ORIGINAL ARTICLE

Abed El Mo^ati. K. El Jarousha.
AL Azhar University-Laboratory Medicine Department, Gaza Strip, Palestine.
Email: amoati2005@yahoo.com .

Influence of Seasonal Environmental Variables on The Distribution of Fecal Indicator Bacteria in Seawater of Gaza Strip.

Abstract

A survey of the occurrence of fecal indicator bacteria (Total Coliform, fecal Coliform and fecal streptococci) in sea water was carried out along the coastline of Gaza strip during the period October 2003 and September 2004. The study was implemented to assess the hygiene of water quality in order to give an indication about the actual magnitude of fecal pollution post the discharge of domestic sewage discharge into the seawater without treatment. This type of baseline study is required to develop monitoring strategies and management plans for the coastline area. The results showed that there are pollution for the seawater, the total Coliform means values ranged from 4,800 CFU/100ml to 15,000 CFU/100ml, fecal Coliform ranged from 2,000 CFU/ 100ml to 12,000 CFU/100ml, fecal streptococci ranged from 1100 CFU/100ml to 6100 cfu/100ml. The highest mean values of bacterial indicators were obtained during winter season, while the lowest mean values were recorded during summer season at all site. Fecal Coliform / Fecal streptococci ratio was 2:1.

Keywords: Bacterial fecal indicator organisms; Total Coliform; Fecal Coliform; Fecal Streptococci.

تأثير المتغيرات الموسمية البيئية على توزيع الميكروبات الدالة على وجود التلوث البرازي في مياة البحر لقطاع غزة.

الملخص:

تم اجراء مسح شامل للبكتيريا المستخدمة ككواشف للتلوث البرازي (مجموعة الكولي فورم الكلي، مجموعة الكولي فورم البرازي، مجموعة المكورات السبحية البرازية) في مياة شواطئ بحر قطاع خلال الفترة ما بين أكتوبر 2003 و سبتمبر 2004، أجريت الدراسة لتقييم مدى وجود التلوث البرازي في مياة البحر في مناطق مصبات المياة العادمة و مياة المجاري الغير معالجة. تعتبر هذه الدراسة ضرورية لعمل استراتيجية على المستوى القومي لوضع أسس عامة للتخفيف و معالجة الأضرار الناجمة عن هذه الظاهرة. أظهرت النتائج أن نسبة التلوث بمجموعة بكتيريا الكولي فورم كانت تتراوح ما بين 4800 إلى 15000 وحدة مكونة للمستعمرات لكل 100مل من مياة البحر، مجموعة الكولي فورم البرازي كانت تتراوح ما بين 2000 إلى 12000 وحدة مكونة للمستعمرات لكل 100مل من مياة البحر، أما مجموعة المكورات السبحية البرازية فكانت تتراوح ما بين 1100 إلى 6100 وحدة مكونة للمستعمرات لكل 100مل من مياة البحر. أثبتت الدراسة أن فصل الشتاء كان يحتوي على أعلى نسبة من التلوث، بينما فصل الصيف فكانت نتائجة أقل. كانت نسبة وجود مجموعة الكولي فورم البرازية إلى مجموعة المكورات البرازية السبحة بنسبة 1:2.

الكلمات المفتاحية: الكواشف البكتيرية للتلوث البرازي، مجموعة الكولي فورم الكلية، مجموعة الكولي فورم البرازية، مجموعة المكورات السبحية البرازية.

Introduction

The pollution is one of the problem being faced in the world all through 20th century, the most important factor for water pollution is the discharge of untreated wastewaters into the seawater**[1]**. Historically fecal Coliform and *E. coli* had been used as indicators of choice when monitoring recreational water quality**[2]**. Also the total Coliform and *fecal Streptococci* had been used as an indicator of human enteric pathogens for many years**[3]**. Recent studies have shown that high densities of *E. coli* and *Enterococci* recovered from recreational waters have a stronger correlation with swimming associated gastrointestinal disease than do densities of fecal Coliform bacteria alone**[4]**, both of these indicators have been referred as being equally acceptable for monitoring freshwater and marine water**[5]**. However it is now well established that *E. coli* one of the total Coliform member is not limited to humans but also exists in the intestine of many other warm blooded animals**[1]**. Consequently its presence in water is not specific to human sources of pollution. This is especially relevant when recognizing that human feces can carry various human enteric pathogens such as *Salmonella spp*., *Shigella spp*., *E. coli*, *Hepatitis A virus* and Norwalk group viruses, *Cryptosporidium parvum*, *Entamaeba histolytica,* and *Giardia lamblia*, in addition most of these pathogens causes a disease to human. In contrast most of these pathogen do not colonize non human species potentially resulting in less risk posed by non human species fecal pollution**[6]**. Therefore it is important to know whether fecal pollution originate from human source or non human source to properly assess risk and researches are needed to determine the characteristics of indicators, to meet this challenge there have been various attempts to develop method that differentiate the sources of fecal pollution, initially the ratio of fecal Coliform to *fecal Streptococci* was proposed where a ratio of baseline 4.0 would indicate human source pollution, whereas a ratio of <0.7 would indicate non human source pollution**[7]**. More traditional method for discriminate bacteria**[8]** have included biochemical tests**[9]**, phage susceptibility**[6]**, outer membrane protein profiles**[1]**, multiple antibiotic resistance**[5,10]** fimbriation**[11]**, bacteriocin production and susceptibility to antibiotics and other methods**[12]**. The survival of human enteric bacteria in the aquatic environment has attracted much interests in view of its public health significance**[13,14]**. Enteric bacteria exposed to the marine environment simultaneously encounter a variety of an abiotic and biotic challenges, among the former, light appears to be critical in affecting see water survival. Previous growth history plays a major part in pre-adaptation of the cells, and stationary phase cells are generally more resistant than exponentially growing ones. Predation mostly by protozoa is probably the most significant biotic factor**[15]**. The rate of *E. coli* die off increases rapidly as solar radiation increases. Conversely the rate of die off of *Enterococci* did not increase as the intensity of sunlight increases**[16]**. Fecal microorganisms differed in their sensitivity to light in sea water and greater sunlight exposure is required to inactivate *Enterococci* compared to fecal Coliform**[16,17]**. Coastal zone management is an important issue for the development of Gaza region. The coastal region and beach of Gaza strip is currently used for disposal of wastewater. In the same time, the anthropogenic activities such as urbanization, Agriculture, and industrial processing have resulted in increase inputs of both chemical and biological pollutants to seawater**[18]**. More information are available on the microbial sanitary conditions in selected area of the Gaza Strip coastal waters including the prevailing microbial communities and pollution. Counts and assessments **[19,20,21]**. Other studies conducted in Arab countries **[22]** in the study of Arabian Gulf seawater pollution reported that bacterial counts had distinct patterns peaking in Spring and Autumn and diminishing during Summer and Winter; total and fecal coliform counts fluctuated depending on the presence of nearby recreation area, while**[23]** in their survey to Al Aqaba seawater reported that occurrence of enteric indicator organisms of total coliform and fecal coliform and enteric pathogenic bacteria. In assessing levels of pollution (Zoffmann et al. 1989) **[24] c**lassified marine beach contamination with fecal coliform as follows: Beaches with cell counts of less than 100/L free from contamination, 100 to 1000 /L, slightly contaminated, 2000 to 10,000/L highly contaminated, and ,more than 10,000/L dangerously contaminated. The goals of this study were to evaluate the rate of microbial pollution in sea water, more detailed information concerning their assemblage with respect to their abundance and to assess their diversity and variability in relation to hydrographic parameters.

Materials and Methods

Seawater Samples: The study was carried out during the period October 2003 and September 2004. A total of 1200 seawater samples were collected from different stations. The seawater samples collection were as the following: 100 water samples per month, 20 samples from each station every month. The sampling period was carried out twice per month. Seawater samples were collected 2 meters from a shore at a depth of 45cm using 10 sterile 100 polypropylene bottles for each Sample to ensure that replication within the container would not affect the results. Samples were placed in the dark on ice and processed within 6 hours of collection in adherence to *APHA(1995) standard methods***[25]**. Five sites (Stations) were selected along the sea shoreline in Gaza strip of Palestine**[26]**. The stations were chosen along the coastline to ensure the samples to be representative for the whole under investigated area. These stations comprised station I (Beitlahia), station II (Jabalia), station III (Soudania), station IV (Beach Camp) and station V (Sheikh Ejleen).

Gaza Strip is located at the Eastern side of the Mediterranean Sea.

Analysis of Seawater Samples The Sea water samples were examined for the detection of total Coliform, fecal Coliform, and *Fecal streptococci*.

1-Total Coliform determination: A 100 ml of seawater was vacuum filtered in duplicate through 0.45 µm sterile membrane filter (Cellulose nitrate filters, type Whatman) **[27]** and placed on M-Endo media. The plates were incubated for 18-24hr at 35^0C , colonies with red color were suspected to be Coliform and were confirmed by using Gram stain and a portion of the colony was inoculated into lactose fermentation test tubes containing lauryl sulphate broth, also API 20E was used for identification.

2-Fecal Coliform determination: A 100ml of seawater was passed through the membranes which were placed on the surface of M-FC media. The plates were incubated for 18-24hr. at 44-45^0C, the suspected colonies with the light blue-blue color were confirmed by EC broth and tubes were incubated for 18-24hr. at 44-45^0C, air bubbles in the Durham tubes indicated positive results for fecal Coliform.

3-Fecal Streptococci determination: A 100ml of Seawater was filtered and the membranes were placed with the forceps on the surface of M-Enterococcus agar media. The plates were incubated for 18-24hrs at 35^0C. The suspected colonies with light red-red color were counted and confirmed by subculture on bile Esculin agar. The colonies of fecal streptococci had a black color and catalase negative test.

Statistical analysis: A computer program was used for data analysis. The descriptive data was given as a log mean, standard deviation, the chi square test and standard error were used for the analysis assessment

Fig (1) A Map for the coastal area of Gaza Strip shows the sampling location
Map key: Station I: Beitlahia, Station II: Jabalia, Station III: Soudania, Station IV: Beach Camp, Station V: Sheikh Ejleen

Results

The bacterial population recorded during the study period are illustrated in the tables (1,2,3).

a-Total Coliform:

The results of the total Coliform by different stations and seasons are recorded in table (1).

Table (1): Seasonal variations of the total Coliform (CFU/100ml^3) mean values at different stations.

	Station I	Station II	Station III	Station IV	Station V
Winter	12000 (10000-14000)	11000 (9000-13000)	14000 (13000-15000)	15000 (14500-15500)	11800 (11600-12000)
Spring	13000 (12000-14000)	14000 (13500-14500)	12000 (11000-13000)	14000 (13000-15000)	12000 (11000-13000)
Summer	6000 (5000-7000)	6500 (6000-7000)	4800 (4400-5200)	6900 (5900-7900)	5800 (4800-6800)
Autumn	4800 (4200-5400)	6200 (5800-6600)	4900 (4500-5300)	7000 (6000-8000)	6200 (5800-6600)

Bacterial count of total Coliform was ranged between 4800 CFU/100ml and 15000 CFU/100ML. The highest mean value was obtained at station **IV during winter season** while the lowest mean value was recorded at station **I during Autumn.**

b-Fecal Coliform

The results of the mean value of the fecal Coliform by different stations and seasons are present in table 2.

Table 2: Seasonal Variation of the fecal Coliform (CFU/100ml^3) mean values at different stations

	Station I	Station II	Station III	Station IV	Station V
Winter	9000 (8500-9500)	8900 (8500-9300)	11000 (1800-11200)	12000 (11800-12200)	8000 (7700-8300)
Spring	7800 (7500-8100)	10000 (9000-11000)	10000 (9500-10500)	11000 (10000-12000)	9000 (8900-9100)
Summer	4800 (4600-5000)	4000 (3900-4100)	4000 (3800-4200)	3000 (2900-3100)	2000 (1800-2200)
Autumn	4300 (4000-4600)	2100 (2000-2200)	3800 (3500-4100)	2300 (2100-2500)	3000 (2900-3100)

The distribution of the fecal Coliform mean value in the seawater was varied , the highest mean value was 12000 CFU/100ml obtained at station **IV during winter** while the lowest mean value was 2000CFU/100ml obtained at station **V during summer season.**

c-Fecal Streptococci

The results of the mean total count of *fecal streptococci* by different stations and seasons are shown in table (3).

Table3: Seasonal variation of the *fecal streptococci* (CFU/100ml^3) mean values at different stations.

	Station I	Station II	Station III	Station IV	Station V
Winter	4200 (4000-4400)	6000 (5900-6100)	6100 (6000-6200)	4000 (3800-4200)	3500 (3300-3700)
Spring	4100 (4000-4200)	6500 (6300-6700)	5000 (4900-5100)	6000 (5800-6200)	4100 (4000-4200)
Summer	2100 (2000-2200)	2000 (1800-2200)	2000 (1900-2100)	1100 (1000-1200)	1000 (900-1100)
Autumn	2500 (2300-2700)	1800 (1600-2000)	1900 (1800-2000)	1200 (1000-1400)	1200 (1100-1300)

The distribution of fecal Streptococci among different stations was varied. The highest mean value was recorded at station **II (6500CFU/100ml)** during spring season while the lowest mean value was recorded during summer season at station **V.**

Discussion

Mediterranean Sea is an open sea and it's relatively higher in osmotic pressure, low in nutrients and quite cold at great depth, the pH also tends to be higher than is optimal for most microorganisms. Much of the microscopic life of the sea water is composed of photosynthetic diatoms and other algae largely independent of preformed organic nutrient sources, these organisms use energy from photosynthesis and atmospheric, carbon dioxide. The basis of the seawater food chain sea bacteria it benefit from the eventual death and decomposition of phytoplankton **[13]**. The lack of operational and effluent wastewater treatment plant makes wastewater the main sources of pollution of the coastal zone of Gaza Strip. The wastewater is discharged untreated or partially treated along the shoreline resulting in pollution of most of the shoreline. In addition to the treatment plants effluents. There are more than 20 individual sewage drains ending either on the beach or a short distance away in the surface zone. Higher percentage of the waste water is generated in Gaza Strip is currently discharged without treatment into the sea (50,000 cubic meters per day) **[18]**. Only about 40% of the sewage generated in the Gaza Strip is properly treated **[18]**. The result of this study demonstrate that localized pollution by pathogenic bacteria from waste and eutrophication effects can be severely along the beach shoreline and such conditions may prevail throughout the coastline of the Gaza Strip, because the results of the total Coliform, fecal Coliform, and *fecal streptococci* recorded exceeds the permissible limit recommended by APHA, US EPA. The pollution resulted in damaging not only the landscape of the sole recreational places in Gaza but also destroying the marine fauna and flora. The potential risk for these contaminants on public health and marine life are indeed of more attention at the national level. The results of the study showed that the highest rate of pollution recorded was during the winter season (total Coliform 15000 CFU/100ml) which is the rainy season in the area. Rainfall can have a significant effect on indicator densities in recreational waters can be increased to high levels because animal wastes are washed from forest and Pasteur land and urban setting or because treatment plants are overwhelmed and causing sewage to bypass treatment, in either case, the effect of rainfall on beach water quality can be quite dramatic. Coliforms and thermotolerant Coliforms are known to have extra-external sources, these two indicators groups can grow to very high densities in industrial wastewaters such as those discharged by pulp and palper millis. *E. coli* and Enterococci are not usually associated with industrial wastewaters but some investigator believe that these indicators can grow in soil in tropical climates, under any of these conditions where the source of the indicator is other than the feces of warm blood animals. Fecal Coliform (FC) and *Fecal streptococci* (FS) ratio was used to differentiate between the source of pollution i.e. the ratio FC/FS > 2.0, this ratio is not prove the pollution of human origin due to greater persistence of Enterococci than of fecal coliforms or *E. coli* in saline water has been documented **[28].** In contrast one study demonstrated that Enterococci from waste stabilization pond effluent had a greater inactivation rate in seawater than fecal coliforms or *E. coli* **[29]**. The results of the preceding studies and work conducted by (Anderson et al. 2005) **[30]** suggest that persistence characteristics of Enterococci are heterogeneous which lead to incomplete understanding. Therefore we have a doubtful the useful of used the ratio of fecal Coliform/fecal streptococci. The natural self purification of sea water is caused by diverse physical, chemical, and biological factors. The period of survival of enteric bacteria and other bacterial indicator of fecal pollution are highly variables ranging from fractions of hours to weeks depending on the specific characteristics of each organism and on several other factors like light in summer **[31]**. Sunlight inactivation in seawater of fecal coliform, Enterococci, *E. coli* and also somatic coliphage was found to be 10 times higher than the corresponding dark inactivation. The overall ranking (from greatest to least inactivation) was as follows, Enterococci, fecal coliform ≥ *E. coli*, and somatic coliphage **[8]**.

The *fecal streptococci* organisms present in water indicate that the water has been polluted with feces (sewage); their incidence was in parallel with the fecal coliform and has the same indicative power of the fecal Coliform. Some environmental organizations believe that the *fecal streptococci* represent the true evidence for fecal Coliform. This is because the differential die off for enterococci is not as great as that for *E. coli* **[32,33,34].**

Other factors playing a role in determining the number of bacteria, are the temperature especially high temperature in summer as it encourages the organisms to multiply and increase rapidly this is supported by experiment conducted by (Carlucci and Pramer, 1960) **[35]** in 48hr experiment in natural seawater (5-40^{o}C) and by (Vasoncelos and Swartz, 1976) **[36]** in a 6 day experiment. The availability of nutrient, particularly phosphates, nitrates by marine bacteria which operates at high rate in summer and the ratio of die off rates of bacteria in the marine environment decreases. In addition there are factors of heavy tidal and flushing coursing waves which accelerate the cleaning and rapid dispersion of wastes. Bacterial activity will exhaust the oxygen (decrease in dissolved in seawater) and increase the BOD (Biological Oxygen Demand), therefore these parameters may be used to express the rate of microbial pollution **[37]**

Conclusion

Contamination of recreational water by fecal pollution is a serious public health concern, and monitoring for actual pathogens is not feasible. We need to rely on an indicator organisms that will not replicate in the environment and is easy to detect despite the limitations. Fecal indicator bacteria may be better than other methods currently in use.

References

1-Carson C. A.; Shear, B. L.; Ellersieck, M. R.; and Asfaw, A. (2001) Identification of feca*l E.coli* from humans and animals by ribotyping. Applied and Environmental Microbiology. 67,(4):1503-1507

2-Chesceri, L. S.; Greenberg, A. E.; and Eaton, A. D. (1998) *Standard methods for examination of water and wastewater* 20th ed. P: 9-32 and 9-75. American Public Health Association Washington D.C.

3-Anderson, K. L.; Whitlock, J. E.; and Harwood, V. J (2005) Persistence and differential survival of fecal indicator bacteria in subtropical waters and sedimentas. Applied and Environmental Microbiology. 71,(6): 3041-3048

4-Bartram J.; and Ress G. (ed). (2000) Monitoring bathing water: Practical guide to the design and implementation of assessments and monitoring programs.113-167 . Routledge, NewYork N. Y.

5-Sayah, R. S.; Kaneene, J. B.; Johnson, Y.; and Miller, R. (2005) Patterns of antimicrobial resistance observed in *E. coli* isolates obtained from domestic-and wild-animal fecal samples, human septage, and surface water. Applied and Environmental Microbiology. 71,(3):1394-1404.

6-Allwood, P. B.; Malik, Y. S.; Hedberg, C. W.; and Goyal, S. M. (2003) Survival of F specific RNA coliphage, Feline calivirus and *E. coli* in water: a comparative study. Applied and Environmental Microbiology. 69,(9):5707-5710

7-Daby, D.; Turner, J.; and Jago, A. (2002) Microbial and Nutrient pollution of coastal bathing waters in Mauritus .Environmental International 27: 555-566

8-Ishii, S.; Ksoll, W. B.; Hicks, R. E.; and Sadowsky, M. J. (2006) Presence and growth of naturalized *E. coli* in temperature soils from lake superior watersheds. Applied and Environmental Microbiology. 72,(1):612-621.

9-Trepeta, R.W.; and Edberg, S. C. (1984) Methylumbelliferyl-β-D glucuronide-based medium for rapid isolation and identification of E. coli. J. clin. Microbiol 19: 172-174.

10-Choi, s.; Chu. W.; Brown, J.; Becker, S. J.; Harwood, V. J.; and Jiang, S. C. (2003) Application of enterococci antibiotic resistance patterns for contamination source identification at Huntington beach. California Mar. Pollut. Bull. 46:748-755.

11-Latham, R. H.; and Stamm, W. E. (1984) Role of fimbriated E. coli in urinary tract infections in adult women correlation with localization studies. J. Infect. Dis. 149: 835-840

12-Govan, J. R.; and Harris, G. W. (1985) Typing of Pseudomonas cepacia by bacteriocin susceptibility and production. J. Clin. Microbiol. 22: 490-494

13-Olsen, I. E.; Brown, D. I.; Baggesen, D. I.; and Bisgaard, M. (1992) Biochemical and molecular characterization of salmonella enteric serovar berta and comparison of methods for typing. Epidemiol Infect. 108: 243-260

14-Davies, R. J.; Bell, R. G.; and Donnison, A. M. (1994) Sunlight inactivation of Enterococci and fecal Coliforms in sewage effluent diluted in seawater. Applied and Environmental Microbiology. 60, 2049-2058

15-Davies, C. M.; and evison L. M. (1991) Sunlight and the survival of enteric bacteria in natural waters. Journal of Applied Bacteriology, 70: 265-274

16-Sinton, L. W.; Hall. C. H.; Lynch. P. A.; and Davies colley, R. T. (2002) Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saliva waters. Applied and Environmental Microbiology. 68,(3):1129-1131.

17-Fong, T. T.; and Lipp, E. K. (2005) Enteric viruses of humans and animals in aquatic environments health risks, detection an potential water quality assessment tools. Microbiol. Mol. Biol. Rev. 69:357-371.

18-Palestinian Ministry of Environmental Affairs (2001) *Assessment of land based pollution Sources.* Gaza Strip.

19- Afifi, S. (1998) *Identification and evaluation of seawater beach quality state in Gaza Governate.* Environmental and Rural Research Center. Islamic University-Gaza.

20-Afifi, S.; El Manama, A. A.; and Shubair, M. E. (2000) Microbial assessment of beach quality in Gaza. Egypt. J. Med. Lab. Sci. 9,(1):51-63

21- Ajour, M.; and Darabih, S. (1997) *Strategy for coastal zone planning and management in Gaza Strip Palestine proceedings of the third international conference on the Mediterranean coastal environment med coast* 97 Nov. 11-14

22- Banat, I. M.; Hassan, E. S.; El Shahawi, M. S.; and Abu Hilal, A. H. (1998) Post-Gulf war assessment of nutrients, heavy metal ions, hydrocarbons, and bacterial pollution levels in the United Arab Emirates Coastal Waters. Environmental International 24,(112):109-116.

23- Al Moghrabi, S. M.; and Horani, H. K. (1997) A follow up on the distribution of coliform bacteria along the jordnian Coastline of the Gulf of Aqaba. M'utah Journal for Research and Studies. 12,(1):221-241.

24- Zoffmann, C.; Rodrigue, Z.; Valera, F.; Peterfillol, M.; Ruia-Bavia, F.; Torreblanca, M.; Colom, F. (1989) Microbial and nutrient pollution along the coast of Alicante, Spain. Mar. Pollut. Bull. 20:74-81.

25- American Public Health Association. (1995) *Standard method for the examination of water and wastewater* 19th ed. American Public Health Association. Washington D.C.

26- GEP (Gaza Environmental Profile) Part one, "*Inventory of resources*". Palestinian Environmental Protection Authority, Euroconsult/Iwaco, 1994

27- Al- Moghrabi, S.; and Horani, H. K. (1997) A follow up on the distribution of Coliform bacteria along the Jordanian coastline of the Gulf of Aqaba. Mu'tah Journal for Research and Studies 12,(1):1-13

28- Davies, C. M.; Long, J. A.; Donald, M.; and Ashbolt, N. J. (1995) Survival of fecal microorganisms in marine and fresh water sediments. Applied and Environmental Microbiology. 61:1888-1896.

29- Sinton, L. W.; Hall, C. H.; Lynch, P. A.; and Davies, R. J. (2002) Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline water. Applied and Environmental Microbiology. 68.1122-1131.

30- Anderson K. L.; Whillock, J. E.; and Harwood, V. J. (2005) Persistance and differential survival of fecal waters and sediments. Applied and Environmental Microbiology. 71,(6):3041-3048.

31- Whitman, R. L.; Nevers, M. B.; Korinek, G. C.; and Byappanahalli, M. N. (2004) Solar and temporal effects on *E. coli* concentration at a lake Michigan swimming beach. Applied and Environmental Microbiology. 70,(7):4276-4285.

32- Keswick, B. H.; Gerbac. P.; Secor, S. L.; and Cech, A. (1982) Survival of enteric viruses and indicator bacteria in groundwater. Journal of Environmental Science and Health. 17,(6):903-912.

33- Omura. T.; Onuma, M.; and Hashimoto, A. (1982) Viability and adaptability of *E. coli* and Enterococci group to salt water with high concentration of sodium chloride. Water Sci. Tech. 14:115-126.

34- Britton, G. Farrah. S.; Rusk, T.; Butner, J.; and Cohn, J. (1983) Survival of pathogenic and indicator organisms in groundwater. Groundwater 21:405-410.

35- Carlucci, A. F.; and Pramer, D. (1960) An evaluation of factors effecting the survival of *E. coli* in seawater. Experimental procedures. Applied and Environmental Microbiology. 8,243-247.

36- Vasconcelos, G. J.; and Swartz, R. G. (1976) Survival of bacteria in seawater using a diffusion chamber apparatus in situ. Applied and Environmental Microbiology. 31.913-920

37- Kataria, H. C. (1996) BOD and COD contents of bores-well water of Bhopal. J. Nature Conserve, 8,(1):69-72.