

# Estimation of bacteriological levels in surface water samples to evaluate their contamination profile

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**Abstract** The present work deals with the assessment of bacteriological contamination along with some physico-chemical parameters of water samples from Lahore canal. ANOVA showed that the observed  $p$  values of log-transformed viable plate counts, total suspended solids, turbidity, and biological oxygen demand are 0.000, 0.000, 0.000,

and 0.000, respectively, which are  $<0.05$ , while the  $p$  value of total coliforms, total fecal coliforms, and *Escherichia coli* are 0.728, 0.827, and 0.081, respectively which are  $>0.05$ . Significant correlation was observed between log-transformed viable plate counts (CFU), biological oxygen demand, total suspended solids, and turbidity. Further regression analysis revealed that simple line regression model is fit for log-transformed viable plate counts and total suspended solids, log-transformed viable plate counts and turbidity, turbidity and total suspended solids, biological oxygen demand and total suspended solids, biological oxygen demand and log-transformed viable plate counts, and biological oxygen demand and turbidity.

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## Introduction

Water sources are of much significance right from the history of natural world as these provide nourishment not only to animals but also to plants. It is also considered a very important source of water supply to many fields like industry, fish culture, public consumption, agriculture etc. Moreover, it is our duty to preserve water resources as these

are also very essential for natural life (Niewolak et al. 1992; Lewandowska et al. 2000). Domestic wastes and agricultural and industrial effluents are polluting water bodies. All these are a major cause for affecting the quality of water (Danielopol et al. 2003). Numerous people suffer from different fatal diseases like typhoid, jaundice etc, due to water pollution (Atlas and Bertha 1997). Furthermore, unfavorable environment also causes trachoma, childhood diarrhea, etc (Daunders and Warford 1976; Teka 1977). Many severe changes have been observed regarding the quality of water during the last few years (Kudesia 1990; Katayal and Rajkumer 1991). Generally, the surface water is not recommended for drinking purposes as it is contaminated mostly by organic, inorganic, and biological pollutants (Kumar et al. 1996). Among the biological pollutants, coliform, fecal coliform, and *Escherichia coli* are considered as main indicators. As *E. coli* dwell in human intestine and other warm-blooded animals, these are most significant indicators of fecal contamination (Edberg et al. 2000).

The Lahore canal (the study area) surroundings are characterized by spacious lawns, many golf courses, dense residential areas, commercial centers, and industrial establishments. Agriculture has been replaced by residential areas and other establishments spread along the sides of the canal. The increased demand for water required greater pumpage from the Lahore canal. The water quality of Lahore canal, that recharges the city's aquifer, is of great concern. The Lahore canal round the clock receives millions of gallons of raw sewage rich in human excreta; animal waste of all sorts; untreated industrial effluents, biologically and otherwise polluted liquid discharges from hospitals, clinics, health care centers, and public toilets sited on either side of the canal. Resultantly, the water in the canal is virtually highly polluted by a host of the pollutants varied in quality and quantity.

Now, it is the responsibility of the environmentalists to prevent water from contamination as the quality of water is diminishing day in and day out. So, here, the aim of present study was to determine the degree of contamination and bacteriological state of water of Lahore canal

flowing through the center of Lahore (city) of Pakistan.

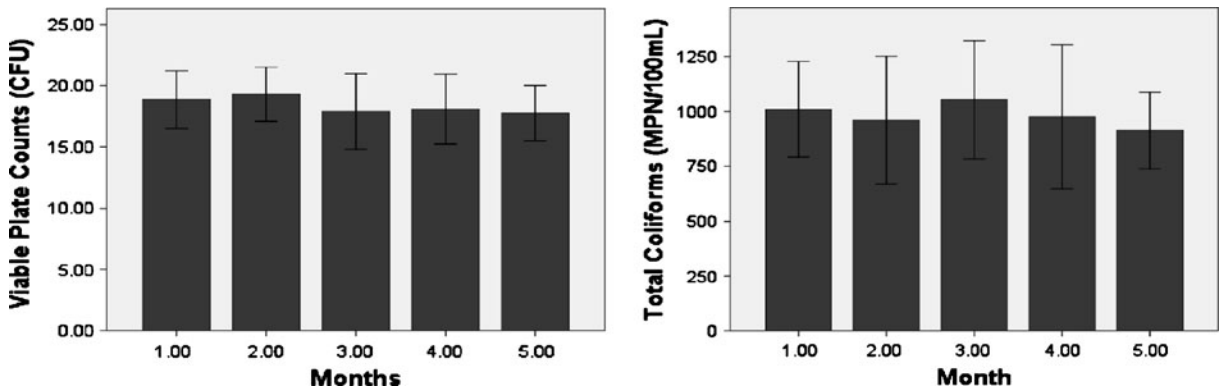
## Materials and methods

In the present study, evaluation of bacteriological contamination of water was carried out by analyzing the water samples from nine defined sampling points of Lahore canal for a study period of 5 months from May 2007 to September 2007. The parameters including the viable plate counts, coliform, fecal coliform, and *E. coli* were studied. All the experiments were performed thrice month wise. From each sampling point, sampling was carried out using fully sterilized sample bottles, which otherwise may interfere with fecal coliform growth (Greenberg et al. 1992). Collected samples were preserved and analyzed immediately to estimate bacteriological contamination of Lahore canal. Well-developed methods were used in this study for the estimation of viable plate counts, coliform, fecal coliform, and *E. coli*.

Colony forming units (CFU) is an indirect approach commonly used for the estimation of microorganisms by counting number of colonies developed on nutrient media (Atlas and Bertha 1997). Spread plate method was used for viable plate counts with the advantage of only counting living bacteria using nutrient agar (nutrient broth 0.8% and agar 1.8%) as a culture media.

Most probable number (MPN) method (Al-Harbi 2003; Feng and Hartman 1982; Hartman 1989; Shadix and Eugene 1991; Greenberg et al. 1992) was used in this study for the measurement of coliform, fecal coliform, and *E. coli* in surface water samples from Lahore canal and results are reported as MPN per 100 ml.

Some physico-chemical parameters (i.e., total suspended solids (TSS), turbidity, and biological oxygen demand, etc) were also studied during the study period. Suspended solids and turbidity were determined by photometric method and attenuated radiation method (HACH 2002), while biological oxygen demand was measured by standard biological oxygen demand (BOD) method (Greenberg et al. 1992). All the statistical analysis



**Fig. 1** Month-wise average log-transformed viable plate counts (CFU) and total coliform (MPN/100 mL) concentration ± standard deviation for nine sampling stations

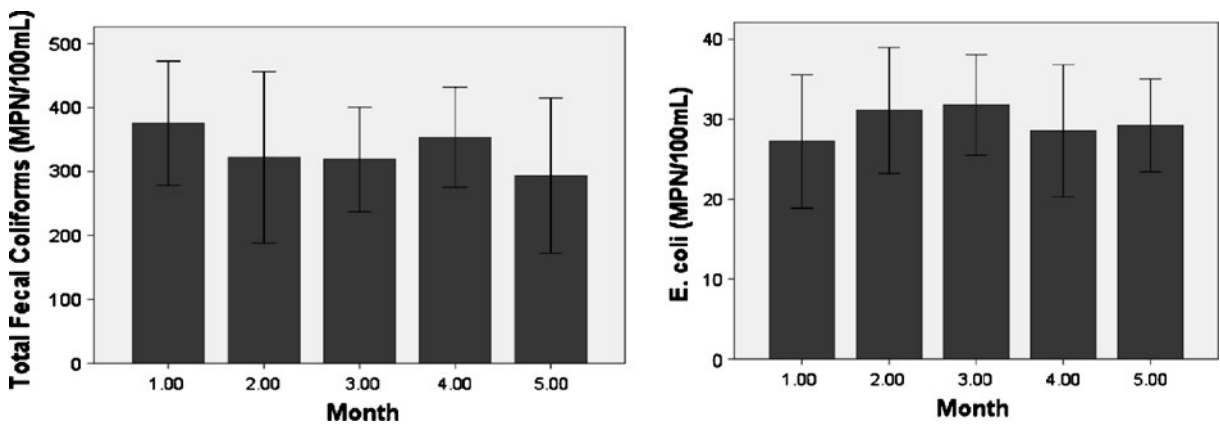
was carried out using SPSS for windows release 10.0 (Norusis 1993).

**Results and discussions**

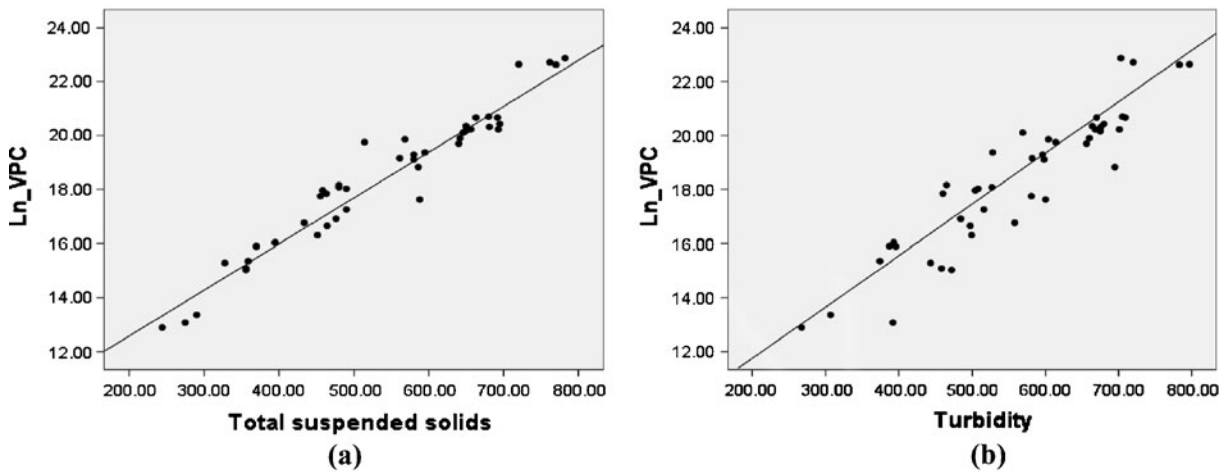
Quantification of coliform and fecal coliform bacteria is one of the most widely used methods to check the quality of surface water (Valiela et al. 1991). Usually runoff from contaminated area results in high number of bacteria in surface water (Greenberg et al. 1992). During present study, quantification of viable plate counts, coliform, fecal coliform, and *E. coli* is carried out and results

are presented as average values with standard deviation along with other statistical analysis.

The observed average log-transformed viable plate counts (CFU) along with their standard deviation during the study period of 5 months, i.e., from May 2007 to September 2007 as shown in Fig. 1 were found to be 19.0 ± 2.0, 19.0 ± 2.0, 18.0 ± 3.0, 18.0 ± 3.0, and 18.0 ± 2.0, respectively, while the observed average total coliform (MPN/100 mL) along with their standard deviation during the study period of 5 months, i.e., from May 2007 to September 2007 were 1,009.0 ± 218.0, 960.0 ± 292.0, 1,053.0 ± 270.0, 975.0 ± 328.0, and 912.0 ± 175.0, respectively.



**Fig. 2** Month-wise average fecal coliform (MPN/100 mL) and *E. coli* (MPN/100 mL) concentration ± standard deviation for nine sampling stations

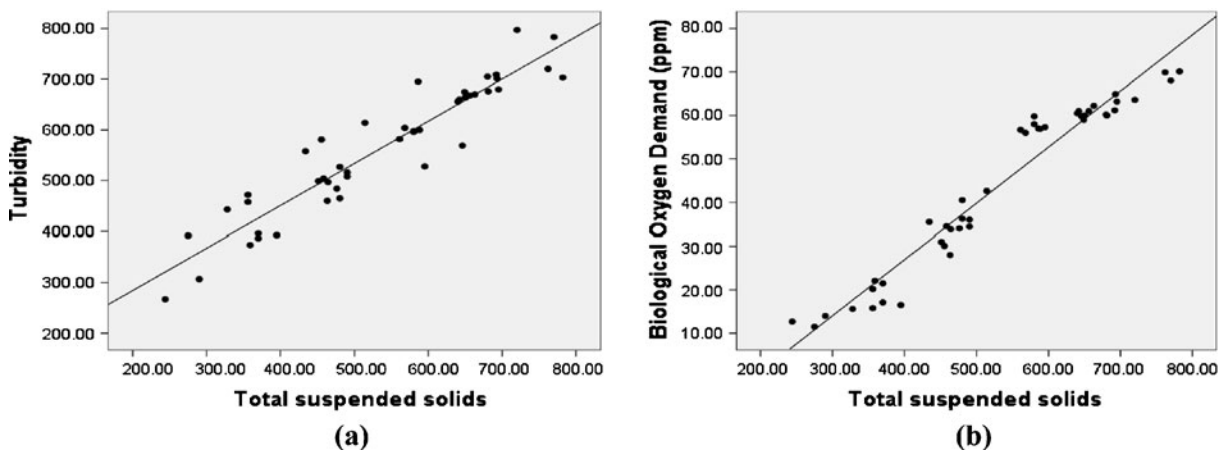


**Fig. 3** **a** Simple line regression model for log-transformed viable plate counts (CFU) and total suspended solids (ppm). **b** Simple line regression model for log-transformed viable plate counts (CFU) and turbidity (NTU)

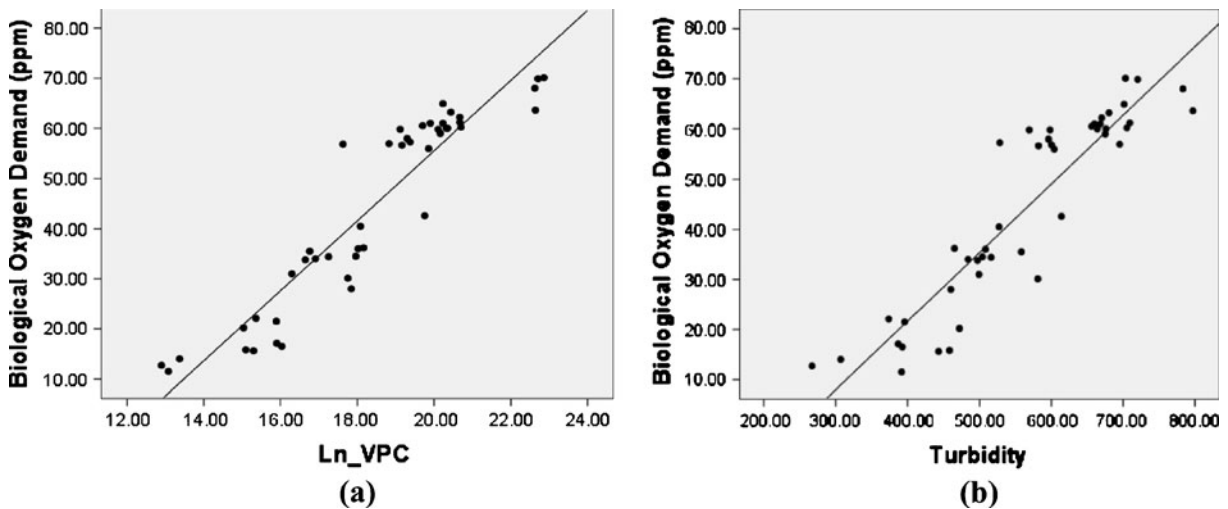
The observed average total fecal coliform (MPN/100 mL) along with their standard deviation during the study period of 5 months, i.e., from May 2007 to September 2007 as shown in Fig. 2 were  $376.0 \pm 97.0$ ,  $322.0 \pm 134.0$ ,  $319.0 \pm 82.0$ ,  $353.0 \pm 78.0$ , and  $293.0 \pm 121.0$ , respectively, while the observed average *E. coli* (MPN/100 mL) along with their standard deviation during the study period of 5 months, i.e., from May 2007 to September 2007 were  $27.0 \pm 8.0$ ,  $31.0 \pm 8.0$ ,  $32.0 \pm 6.0$ ,  $29.0 \pm 8.0$ , and  $29.0 \pm 6.0$ , respectively.

Significant correlation was observed between log-transformed viable plate counts (CFU), bio-

logical oxygen demand, total suspended solids, and turbidity. Apart from this, regression analysis was performed to test the fitness of simple line regression model for log-transformed viable plate counts, total suspended solids, turbidity, and biological oxygen demand. TSS, turbidity, and BOD entered in the regression equation and thus participated in deciding the abundance of bacteria. Studies related to water quality are also reported by Anesio et al. (1997), Mohamed et al. (1998), Kirschner et al. (1999), Mitsuru et al. (2000), Lindstrom (2001), Heidelberg et al. (2002), and Castillo et al. (2004).



**Fig. 4** **a** Simple line regression model for turbidity (NTU) and total suspended solids (ppm). **b** Simple line regression model for biological oxygen demand (ppm) and total suspended solids (ppm)



**Fig. 5** **a** Simple line regression model for biological oxygen demand (ppm) and log-transformed viable plate counts (CFU). **b** Simple line regression model for biological oxygen demand (ppm) and turbidity (NTU)

For log-transformed viable plate counts (CFU) and total suspended solids,  $R^2 = 94.1\%$ , the  $p$  value is  $<0.05$ , the simple line regression model is fit for the data as shown in Fig. 3a. The fitted regression line is log-transformed viable plate counts =  $9.184 + 0.017$  TSS, while for log-

transformed viable plate counts (CFU) and turbidity  $R^2 = 85.6\%$ , the  $p$  value is  $<0.05$ , the simple line regression model is fit for the data as shown in Fig. 3b. The fitted regression line is log-transformed viable plate counts =  $7.957 + 0.019$  turbidity.

**Table 1** ANOVA table for bacteriological and physiochemical variables with respect to different sampling points

Bacteriological and physiochemical variables	Source of variation	Sum of squares	df	Mean square	F	p value
Total coliform (MPN/100 mL)	Between sampling points	362,525.378	8	45,315.672	0.653	0.728
	Within sampling points	2,497,595.200	36	69,377.644		
	Total	2,860,120.578	44			
Total fecal coliform (MPN/100 mL)	Between sampling points	50,160.000	8	6,270.000	0.528	0.827
	Within sampling points	427,320.000	36	11,870.000		
	Total	477,480.000	44			
<i>E. coli</i> (MPN/100 mL)	Between sampling points	701.778	8	87.722	1.955	.081
	Within sampling points	1,615.200	36	44.867		
	Total	2,316.978	44			
Log transformed viable plate counts	Between sampling points	159.913	8	19.989	5.809	0.000
	Within sampling points	123.885	36	3.441		
	Total	283.798	44			
Total suspended solids	Between sampling points	512,196.711	8	64,024.589	5.875	0.000
	Within sampling points	392,307.200	36	10,897.422		
	Total	904,503.911	44			
Turbidity	Between sampling points	442,216.000	8	55,277.000	7.444	0.000
	Within sampling points	267,309.200	36	7,425.256		
	Total	709,525.200	44			
Biological oxygen demand (ppm)	Between sampling points	9,428.352	8	1,178.544	6.565	0.000
	Within sampling points	6,462.600	36	179.517		
	Total	15,890.952	44			

For turbidity and total suspended solids,  $R^2 = 88.2\%$ , the  $p$  value is  $<0.05$ , the simple line regression model is fit for the data as shown in Fig. 4a. The fitted regression line is turbidity =  $118.080 + 0.832$  TSS, while for biological oxygen demand (BOD) and total suspended solids  $R^2 = 94.4\%$ , the  $p$  value is  $<0.05$ , the simple line regression model is fit for the data as shown in Fig. 3b. The fitted regression line is BOD =  $-24.669 + 0.129$  TSS.

For biological oxygen demand and log-transformed viable plate count (CFU),  $R^2 = 87.3\%$ , the  $p$  value is  $<0.05$ , the simple line regression model is fit for the data as shown in Fig. 5a. The fitted regression line is BOD =  $-84.289 + 6.993$  log-transformed viable plate counts, while for biological oxygen demand and turbidity,  $R^2 = 84.3\%$ , the  $p$  value is  $<0.05$ , the simple line regression model is fit for the data as shown in Fig. 5b. The fitted regression line is BOD =  $-33.138 + 0.137$  turbidity.

After applying analysis of variance (Table 1) to test the significant difference of average log-transformed viable plate counts, total coliform, total fecal coliform, *E. coli*, total suspended solids, turbidity, and biological oxygen demand with respect to sampling stations, the observed  $p$  values of log-transformed viable plate counts, total suspended solids, turbidity, and biological oxygen demand 0.000, 0.000, 0.000, and 0.000 which are  $<0.05$ . It means log-transformed viable plate counts, total suspended solids, turbidity, and biological oxygen demand differ significantly on the average at different sampling stations. The  $p$  value of total coliform, total fecal coliform, and *E. coli* are 0.728, 0.827, and 0.081, respectively which are  $>0.05$ . It means total coliform, total fecal coliform, and *E. coli* do not differ significantly on the average at different sampling stations.

## Conclusion

Based upon observations, it is found that Lahore Canal water is bacteriologically highly contaminated. At different sampling stations, the concentration of bacteria, i.e., total coliform, total fecal coliform, and *E. coli* exceeds the limits as defined

by National Environmental Quality Standards of Pakistan.

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