

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research Vol. 8, Issue, 11, pp. 21964-21968, November, 2017 International Journal of Recent Scientific Rerearch

DOI: 10.24327/IJRSR

Research Article

SCREENING OF PHYSIO-CHEMICAL PARAMETERS AND BACTERIAL POPULATION DENSITY OF WATER AND SEDIMENT SAMPLES FROM MANDAPAM COASTAL AREA (SOUTH EAST COAST) OF INDIA

Vishnuvardhan V¹., Mahalakshmi C² and *Jaikumar M³

¹Department of Humanities and Sciences, Annamacharya Institute of Technology and Sciences (Autonomous), Rajampet – 516 126, Andhrapradesh, India ²Sri Chaitanya Junior College, Anantapur – 515 001, Andhrapradesh, India ³CCAMP Incubator, NCBS-TIFR, SEA6 Energy Pvt Ltd., Bangalore – 560 065, Karnataka, India

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0811.1172

ARTICLE INFO

ABSTRACT

Article History:

Received 05th August, 2017 Received in revised form 21st September, 2017 Accepted 06th October, 2017 Published online 28th November, 2017

Key Words:

Total heterotrophic bacteria, Physicochemical parameters, South east coast, Mandapam coastal area. The present paper deals the total heterotrophic bacterial population and physico-chemical parameters of the Mandapam coastal area. Water and sediment samples were collected season wise from two different stations for analyzing the total heterotrophic bacterial population and various physico-chemical parameters such as Temperature, pH, Salinity, Dissolved Oxygen, Nitrite, Nitrate, Sulphate and Phosphate. The work highlights the condition of this coastal water in various seasons with respect to the parameters mentioned above.

Copyright © **Vishnuvardhan V., Mahalakshmi C and Jaikumar M, 2017**, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Explosion of human population in the last few decades has led to the fast reduction of land based resources at a distressing velocity and optimistically focusing on the utilization of marine living resources. The Marine ecosystem, which is considered as the 'Mother of origin of life', is also a source of structurally unique natural products which are mainly accumulated in living organisms. However, People perceive the ocean as a bottomless pit, which can accumulate and assimilate unlimited quantity of pollutants and therefore, they go on releasing waste into it, causing harm to marine ecosystem and human health.

Numerous human diseases having bath in rivers, lakes, ponds and coastal sea waters in the area of river and sewage inflow, swimming pools are associated with the presence of opportunistic pathogenic microorganisms, being able to generate infections by contact with skin, mucous membrane, nasopharyngeal cavity, respiratory ducts, eyes, ears and urogenital passages. Pyogenic infection of injuries, meningitis, urinary system, respiratory system, inflammation of the middle ear and eyes are typical diseases caused by contaminated water where pathogenic microorganisms are found (Pellet *et al*, 1983; Wheater *et al*, 1979)

Pathogenic bacteria which can cause diseases like typhoid, dysentery, diarrhoea and cholera etc., are found in the coastal region. Generally, polluted water contains large number of pathogens. The most common indicator organisms are a group of microbes called as faecal coliforms, the presence of which simply indicates that the pathogens are expected to be present (Pisciotta *et al*, 2002). As coastal communities continue to expand rapidly, accurate quantification of faecal contaminants in near shore marine waters will continue to be a pressing public health and environmental concern (Nallathambi *et al*, 2002).

Microbiological quality and safety of sea food, is a must and should to save the risk of contamination of the systems for drinking water and harvesting of sea food specially from human health. It is crystal clear that economically loss is apparent owing to closure of beaches and stopping the shellfish

^{*}Corresponding author: Jaikumar M

Department of Humanities and Sciences, Annamacharya Institute of Technology and Sciences (Autonomous), Rajampet - 516 126, Andhrapradesh, India

Vishnuvardhan V., Mahalakshmi C and Jaikumar M., Screening of Physio-Chemical Parameters And Bacterial Population Density of Water And Sediment Samples From Mandapam Coastal Area (South East Coast) of India

harvesting areas. Contamination of water with human faeces has been considered as a risky factor to human health, because they are supposed to contain human specific enteric pathogens. This is imperative to conduct programmes for water quality surveillance to evolve, regulate on coastal environmental protection (Nallathambi et al, 2002). Worldwide the scientists used to take bacterial indicators in finding the quality and to monitor. Total heterotrophic bacteria (THB) are the major components of marine ecosystem and they play an important role in the degradation of organic matter and in the recycling of nutrients in the sea and their abundance is influenced by biotic and abiotic environmental factors. The present investigation was carried out to provide the basic insights into the abundance, distribution and variations of total heterotrophic bacterial population in relation to physico-chemical parameters from Mandapam Coast (Palk Bay).

MATERIALS AND METHODS

Description of the study area

Mandapam (palk bay)

The present investigation was carried out from Mandapam, a doorway to the sacred island of Rameswaram, a quiet and beautiful sea beach in the Ramanathapuram district of Tamil Nadu (Fig 1). Once a ruling ground of British personnel, the place today attracts huge number of visitors from all over the world. Connecting a hall in Tamil, Mandapam is characterized by natural coral reefs. Mandapam is situated ($79^\circ 8E - 9^\circ 17'N$) on a narrow tongue of land projecting from the southern part of the east cost of India (Fig 1). To the north of this peninsular extension is the Palk bay and to the South the Gulf of Mannar. In the present investigation two stations situated in Palk bay (Mandapam Coast) was selected (Station-1 and Station-2) to study the ecological parameters and total microbial density.

For the sake of convenience and interpretation, a calendar year of study was divided into four seasons in Tamilnadu. This kind of grouping viz;

Monsoon (October-December) Postmonsoon (January - March) Summer (April-June) Premonsoon (July-September).



Figure 1 Study Area: Mandapam coastal area Staion 1 and 2

Collection of samples for Bacteriological and Physicochemical analysis

Collection of samples of water and sediments were made every month for a period of one year (July 2004 to June 2005) from two different Stations 1 and 2 of Mandapam coast. For bacteriological and physico-chemical analysis, water samples were collected in sterile capped bottles just below the water surface leaving enough air space in the bottles to allow thorough mixing. Precautionary measures were taken to minimize the contamination through handling.

Sediment samples were collected by employing an alcohol rinsed and air-dried small Peterson grab. The central portion of the collected sample was aseptically transferred into new sterile polyethylene bags using a sterile spatula for bacteriological and physico-chemical analysis.

Collection of rainfall data

Rainfall data of Mandapam (Palk Bay) was obtained from the Meteorological Department of Pampan (Rameswaram Island).

RESULTS AND DISCUSSION

Monthly variation in rainfall is shown in Fig 2. The total annual rainfall was 1626.6 mm. The rainfall recorded during the study period was 1487.01 mm in monsoon, 83.47 mm in post monsoon, 9.3 in summer and 53.8 in premonsoon season. The present study area received the highest rainfall during the northeast monsoon period. The rainfall during the premonsoon and summer months was very poor.



Figure 2 Monthly variation in rainfall recorded from June 2004 to July 2005 at Pampan.

Total heterotrophic bacterial population density in water varied from 5.6 to 14.6 $\times 10^{6}$ CFUml⁻¹. At station 1, the minimum population density of THB (5.4 $\times 10^{6}$ CFUml⁻¹) was recorded during the premonsoon season (August) and the maximum (14.6 $\times 10^{6}$ CFUml⁻¹) was recorded during the monsoon season (November). At station 2, the minimum density (5.4 $\times 10^{6}$ CFUml⁻¹) was recorded during the premonsoon season in the month of September and the maximum density (11.6 CFUml⁻¹) during the monsoon season in the month of November.

Seasonal mean population density of total heterotrophic bacteria in water was worked out at station 1, the minimum density $(5.9 \times 10^6 \text{ CFUml}^{-1})$ was recorded during the premonsoon season and the maximum density (11.3 $\times 10^6 \text{ CFUml}^{-1}$), was noticed during the monsoon season. At station 2, the minimum density (5.5 $\times 10^6 \text{ CFUml}^{-1}$) was recorded during the premonsoon season and the maximum density (8.46 $\times 10^6 \text{ CFUml}^{-1}$) was observed during the monsoon season.

In general, primary peak-point was observed during the monsoon season at both the stations.

Population density of total heterotrophic bacteria in sediments fluctuated between 1.9 and 8.1x10⁸ CFUg⁻¹. At station 1, the minimum population density (2.3 x10⁸ CFUg⁻¹) was recoded during the premonsoon season in the month of September and the maximum density (8.1x10⁸ CFUg⁻¹) was observed during the monsoon season in the month of November. At station 2, the minimum population density $(1.9 \times 10^8 \text{ CFUg}^{-1})$ was noticed during the premonsoon season in the month of August and the maximum density $(5.9 \times 10^8 \text{ CFUg}^{-1})$ during the monsoon season in the month of November. Seasonal mean population density of total heterotrophic bacteria in sediments was also worked out. It ranged between 2.1 and 7.6 x10⁸ CFUg⁻¹. At station 1, the minimum density (2.7 x10⁸ CFUg⁻¹) was recorded during the premonsoon season and the maximum density $(7.6 \times 10^8 \text{ CFUg}^{-1})$ was registered during the monsoon season. At station 2, the minimum density $(2.1 \times 10^8 \text{ CFUg}^{-1})$ was recorded during the premonsoon season and maximum density $(5.5 \times 10^8 \text{ CFUg}^{-1})$ was recorded during the monsoon season. In general, station 1 recorded higher THB population density than station 2. The primary peak of THB population density in sediments was observed during the monsoon season and the secondary peak was noticed during summer season.

The marine environment is a complex system mainly influenced by a variety of physical, chemical and biological processes. One of the basic goals of ecology is to understand the factors which play their role in the distribution pattern of organisms (Varadachari et al, 1987). Environmental conditions also play an important role in promoting the occurrence and abundance of commercially exploitable marine resources (Kannan and Kannan, 1996). In the present study the percentage composition of THB of the coastal waters were examined (Fig 3 and 4). It was found to be very diverse and complex. However the ecological factors such as salinity, pH, dissolved oxygen, temperature, rainfall and nutrients may influence the growth of all microorganisms (Table 1 and 2). The interwoven factors for the physico-chemical parameters are involved in determining the distribution of living components of an ecosystem. Knowledge on the environment is of utmost importance to understand the distribution and colonization of marine microorganisms.







Fig 4 Percentage Composition of THB genera isolated from water and sediment samples from Station 2.

Figure 4 Percentage Composition of THB genera isolated from water and sediment samples from Station 2 Mandapam coast (Palk Bay).

Atmospheric temperature varied between 26 and 33.5 °C at both the stations. The maximum atmospheric temperature (33.5 °C) was recorded during the summer season and the minimum atmospheric temperature (26.0 °C) was recorded during monsoon season. In general, atmospheric temperature increased during the summer and premonsoon seasons and decreased during the other seasons. This could be attributed to ocean's large thermal inertia which causes a lag between the absorption and the subsequent release of solar energy to the atmosphere (Rajapandian et al, 1990) during the summer season. Surface water temperature was largely determined by the amount of incoming solar radiation. Water temperature variation seems to be influenced by many factors such as freshwater influx, solar radiation, warming, evaporation and cooling and mix up with the ebb flow from the adjoining neritic waters (Bowman et al, 1982). It ranged between 25.5 and 33.0 °C.

Hydrogen-ion concentration in water remained alkaline throughout the study period. Monsoon season recorded low pH while summer and premonsoon seasons recorded high pH. Station 1, recorded relatively higher pH than station 2. It may be due to the removal of CO_2 by photosynthesis, and the low pH, due to the dilution of sea water by fresh water flow during the monsoon season (Gopinath *et al*, 2000). The pH varied between 7.8 and 8.8. The values obtained in the present study are comparable with the values reported by earlier workers (Goyal *et al*, 1977).

Salinity influence the distribution of bacteria in tropical estuaries, bays and coastal ecosystems, since these water bodies are characterized by distinct short term (diurnal) and long term (seasonal) fluctuations. At station 1 and 2, minimum salinity was recorded during the monsoon season and the maximum during the summer season. It varied between 27.68 and 34.79 ppt. During the monsoon period almost the rain water were added to the sea and it reduces the salinity concentration. The earlier study reports of physico-chemical parameters from Mangalore waters showed that the salinity values were observed lower during the monsoon season. This may be due to the influence of southwest monsoon (Mridula *et al*, 2002).

Vishnuvardhan V., Mahalakshmi C and Jaikumar M., Screening of Physio-Chemical Parameters And Bacterial Population Density of Water And Sediment Samples From Mandapam Coastal Area (South East Coast) of India

 Table 1 Ecological parameters and total heterotrophic bacteria of water and sediment samples from Station 1 Mandapam, coast (Palk Bay).

Parameter	Tempera	ture (°C)	pН	Salinity (ppt)	D.O. (mg/l)	NO ₂ (µg/l)	NO3 (μg/l)	SiO ₃ (µg/l) ^I	n org. PO (µg/l)	⁴ THB-W	THB-S
July 2004	32.0	30.5	8.53	30.67	6.98	0.19	2.76	12.40	0.21	165.7	118
August	31.5	31.0	8.45	32.45	7.10	0.23	3.14	11.12	0.16	165	123
September	32.0	30.6	8.55	32.64	6.72	0.24	2.17	8.07	0.17	154.9	93.1
October	30.5	29.5	8.30	28.87	4.78	0.14	1.54	8.60	0.16	192	259
November	28.5	28.0	8.26	27.90	5.26	0.18	1.91	10.20	0.36	296.2	331
December	27.0	26.4	8.10	28.32	5.88	0.23	1.59	13.60	0.24	184.3	282
January 2005	30.5	29.0	8.45	30.44	6.11	0.21	1.78	12.50	0.23	105.3	107
February	31.5	30.5	8.40	32.54	6.78	0.15	1.41	10.2	0.21	181.7	169
March	30.0	30.0	8.75	33.20	7.83	0.11	0.81	8.70	0.23	128.5	113
April	32.7	31.5	8.82	33.52	8.22	0.12	0.98	7.62	0.18	234.4	134
May	33.5	33.0	8.50	34.79	8.75	0.14	1.47	6.03	0.16	201.8	181
June	31.5	30.6	8.40	32.86	8.48	0.21	2.34	10.09	0.19	257.1	146

W-water, S-sediment

 Table 2 Ecological parameters and total heterotrophic bacteria of water and sediment samples from Station 2 Mandapam coast (Palk Bay).

				G 1' ''		NO	NO	G:O	т		
Parameter	Temperat	ture (°C)	pН	Salinity (nnt)	D.O. (mg/l)	NO ₂ (μσ/l)	NO3 (μσ/l)	S1O3 (119/1)	In org. PO.(ug/L)	THB-W	THB-S
X 1 2004	22.5	22.0	0.40		Z 02	(µ <u>s</u> /1)	(µ <u>s</u> /1)	(µ ₅ /1)	104(µg/L)	1.40	0.1
July 2004	32.5	32.0	8.40	32.15	7.82	0.17	2.09	12.32	0.18	142	94
August	31.6	31.2	8.30	32.40	7.63	0.21	3.02	10.08	0.11	138.3	81.1
September	31.5	30.0	8.46	32.55	6.52	0.22	2.18	8.01	0.12	129.4	86
October	30.5	29.5	8.10	27.68	4.88	0.13	1.34	8.54	0.13	260	189
November	28.5	28.0	8.00	28.01	5.19	0.15	1.41	12.14	0.31	176.6	188
December	26.0	25.5	7.88	29.48	5.68	0.20	1.29	13.52	0.19	158.9	150
January 2005	32.0	31.5	8.54	30.34	6.76	0.18	1.61	10.43	0.18	95.4	96.7
February	31.5	30.5	8.50	32.45	6.88	0.12	1.01	10.11	0.17	123.5	102
March	30.5	30.0	8.58	33.10	7.63	0.10	0.61	8.62	0.20	114	111
April	32.0	31.2	8.77	33.46	8.32	0.11	0.90	7.32	0.16	206	150
May	33.0	32.5	8.48	34.04	8.76	0.14	1.40	6.01	0.12	164.3	114
June	31.5	30.0	8.20	33.80	8.85	0.20	1.63	10.02	0.14	194	169

The quantity of dissolved oxygen in the sea-water was a main factor that influences the microbial growth. Dissolved oxygen concentration was high during the summer season probably due to the photosynthetic activity of the organisms (Johannes and Webb, 1970). The DO ranged between 4.78 and 8.85 ml 1^{-1} . At station 1 and 2, the minimum DO recorded during the monsoon season and the maximum during the summer season. Lower dissolved oxygen concentration recorded during the monsoon season was due to the low rate of photosynthesis, high rate of oxidation of detritus and respiration of bottom communities along with the slow diffusion of dissolved gases. The primary production was a main factor that controls the level of dissolved oxygen. Previous studies reported that the primary production in most of the estuaries was less during the monsoon period due to low light intensity and dissolved oxygen (Bhattathiri, 1992).

Availability of nutrients is one of the primary factors regulating the growth, reproduction and biochemistry of all marine organisms (Kannan and kannan, 1996). Nutrients showed, an increasing trend from surface to the bottom particularly at farthest stations in the coastal region, which was due to their uptake at surface and demineralization in the water column (Padmavathi and Satyanarayana, 1999). In the present study the nutrients like nitrate, nitrite, inorganic phosphate and reactive silicate were analysed. Earlier studies reported that various nutrient concentrations studied were greater in the monsoon or post- monsoon seasons (Gopinath *et al*, 2000).

Dissolved inorganic phosphorus concentration varied between 0.11 and 0.36 μ g/l at both the stations.

High concentration of inorganic phosphate observed during the monsoon and post monsoon seasons were due to the monsoonal flow of fresh water and land runoff. This was followed by a sudden decrease in nutrient concentration during the summer season, probably due to the utilization by micro and macro phytobenthic communities (Bowman et al, 1982). Present study nitrate concentration varied from 0.61 to 3.14 μ M. At both the stations, the minimum nitrate was observed during the summer season and the maximum were recorded during the monsoon and pre monsoon respectively. Nitrite concentration in water fluctuated between 0.10 and 0.24 µM. The minimum concentration of nitrite was recorded during the summer season and the maximum concentration during the monsoon season at both the stations. The earlier reports shows that nitrate concentrations were the highest and comparatively higher concentrations were observed in the bottom water of sea due to the decomposition of organic matter resulting in the release of the thermodynamically stable nitrogen species (Gopinath et al, 2000).

In the present investigation silicate concentration varied from 6.01 to $13.60 \ \mu g/l$ at both the stations. The minimum concentration of reactive silicate was recorded during the summer season in the month of May and the maximum concentration of reactive silicate was recorded during monsoon season in the month of December. Previous study reports showed that high silicate concentration was due to the addition of silica material by land run-off, which was caused by flood during the monsoon and subsequent post monsoon seasons (Kannan and Kannan 1996). During the summer season, reactive silicate concentration was poor due to the sizeable

reduction in the fresh water input and greater utilization of this nutrient by the marine fauna and flora for their biological activity.

The total heterotrophic bacterial population showed an ascending trend in counts in surface waters were recorded between July and December (Premonsoon and Monsoon). This could be ascribed to southwest and northeast monsoon rains, resulting in land drainage which gets discharged into the coastal environment. This is in concordance with the findings reported earlier at various situations (Goyal *et al*, 1977; Matson *et al*, 1978). Also concluded that storm water runoff brings more indicators and other bacteria, with factors accentuating their survival (Sinton *et al*, 2002).

CONCLUSION

In the present study, Station 1 reported more bacterial load than Station 2 because Station 1 is a famous south Indian tourist centre of Rameswaram, this area was polluted by the tourists from different parts of the country through faecal contamination and dumping waste. In addition to most of the hotels and house hold wastes are discharged into the sea and runoff water during monsoon season brings the animal wastes and human wastes that contain pathogenic bacteria.

References

- Bhattathiri, P.M.A. Primary production of tropical marine ecosystems. In: K.P. Singh and J.S. Singh (eds.), Tropical Ecosystems Ecology and Management, Wiley-Eastern Ltd., Delhi, India, 269-276, (1992).
- Bowman, M.J., Foster, B.A., and Lapennas, P.P. Development of Environmental Study Report: Part I, Environmental Information Natural Resources Institute Contribution No.446, University of Maryland, pp. 91, (1982).
- Gopinath, A., Joseph, N., Sujatha, C.H., and Nair, S.M. 2000. Forms of nitrogen (NO sub (3) super (-) -N; NO sub (2) super (-) -N and NH sub (2) CONH sub (2)-N) and their relations to A.O.U. in the Indian coastal waters of the Arabian Sea. *Chemistry in Ecology*, 18, 233-244.
- Goyal, S.M., Gerba, C.P., and Melnick J.L. 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. *Applied and Environment Microbiology*, 34, 139-149.
- Hari Muraleedharan, P., Abhilash., and Ramasubbu, R. 2010. Physio-chemical parameters and planktons analysis of Sea water of Thondi of Palk Bay, Tamil Nadu. *Journal of Bioscience Research*, 1, 20-24.
- Huttly, S.R. 1990. The impact of inadequate sanitary conditions on health in developing countries, World Health Statistics Quarterly, 43, 118-126.
- Johannes, R.E., and Webb, K.L. Release of dissolved organic compounds by marine invertebrates. In: Organic matter in natural waters (ed. O. Hood), University of Alaska Press, 13-51, (1970).
- Kannan, R., and Kannan, L. 1996. Physio-chemical characteristics of seaweeds beds of the Palk Bay, Southeast coast of India. *Indian Journal of Marine Sciences*, 25, 358-362.

- Matson, E.A., Horner, S.G., and Buck, J.D. 1978. Pollution indicator and other microorganisms in river sediment, *Journal of Water Pollution Control Fedaration*, 50, 13-20.
- Mridula, R.M., Katti, R.J., and Rajesh, K.M. 2002. Hydrography of the near shore waters of Panambur (Mangalore), South-west coast of India. *Indian Journal of Environmental Sciences*, 6, 61-68.
- Nallathambi, T., Eshwar, M., and Kuberaj, K. 2002. Abundance of indicator and general heterotrophic bacteria in Port Blair bay, Andamans, *Indian Journal of Marine Sciences*, 31, 65-68.
- Padmavathi, D., and Satyanarayana, D. 1999. Distribution of nutrients and major elements in riverine, estuarine and adjoining coastal waters of Godavari, Bay of Bengal, *Indian Journal Marine Sciences*, 28, 345-354.
- Pellet, S., Bigley, D.V., and Grimes, D.J. 1983. Distribution of *P.aeruginosa* in iverine ecosystem, *Applied Environmental Microbiology*, 45, 328.
- Pisciotta, J.M.D., Rath, F, Stanek, P.A., Flanery, D.M., and Harwood, V.J. 2002. Marine bacteria cause false positive results in the Colilert-18 rapid identification test for Escherichia coli in Florida waters, *Applied and Environmental Microbiology*, 68, 539-544.
- Rajapandian, M.E., Gopinathan, C.P., Rodrigo, J.X., and Gandhi, A.D. 1990. Environmental characteristics of edible oyster bed in and around Tuticorn. *Journal of Marine Biological Association of India*, 32, 90-96.
- Robert Earle Buchanan., and Gibbson, N.E. Bergey's manual of determinative bacteriology, 8th Edn, The Williams and Wilkins Company, Baltimore, 1268, (1974).
- Rose, J.B., Daeschmer, D.R., Easterling, F.C., Curriern, S., Lele, Patz, J.A. 2000. Climate and Water borne disease outbreaks. *Journal of the American Water Works Association*, 92, 77-87.
- 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, *Applied and Environmental Microbiology*, 68, 1122-1131.
- Strickland, J.D.H., and Parson, T.R. 1972. A practical handbook of seawater analysis, Bulletin of Fisheries Research Board of Canada, 167, 310.
- Varadachari, V.V., Kesava Rao, R., and Sen Gupta, D. 1987. Oceans and the Indian summer and monsoon: a review, contribution in marine sciences (Dr.S.Z. Quazim Sastyabdapurti felicitation volume), 141-174.
- Wheater, D.W.F., Mara, D.D., and Oragui, J.I. 1979. Indicator systems to distinguish sewage from storm water run-off and human from animal fecal material. In: A. James and L. Evison (Eds.), Biological indicators of water quality, John Wiley and sons, Chichester, England, pp. 21-27.
- World Health Organization. Cholera annual report 2000. Weekly Epidemiological Record, Vol 76, 233-240, (2001).
