.73, -0.36$) at all the experimental stations. The present observations very clearly indicate that there is a perceptible increase in MPN values with increase in dissolved oxygen. DO recorded a significant relationship ($p<0.05$) with MPN at Station I, II & III whereas insignificant ($p>0.05$) relation was observed at Station IV.

Free carbon-dioxide (FCO_2) was found to be absent at Station I (with minimum pollution level), so no correlation was noticed between SPC and FCO_2 where as negative correlation ($r = -0.15, -0.23, -0.72$) was observed between the two at Station II, III & IV. SPC was significantly ($p<0.05$) correlated with FCO_2 at Station IV and insignificantly ($p>0.05$) at Station II & III. MPN showed negative correlation ($r = -0.30, -0.26, -0.55$) with FCO_2 at all the stations (II, III & IV) except Station I where no correlation was observed between MPN & FCO_2 due to the complete absence of FCO_2. With FCO_2, MPN correlated significantly ($p<0.05$) at Station IV whereas at Station II & III it showed insignificant ($p>0.05$) correlation.



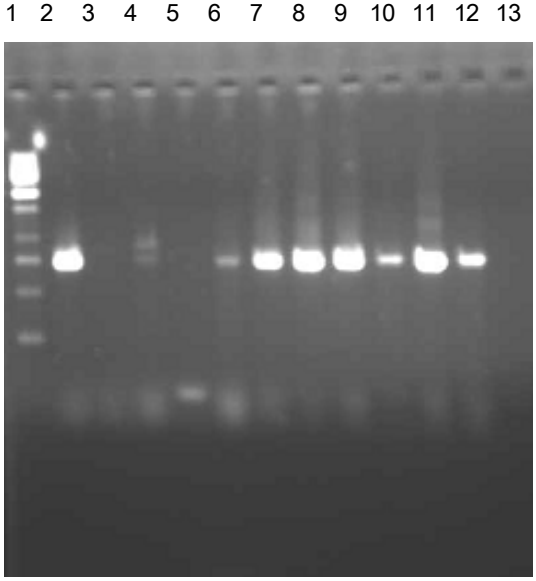
Lane 3 *Pseudomonas*
Lane 4 *E. coli*
Lane 5 *Salmonella*

Fig. 1. 1% Agarose Gel Showing DNA Isolated From Bacterial Isolates



Lane 2 DNA Ladder
Lane 3 *E. coli*
Lane 5 Negative Control

Fig. 2. 1% Agarose Gel Showing DNA Isolated From Bacterial Isolates



Lane 1 DNA Ladder
Lane 3 Negative control
Lane 6-10 *Salmonella*

Fig. 3. 1% Agarose Gel Showing DNA Isolated From Bacterial Isolates

A negative correlation (r = -0.46, -0.18, -0.26) was recorded between SPC and Alkalinity at Station I, II & III whereas at Station IV a positive correlation (r=0.35) was observed. An insignificant ($p>0.05$) correlation was noticed between the two at all the selected stations (I–IV). At Station II & IV positive correlation (r = 0.12, 0.25) and at Station I & III negative correlation (r = -0.59, -0.13) was observed between MPN & Alkalinity. At Station I, MPN showed significant correlation ($p<0.05$) with Alkalinity whereas at Station II, III & IV, an insignificant ($p>0.05$) correlation was recorded between the two.

Bacterial isolates

During the present study period, 141 bacteria were isolated as pure cultures. Out of these, 97 bacteria were isolated from water and 44 were isolated from fish samples. Among 97 isolates (from water), 34 were unidentified and 65 were identified whereas from 44 isolates of fish samples, 16 were unidentified and 28 were identified. Out of 65 identified isolates of water, 33 were *E. coli*, 19 were *Salmonella* sp., 11 were *Pseudomonas* sp. Among 28 identified isolates of fish, 21 were *E. coli*, 6 were *Salmonella* sp. and 1 belonged to genus *Pseudomonas* (Table 5). The organwise distribution of bacterial isolates is given in Table 7. Bacterial isolates were identified on the basis of biochemical tests (Table 8) and further confirmation was with the help of PCR (Fig. 1, 2 & 3). The primers used for *Salmonella* sp. yielded a specific PCR product of 752 bp and the primers used for *E. coli* yielded PCR product of 293 bp. The method presently used has already been developed and validated by Biotechnology division, IIIM (Indian Institute of Integrative Medicine), Jammu (J&K).

Identified bacterial species i.e. *E. coli, Salmonella* sp. and *Pseudomonas* sp. (Fig. 4, 5 & 6) were isolated from water as well as from resident fishes of river Tawi, thereby suggesting the impact of surrounding environment on the composition of the microflora of fish. Also, faecal coliforms in fish reflect the level of pollution of their environment, as the normal flora of fish does not include coliforms. This observation gets support from the findings of Shewan and Hobbs (1967), Cohen and Shuval (1973), Sivakami *et al.* (1996) and Apun *et al.* (1999), who also suggested the impact of aquatic environment on the microflora of inhabiting fish fauna.

Fig. 4. *Escherichia coli* showing green metallic sheen on EMB detection medium

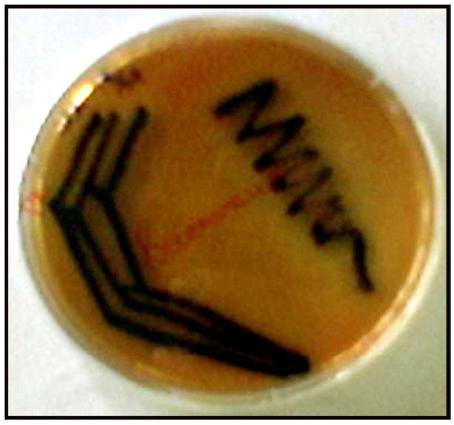


Fig. 5. *Salmonella* showing black growth on DCA detection medium

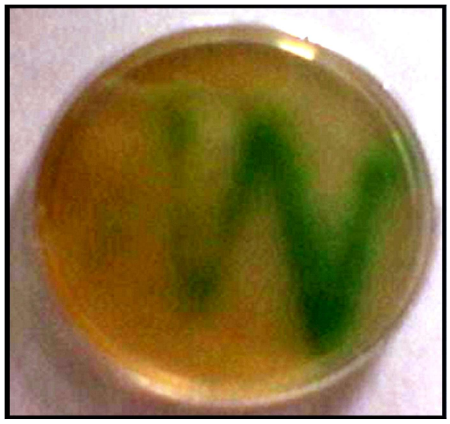


Fig. 6. *Pseudomonas* showing fluorescent growth on CAB detection medium

During the present study, *Salmonella* sp. was recorded both from Station III and Station IV (polluted areas) and no record was observed from Station I or Station II (less polluted). The data further revealed that its number was highest in Station IV (highly polluted station) as compared to Station III (moderately polluted) -Table 6. Present observations are supported by the findings of Efstratiou *et al*. (1998), who also reported maximum *Salmonella* from highly polluted sites.

CONCLUSION

Both physico-chemical and bacteriological parameters revealed an alarming trend of increase as we move from Station I to Station IV, thereby indicating deteriorating quality of river water & its edible fish fauna. The increasing pollution at Station III & IV also indicate the maximum load of pathogens in these spots which may prove health hazardous. Thus, keeping in view the public health, there is an urgent need for regular monitoring of river Tawi in order to avoid water borne diseases.

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REFERENCES

1. Agbogu, V.N., Umoh, J.V., Okuofu, C.A., Smith, S.I., Ameh, J.B. Study of the bacteriological and physico-chemical indicators of pollution of surface waters in Zaria, Nigeria. *African Journal of Biotechnology*, 2006; **5**(9): 732-737.
2. Al-Harbi, A.H. Faecal coliforms in pond water, sediments and hybrid tilapia *Oreochromis niloticus* × *Oreochromis aureus* in Saudi Arabia. *Aquaculture Research*, 2003; **34**: 517-524.
3. APHA, American Public Health Association. Standard Methods for the Examination of Water and Wastewater, 19th ed., Washington, DC, 1998.
4. Apun, K., Yusof, A.M., Jugang, K. Distribution of bacteria in tropical freshwater fish and ponds. *International Journal of Environmental Health Research*, 1999; **9**: 285-292.
5. Austin, B., Austin, D.A. Microbial quality of water in intensive fish rearing. *Journal of Applied Bacteriology*, Symposium supplement, 1985; 2075-2265.
6. Austin, B., Austin, D.A. Bacterial fish pathogen. Eillis Horwood limited, Chichester, 1987; 364.
7. Bergey's Manual of Determinative Bacteriology 8th Ed. The William & Wilkins Co. Baltimore, 1974; 1268.
8. Brenner, K.P., Rankin, C.C., Roybal, Y.R., Jr. Stelma, G.N., Scarpino, P.V. Dufour, A.P. New medium for the simultaneous detection of total coliforms and *Escherichia coli* in water. *Appl. Environ. Microbiol*., 1993; **59**: 3534-3544.
9. Byamukama, D., Kansiime, F., Mach, R.L., Farnleitner, A.H. Determination of *Escherichia coli* contamination with chromocult coliform agar showed a high level of discrimination efficiency for differing faecal pollution levels in tropical waters of Kampala, Uganda. *Appl. Environ. Microbiol*., 2000; **66**: 864-868.
10. Cohen, J., Shuval, H.I. Coliforms, faecal coliform and faecal streptococci as indicators of water pollution. *Water Soil Pollution*, 1973; **2**: 85-95.
11. Collins, C.H., Lyne, P.M. Microbiological Methods 5th Ed., Butterworth and Co. Ltd., London, 1985; 450.
12. Efstratiou, M.A., Mavridou, A., Richardson, S.C., Papadakis, J.A. Correlation of bacterial indicator organisms with *Salmonella* spp., *Staphylococcus aureus* and *Candida albicans* in seawater. *Letters in Applied Microbiology*, 1998; **26**: 342-346.
13. Grant, M.A. A new membrane filtration medium for simultaneous detection and enumeration of *Escherichia coli* and total coliform. *Appl. Environ. Microbiol*., 1997; **63**: 3526-3530.
14. Gupta, A.K. Nutritional & Microbial studies on the quality of fish flesh of Jammu region. *Ph.D. Thesis,* University of Jammu, Jammu, 1996.
15. Sharma, J.P. Some microbial relations in fishes of Jammu. *Ph.D. Thesis,* 1993: 1-282.
16. Shewan, J.M., Hobbs, G. The bacteriology of fish spoilage and preservation. In: Progress in Industrial Microbiology (ed. By D.J.D. Hockenhull). Illffe Books, London, 1967.
17. Sivakami, R., Premkishore, G., Chandran, M.R. Occurrence and distribution of potentially pathogenic Enterobacteriaceae in carps & pond water in Tamil Nadu, India. *Aquacult. Res*., 1996; **27**(5): 375-378.