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# CELLULOLYTIC, ENDOSPORE FORMER AND COLIFORM BACTERIAL PROFILES IN THE BOTTOM MUD OF LAHORE CANAL AT DIFFERENT LOCATIONS IN THE CITY

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**Abstract:** The study reports the bacterial profiles of four locations of the canal. Mud of the given localities were sampled, when the water supply was closed. Bottom soils of canal were sampled in sterile containers, from four different locations in the urban area. In the laboratory, l0gm of a soil sample was suspended in 100ml of autoclaved water and processed for; enumeration of colony forming units (CFU) representing endospore formers on nutrient agar plates, and cellulose degrading bacteria on a selective agar medium. While for the coliforms MacConkcy agar was used. Pure cultures of coliforms and endospore formers were processed for determining their antibiotics and heavy metal sensitivity/resistance. Too many CFUs of cellulose degrading bacteria were obtained for the first three samples. These bacterial populations appeared to degrade the sediment cellulose material and useful for providing relatively less turbid and of low biological oxygen demand water to the downstream areas. For the fourth sampling locality CFU of cellulose degrading bacteria reduced drastically, which indicated the lesser amounts of the available substrate (cellulose material) at this place, downstream. Hemolytic endospore formers were isolated from all samples. Fecal contamination of the canal bed became evident from the presence of coliform bacteria in all the samples. It is concluded that different types of bacteria are inhabitants of the canal bed, performing their ecological roles. The bed is also a good indicator, as assessed by the presence of hemolytic endospore formers and coliform bacteria of pathogen contamination and fecal pollution of the habitat.

**Keyword:** Surface water pollution; stream pollution; cellulose degrading bacteria; endospore formers and coliforms in canal mud.

## INTRODUCTION

rban environments are big sources of different sorts of pollutions, which are altering the surrounding natural habitats, in general, proportionate to the pollution load they create. Conveniently, various pollutants are described as atmospheric, aquatic and soil, despite of the fact that they enter routinely from one phase to others. Water pollution, both the surface and ground water habitats is of immediate concern to humans. Depending upon its physiochemical characteristics. Water is a reservoir of diverse forms of microorganisms and depending upon nature and amount of chemical contaminants it may serve as medium for microbial growth. The microorganisms in water include several harmless but useful bacteria. However, contaminated water may harbour bacteria capable of causing diseases. Such microorganisms may be present in water

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bodies, contaminated by domestic and other wastes (Borchardt *et al.,* 2004; Dorner *et al.,* 2004). In this regard, fecal contamination of drinking or potential drinking water, which may bring enteric pathogens, has been worked out extensively. An enumeration of *E.coli* and/or either coliforms in water samples is an established protocol for assessing the fecal contaminations of water sources, storage vessels and nature of pipes transporting water from a treated/untreated water reservoir (Welch *et al.* 2000; Grandjean *et al.,* 2005; Tandon *et al.,* 2005).

Presence of different types of chemical substances in a water body influences the nature of microbial communities, which are allowed to make their presence and prevalence. Owing to such links between physiochemical parameters and the microbial communities several microorganisms especially bacteria have been considered as bioindicator of specific pollutants. Sampling and subsequent microbiological analyses of running waters may indicate immediate as well as upstream contaminations. A very interesting example of such a habitat is the canal passing through the city Lahore. Apart from atmospheric fallout and road dust, sewage leakage and solid wastes such as bagasse, waste fruits and vegetables contaminate the Lahore canal water at various locations along its way through the city. Thus, the canal water faces radical physiochemical changes which might had been affecting microbial community of the running water. Samplings of water at a given location around the same time are thus expected to yield variable results. However, the bottom mud environment may indicate certain stable bacterial inhabitants that may relied the influence of different pollutants at different locations of the canal. In fact, more than 90% microbial species arc found in water habitats while attached to some solid surfaces (Sinton *et al.,* 2002; Trevett *et al.,* 2004). The present survey refers to bacterial profiles of the canal bottom muds, collected from its different locations within the city. The information is relevant to assess nature and load of pollutants being added to the canal water for the public health authorities.

## MATERIALS AND METHODS

*Sample collection*

Mud samples of the Lahore canal, were collected from four different sites in sterile glass containers in May 2001. On the sampling day canal did not contain running water, as supply was cut many days before. The canal bottom appeared to harbour ditches containing 1 to 6 inches deep water. The ditches of varying sizes were interrupted with naked mud patches. The first sample was collected from the vicinity of Jalo Park while, the $2^{nd}$, $3^{rd}$ and $4^{th}$ samples from 7, 14 and 21 kms downstream, respectively, from the first sampling point. For sampling, a suitable place was selected, where about 1 inch of water was present. For each sample sterilized open mouth bottle was approached to the mud surface in inverted position. It was then opened by removing the metallic cover and

pressed down on collecting location. Then the bottle containing mud and upper thin layer of water was taken out of the sediment by digging out some mud around it and the lid was immediately closed, while keeping the bottle inverted. Each sample represented 4 to 5 inches deep mud. These were brought to the laboratory and processed for the examination of bacterial contents. Temperature of the soil to be sampled was also noted *in situ* by immersing a decontaminated thermometer to the nearest sampling location.

*Microbiological processing of the samples*

Ten g of a sample was suspended in 100ml of distilled sterilized water. From this three dilutions i.e., $1:10^2$, $1:10^4$ and $1:10^6$ were prepared. These dilutions were streaked on MacConkey agar (Merck, 1996) and the plates were incubated for 24 hours at 37°C. The bacterial colonies from the MacConkey agar were then subcultured onto nutrient broth and nutrient agar. The growth was processed for characterizing the isolates. For culturing endospore formers, portions of dilutions were given a heat shock at 80°C for 10 minutes. Then 2 and 5µl of each dilution were spreaded on nutrient agar plates and incubated at 37°C for 24 hours. The bacterial colonies were subcultured on nutrient agar for pure culture and the growth was processed for endospore staining and biochemical characterization.

For estimation of cellulose degrading colony-forming units (CFU) 2µl from the suspension of 10g of a sample/100ml of autoclaved distilled water, was spreaded on a selective medium (Pelczar *et al.*, 1993) and the plates were incubated at 37°C for 24 hours. CFU indicated the cellolulytic bacterial profiles of the samples. Well-separated representative colonies of the three categories of the bacteria were pure cultured, routinely, screening at least once by growing them on nutrient agar. The isolates were then grown on nutrient agar slants for further use. The bacteria were then characterized by performing various test viz., gram staining, endospore staining, hemolytic activity, indole, methyl red, oxidase, motility, catalase and VP tests, according to Benson (1994) and Holt *et al.* (1994). The isolates of endospore formers and coliforms were also tested for their antibiotics and heavy metals susceptibility resistance patterns. Commercially available antibiotic discs were used for this purpose. While for the heavy metals 1% aqueous solutions of Cu Ag and Hg were used. Sterile discs of Whatman's filter paper No. 1 (0.6 mm diameter) were immersed in sterile solutions of these metals. They were dried and then placed on inoculated nutrient agar plates.

## RESULTS AND DISCUSSION

The Lahore canal's water enters the city with relatively better look. Then along its way through the city it receives heavy loads of solid wastes of varying characteristics, mainly comprising of kitchen left overs, fruit and sugarcane residues and even sewage

effluent. Thus the running water is contaminated with particulate organic matter and myriad of microbial species. Many of the organic matter degrading bacteria might be considered playing their roles to render the water less turbid. However, running waters microbial communities remain highly variables and show fluctuating trends (Davikar and Saxena, 1998). Thus the present report is an attempt to look into the bottom mud bacterial profiles of four different locations. However, the bottom mud contained free water. The sampling points represented mud patches having free water containing ditches around. Temperatures of the muds of first two localities turned out to be the same. The parameter did not fluctuate much for the other two localities (Table 1). Plating of different amounts of different dilutions of primary soil suspension on the selective medium for cellulolytic bacteria yielded too much colonies to be counted for the first three localities. Bacterial population dynamics are known to follow in general, closely the availability of substrate (nutrition) and removal efficiency of the metabolic wastes. This is all that is tuned, while growing continuous cultures of bacteria (Pelczar *et al.,* 1993). It is likely that the (lowing water of the canal and more or less continuous loading of cellulose material and its sedimentation have rendered continuously growing populations of cellulose degrading bacteria to remain attached and entrapped in the submerged soil particles. Such bacterial populations are apparently responsible for reductions in the turbidity and biochemical oxygen demands of the water in downstream localities. Lesser amounts of the substrate are also expected to be translated with decline in the bacterial population. This became suggestive when colony-forming units (CFU) of cellulolytic bacteria turned out to be within the countable limits for the last (fourth) sampling point. For this sample spreading of 20µl a dilution gave 45 CFU a value within acceptable range for bacterial CFU determination (Black, 1996). Regarding the colonial characteristics of cellulose degrading bacteria, surprisingly all the colonies obtained from the samples expressed consistently similar features. The colonies were round in configuration having smooth margins with convex elevations and transparent look. Well separated colonies were of 1mm diameter. Regarding the endospore formers, astonishingly gram-positive bacteria were isolated from all the samples (Table 2). All the isolates were also found positive for catalase and VP tests, while negative for indole test. However, they varied in other characteristics (Table 2). Alarming results appeared when the endospore formers were screened for their hemolytic activity on blood agar. All of the isolates were found hemolytic. This indicates that the streambed is at least receiving pathogenic endospore formers. It may be speculated that these bacteria might have arrived to the canal bed via contaminated food, especially foods sold by the street venders along the riverbank, who frequently use the canal water for washing the utensils etc. Many of the endospore formers were also found resistant to some antibiotics and heavy metals (Table 2). These characteristics indicate anthropogenic-associated effects on them.

**Table 1: Sampling locations and estimates of cellulose degrading bacteria in the canal's bottom muds.**

| Sample No. | Locality | Temperature | pH | CFU of cellulolytic bacteria/g of mud* |
|---|---|---|---|---|
| S-1 | Jallo Park | 32.6°C | 8.36 | T.N. |
| S-2 | Harbans Pura | 32.6°C | 8.08 | T.N. |
| S-3 | Mall Road | 32.3°C | 8.78 | T.N. |
| S-4 | Campus | 33.0°C | 8.26 | 2250 |

T.N. = too numerous to count
* = Wet weight

**Table 2: Biochemical characteristics of endospore forming bacteria isolated from the different samples.**

| Sample No. | Strain's Code | Endospore Staining | Oxidase Test | Methyl Red Test | Pathoge-nicity Test | Motility Test | Susceptibility to Cu, Ag, Hg, Stp, Ert, Bac |
|---|---|---|---|---|---|---|---|
| **S-1** | 1EI(0.1) | +ve | -ve | +++ | +ve | -ve | 2.1[a], 2, 3.8*, 3.3, 3.8, R |
| **S-2** | 2E1(0.1) | +ve | +ve | +++ | +ve | -ve | 3, 2.2, 3, 2.2, 3.4, 0.9 |
| | 2E2(0.1) | +ve | +ve | +++ | +ve | +ve | 3.7, 2.7, 1.9, 1.9, 3.9, R |
| | 2E3(0.1) | +ve | +ve | ++ | +ve | -ve | R, R, 2, R, R, R |
| | 2E4(0.1) | +ve | +ve | -ve | +ve | +ve | 3.4, 2.1, 3.2, 2, 1.6*, R |
| | 2E5(0.1) | +ve | -ve | +++ | +ve | -ve | 2.1, 2, 3.8, 3.4, 2.7, 0.8 |
| **S-3** | 3E1(0.1) | +ve | -ve | ++++ | +ve | -ve | 2, 2, 3.7, 3.7, 2.6, 0.8 |
| | 3E2(0.1) | +ve | -ve | ++++ | +ve | -ve | 3.7, 2.1, 4.2, 2.5, 3.4, 1.6* |
| | 3E3(0.1) | +ve | +ve | +++ | +ve | -ve | 2.2, 2.1, 3.4, 3.2, 2.9, R |
| | 3E4(0.1) | +ve | +ve | ++ | +ve | -ve | 2, 2, 3.3, 3, 2.6, 0.7 |
| **S-4** | 4E1(0.1) | +ve | +ve | -ve | +ve | -ve | R, R, 1.8, R, R, R |
| | 4E2(0.1) | +ve | -ve | -ve | +ve | -ve | R, 1.9, 3.5, 3, 3.1, 1.3 |
| | 4E3(0.1) | +ve | +ve | ++ | +ve | -ve | 3.5, 3.9, 3.7, 2, 4, 1.9 |
| | 4E4(0.1) | +ve | -ve | ++++ | +ve | +ve | 3.6, 1.8, 3.3, 2, 1.6, 0.7 |

a=Diameter in cm of growth inhibition zones: *=growth retarding zone, R=The isolate appeared resistant to the application.
Cu=Copper, Hg=Mercury, Ag=Silver, Strp=Streptomycin, Ert=Erthromycin, Bac=Bacitracin

Coliform picture also revealed that the stream bed is contaminated with these bacteria. Their presence definitely indicates fecal contamination of the water habitat. Many of the isolates were found resistant to some antibiotics and heavy metals (Table 3).

All the isolates were found positive for catalase, methyl red and VP tests. All were found negative for the hemolytic test. Other characteristics are summarized in Table 3. Presence of coliform in the canal bed should be taken seriously by public health authorities. Indeed canal water itself is contaminated by fecal pollution. It is proposed that this water might be carrying enteric pathogens downstream. In this connection use of this water for human recreational purposes is recommended to be controlled. Moreover, the canal water is used

**Table 3: Biochemical characteristics of coliform bacteria isolated from the different samples.**

| Sample No. | Strain's Code | Gram Staining | Endospore Staining | Oxidase Test | Methyl Red Test | Pathogenicity Test | Motility Test | Susceptibility to Cu, Ag, Hg, Stp, Ert, Bac |
|---|---|---|---|---|---|---|---|---|
| S-1 | 1C1 | -ve | -ve | -ve | ++++ | -ve | -ve | 1.9[a], 1.5, 2.6, 2.4, R, R |
| | 1C2 | -ve | -ve | -ve | ++++ | +ve | -ve | R, 1.3, 1.5, R, R, R |
| | 1C3 | -ve | +ve | -ve | ++++ | -ve | -ve | 1.5, 1.4, 3, 1.8, 2.2, 0.8 |
| S-2 | 2C1 | +ve | -ve | +ve | ++ | +ve | +ve | R, 2, 3.2, 1.3, 1.3*, 3, R |
| | 2C2 | +ve | +ve | -ve | ++++ | +ve | -ve | R, 1.9, 1.5, 3.2, 1.3, 1.9 |
| S-3 | 3C1 | -ve | +ve | -ve | ++++ | +ve | -ve | 3, 2, 3.3, 3.4, 3, 2.5 |
| | 3C2 | +ve | +ve | -ve | ++++ | -ve | -ve | R, 1.4, 1.4, R, R, R |
| | 3C3 | -ve | +ve | -ve | ++++ | -ve | -ve | 1.6, 1.6, 3, 2, 2.2, 2.7, 0.9 |
| | 3C4 | +ve | -ve | -ve | ++++ | +ve | -ve | 1.9, 2, 3, 2, 1.7*, R |
| S-4 | 4C1 | +ve/-ve | -ve | -ve | ++++ | +ve | -ve | 1.8, 1.7, 3, 2.2, 2, 2.2 |
| | 4C2 | +ve | +ve | -ve | ++++ | -ve | +ve | 2, 1.7, 3, 1, 2.4, 0.8 |

a=Diameter in cm of growth inhibition zones; *=Growth retarding zone, R=The isolate appeared resistant to the application.
Cu=Copper, Hg=Mercury, Ag=Silver, Strp=Streptomycin, Ert=Erthromycin, Bac=Bacitracin

for irrigation purposes for different crops including vegetables. It is known that various pathogens occur on vegetables harvested from soil irrigated with contaminated water (Okafo *et al.,* 2003; Attikat-Ur-Rehman, 2005). Thus the vegetables being irrigated with the canal water, especially those used as salad, might have been involved in the spread of enteric bacterial pathogens.

## REFERENCES

ATIKAT-UK-KLHMAN, 2005. *Periphytic coliform contents of a commonly used salad, vegetables, Brasica oleracea.* M.Sc. Thesis, University of the Punjab, Lahore, Pakistan.

BENSON, H.J. 1994. *Microbiological Application, Laboratory manual in General Microbiology.* W.M.C. Brown Publishers, Dubuque, USA.

BLACK, J.G., 1996. *Microbiology: Principles and Applications.* Prentice Hall, Upper Saddle River. New Jersey. USA.

BORCHARDT, M.A., HAAS. N.I, AND HUNT. R.J., 2004. Vulnerability of drinking water wells in La crosse, Wisconsin to enteric-virus contamination from surface water contribution. *Appl. Environ. Microbiol.,* **70:** 5937-5946.

DAVIKAR. R.P. AND SAXLNA. P.N., 1998. Fluctuating trend in microbial population an index of water quality. *Proc. Natl. Frontiers in Appl. Environ. Microbiol.,* 11-13 Dec. 1995 SES, CUSAT. Cochin, pp. 225-229.

DORNLR, S., HUCK, P., SLAWSON, R. AND ANDLRSON, W., 2004. Assessing level of pathogenic contamination in a heavily impacted river used as a drinking water source. *J. Toxicol. Environ. Hlth.,* **67:** 1813-1823.

GRANDJEAN, D., FASS, S., TOZZA, D., CAVARD, J., LAHOUSSINE, V., SABY, S., GUILLOTLAU, H. AND BLOCK, J.C., 2005. Coliform culturability in over- versus understand drinking waters. *Water Res.,* **39:** 1878-1886.

HOLT, J.G., KR1LY, N.R., SNLATIL P.H.A., STALLY. J.T AND WILLIAMS, S.T., 1994. *Bergey's Manual of Determinative Bacteriology,* pp. 562. Lippincott Williams and Wilkins, philandelphia, USA.

MLRCK, 1996-1997. *Microbiology Manual Merck kgaA*, Darmstadt, Germany.

OKAFO, C.N., UMOIL VJ AND GALADIMA, M. 2003. Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. *Sci. Total Environ.,* 311: 49-56.

PELCZAR, M., CHAN, E.C.S. AND KRIEG, N.R., 1993. *Microbiology: concepts and Applications.* McGraw Hill Book Company, New York, USA.

SINTON, L.W., HALL, C.H., LYNCH, P.A. AND DAVIES-COLLEY, R.J., 2002. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Appl. Environ. Microbial.,* **68:** 1122-1131.

TANDON, P., CHHIBBER, S. AND REED, R.H., 2005. Inactivation of *Escherichia coli* and coliform bacteria in traditional brass and earthenware water storage vessels. *Antonie Van Leeuwenhoek.,* **88:** 35-48.

TREVETT, A.F., CARTER, R. AND TYRREL, S., 2004. Water quality deterioration: a study of household drinking water quality in rural Houduras. *Int. J. Environ. Hlth. Res.,* **14:** 273-283.

WELCH, P., DAVID, J., CLARKE, W., TRINDADE, A., PENNER, D., BERSTEIN, S., MCDOUGALL, L., AND ADESIVUN, A. A., 2000. Microbial quality of water in rural communities of Trinidad. *Rev. Panam. Salud. Publica.,* **9:** 172-180.

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